GS1-01: Imlunestrant, an Oral Selective Estrogen Receptor Degrader (SERD), as Monotherapy & Combined with Abemaciclib, for Patients with ER+, HER2- Advanced Breast Cancer (ABC), Pretreated with Endocrine Therapy (ET): Results of the Phase 3 EMBER-3 trial

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Background: Imlunestrant is a next-generation, brain-penetrant, oral SERD and pure estrogen receptor (ER) antagonist that delivers continuous ER inhibition, including in ESR1-mutant cancers.

Methods: This phase-3, randomized, open-label trial (NCT04975308) enrolled patients (pts) with ER+, HER2- ABC that recurred or progressed on/after an aromatase inhibitor, alone or with a CDK4/6 inhibitor (CDK/6i). No other prior therapy for ABC was allowed. Pts were randomized 1:1:1 to imlunestrant (400 mg once daily [QD]), physician's choice standard-of-care (SOC) ET (fulvestrant or exemestane per label), or imlunestrant (400 mg QD) + abemaciclib (150 mg twice daily). Primary endpoints were investigator-assessed PFS of imlunestrant vs SOC in pts with ESR1 mutations (ESR1m) and all pts and of imlunestrant + abemaciclib vs imlunestrant in all concurrently randomized pts. Secondary endpoints included OS (tested if the corresponding PFS was statistically significant), PFS by BICR, ORR, and safety.

Results: Overall, 874 pts were randomized (imlunestrant, n=331; SOC, n=330; imlunestrant + abemaciclib, n=213), 60% received prior CDK4/6i (imlunestrant, 59%; SOC, 57%; imlunestrant + abemaciclib, 65%). A total of 256 pts had ESR1m (imlunestrant, n=138; SOC, n=118). Imlunestrant significantly improved PFS vs SOC in pts with ESR1m (HR, 0.62; 95% CI, 0.46-0.82; P<0.001; median PFS [mPFS] 5.5 vs 3.8 months). Imlunestrant did not significantly improve PFS in the overall population (n=661; HR, 0.87; 95% CI, 0.72-1.04; P=0.12). Imlunestrant + abemaciclib significantly improved PFS vs imlunestrant in all pts (n=426; HR, 0.57; 95% CI, 0.44-0.73; P<0.001; mPFS 9.4 vs 5.5 months), with benefit observed regardless of ESR1m or PI3K pathway mutation status and in CDK4/6i pretreated pts. Investigator and BICR assessments were consistent across all endpoints. In all pts with measurable disease, ORR was 12% for imlunestrant; 8% for SOC; and 27% for imlunestrant

+ abemaciclib. All OS analyses were immature and ongoing; favorable trends were observed for imlunestrant vs SOC in pts with ESR1m (31% events; HR, 0.55; 95% CI, 0.35-0.86; P<0.01 [not statistically significant]) and in all pts (23% events; HR, 0.69; 95% CI, 0.50-0.96; [not inferentially tested]). OS analyses were less mature for imlunestrant + abemaciclib vs imlunestrant (15% events; HR, 1.34; 95% CI, 0.81-2.21; P=0.25). In post-hoc analyses, imlunestrant had lower 12-month cumulative incidence of central nervous system progression vs SOC in pts with ESR1m (2% vs 7%; HR, 0.18; 95% CI, 0.04-0.90) and all pts (2% vs 3%; HR, 0.47; 95% CI, 0.16-1.38), though absolute numbers were small.

Common all-grade TEAEs with imlunestrant were fatigue (23% vs 13% SOC), diarrhea (21% vs 12%), and nausea (17% vs 13%), mostly grade 1. Notably, all-grade bradycardia (2% vs 0% SOC), photopsia (0% each), and dyslipidemia (7% vs 9%) were infrequent or not observed with imlunestrant. Common grade ≥3 TEAEs with imlunestrant were anemia (2% vs 3% SOC), and neutropenia (2% each). Common all-grade/grade ≥3 TEAEs with imlunestrant + abemaciclib were diarrhea (86%/8%), nausea (49%/2%), and neutropenia (48%/20%). Grade ≥3 TEAEs rates were 17% for imlunestrant, 21% for SOC and 49% for imlunestrant + abemaciclib. Discontinuation of imlunestrant and imlunestrant + abemaciclib due to AEs was low (4% and 6%, respectively). Conclusion: Imlunestrant significantly improved PFS vs SOC in pts with ESR1m, and imlunestrant + abemaciclib significantly improved PFS vs imlunestrant in all pts regardless of ESR1m status. Imlunestrant had a favorable safety profile alone and combined with abemaciclib, thus providing an all-oral targeted therapy option for ET-pretreated pts with ER+, HER2- ABC.

GS1-03: Pyrotinib or placebo in combination with trastuzumab and docetaxel for untreated HER2-positive metastatic breast cancer (mBC): prespecified final analysis of progression-free survival (PFS) of the phase 3 PHILA trial

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Background: The interim analysis of the PHILA trial (NCT03863223) revealed that pyrotinib (an irreversible tyrosine kinase inhibitor targeting EGFR, HER2, and HER4) in combination with trastuzumab and docetaxel (PyroHT) significantly improved PFS compared to placebo in combination with trastuzumab and docetaxel (HT) in untreated HER2-positive mBC patients (Ma et al., BMJ, 2023). However, the overall survival (OS) data was immature at that time. Here, we present the prespecified final analysis of PFS, as well as long-term efficacy and safety outcomes from the PHILA trial following an additional 2-year of follow-up.
Methods: The PHILA study was a randomized, double-blind, multicenter, phase 3 trial

conducted at 40 centers in China. Eligible patients were randomly assigned in a 1:1 ratio to receive either oral pyrotinib (400 mg once daily) or placebo, both in combination with intravenous trastuzumab (8 mg/kg in the first cycle and 6 mg/kg in subsequent cycles) and docetaxel (75 mg/m2) on day 1 of each 21-day cycle. The primary endpoint was investigator-assessed PFS. The data cutoff date for this final analysis of PFS was April 30, 2024.

Results: Between May 2019 and January 2022, 590 eligible patients were randomized and received their allocated treatment (297 patients in the PyroHT group and 293 patients in the HT group). The median follow-up duration was 35.7 months for the PyroHT group and 34.3 months for the HT group. The benefit of investigator-assessed PFS associated with the PyroHT group, compared to the HT group, was sustained in this final analysis (22.1 months [95% CI 19.3–27.8] vs 10.5 months [95% CI 9.5–12.4], HR 0.44 [95% CI 0.36–0.53]; 1-sided P<0.0001), meeting the protocol-prespecified criteria for statistical significance. The PFS rates were 92.8% in the PyroHT group and 84.1% in the HT group at 6 months, 74.3% and 46.8% at 1 years, 47.6% and 20.2% at 2 years, and 39.7% and 9.9% at 3 years, respectively. Additionally, the benefits in PFS with PyroHT were observed across nearly all analyzed subgroups. As of the data cutoff, 59 patients (19.9%) in the PyroHT group and 87 patients (29.7%) in the HT group had died. The OS exhibited superiority in the PyroHT group compared to the HT group, with a HR of 0.64 (95% CI 0.46–0.89; 1-sided P=0.0038). The estimated Kaplan–Meier OS rates were 96.6% in the PyroHT group and 94.5% in the HT group at 1 year, 88.7% and 84.1% at 2 years, 80.9% and 72.4% at 3 years, and 74.5% and 64.3% at 4 years, respectively. The safety profiles in the updated analysis remained consistent with those reported in the previous interim analysis in terms of frequency, severity, and specificity. No new safety signals were identified. Grade ≥3 treatment-related adverse events were reported in 270 patients (90.9%) in the PyroHT group and 227 patients (77.5%) in the HT group, with decreased neutrophil count (63.0% vs 64.8%) and decreased white blood cell count (53.2% vs 50.9%) being the most frequent. Grade 3 diarrhea was predominantly observed during the first cycle and notably decreased in subsequent cycles, with no grade 4 or 5 diarrhea events reported. Treatment-related serious adverse events were experienced by 27.3% vs 7.5% of patients, and treatment-related deaths occurred in 0% vs 0.3% of patients in the PyroHT and HT groups, respectively.

Conclusion: After an extended follow-up period, the updated analysis demonstrates that PyroHT provides sustained longer PFS compared to HT, which translates into longer OS for PyroHT than HT in the first-line treatment of HER2-positive mBC. The safety profile of PyroHT remained manageable throughout the extended treatment duration. This updated analysis further reinforces PyroHT as a well-established and efficacious therapeutic strategy for this patient population.

GS1-04: HER2-Directed Antibody-Drug Conjugate SHR-A1811 in the Neoadjuvant Treatment of HER2-positive Early Breast Cancer: a Prospective, Randomized, Open-label, Phase 2 Trial

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Background: Standard neoadjuvant regimens for HER2 positive breast cancer include trastuzumab and pertuzumab combined with chemotherapy. No data has been reported for the efficacy and safety of HER2-direted Antibody-drug conjugate (ADC) monotherapy or combined with Tyrosin kinase inhibitor (TKI) in the neoadjuvant setting. We assessed the antitumor activity and safety of a novel ADC SHR-A1811 in neoadjuvant treatment of HER2+ breast cancer.

Methods: This open label, randomized, phase 2 study enrolled 265 HER2 positive patients, aged 18 years or older with stage II–III disease. Patients were randomly assigned (1:1:1) to receive nab-paclitaxel (100mg/m2 i.v. day 1, day 8, and day 21 in a 28-day per cycle) combined with carboplatin(AUC 1.5 i.v. day 1, day 8, and day 21 in a 28-day per cycle), trastuzumab(initial dose 8mg/kg, subsequent dose 6mg/kg i.v. every 3weeks), and pertuzumab (initial dose 840mg, subsequent dose 420mg i.v. every 3weeks) for 6 cycles (PCbHP), SHR-A1811 monotherapy (4.8mg/kg i.v. day 1 every 3weeks) for 8 cycles, or SHR-A1811 with pyrotinib (240mg orally once a day daily) for 8 cycles. SHR-A1811 is composed of anti-HER2 antibody trastuzumab, a cleavable linker, and the topoisomerase I inhibitor payload SHR169265 with a DAR of 5.7. Primary endpoints were pathological complete response (pCR) in all randomly assigned patients in the intention-to-treat population. This study is registered with ClinicalTrials.gov (NCT05582499).

Findings: 265 patients were randomly assigned, 90 to PCbHP, 87 to mono-SHR-A1811, 88 to SHR-A1811 plus pyrotinib. Baseline characteristics were well balanced between the treatment groups, about 45% were Hormone receptor (HR) positive, 70% were stage III. The pCR rate was 63% in mono-SHR-A1811 (44% in HR+ and 78% in HR-), 62% in SHR-A1811 plus pyrotinib (44% in HR+ and 76% in HR-) and 66% in PCbHP (54% in HR+ and 75% in HR-), with no statistical difference between each group. Grade 3 or higher adverse events occurred 45% in mono-SHR-A1811, 72% in SHR-A1811 plus pyrotinib and 34% in PCbHP. 1 patient occurred grade 2 interstitial lung disease in SHR-A1811, 9% patients occurred grade 3 diarrhea in SHR-A1811 plus pyrotinib, no treatment related death was occurred.

Interpretation: This is the first study to report the efficacy of third generation HER2-directed ADC in the neoadjuvant setting of HER2 positive breast cancer. SHR-A1811 exhibited promising antitumor activity and acceptable tolerability, with monotherapy pCR rate up to 63%.

GS1-06: PRO B - a superiority randomized controlled trial evaluating the effects of symptom monitoring in metastatic breast cancer patients

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Despite improved treatment options up to 30% of patients with breast cancer develop distant metastases. With their detection the treatment focus changes from cure to prolonging survival and maintaining the best possible quality of life (QoL). There is increasing evidence that the use of Patient Reported Outcomes (PRO) can help to achieve these goals. The PRO B study examined the impact of a weekly PRO monitoring combined with an automated alert system in advanced breast cancer patients within the routine care in the German health care system.

Methods: The PRO B trial, a multi-center, superiority, randomized controlled trial, enrolled 924 patients from 52 certified breast centers between 05/2021 and 06/2023. Patients were randomized in a 1:1 ratio, stratified by hormone receptor (HR) status and localization of metastases present at time of randomization. Patients in the intervention arm received a weekly questionnaire to their smartphone consisting of items selected from the EORTC CAT Core item banks for optimized assessment of QLQ-C30 domains. In case of deteriorating PRO values, an automated alert was sent to the treating breast center, which then contacted the patient within 48 hours to inquire about the reported symptoms and to intervene, if necessary. Patients in the control arm received PRO questionnaires every 3 months, but were not connected to the alert system. Primary endpoint was patient-reported fatigue at 6 months post-randomization. Secondary endpoints included physical functioning (PF), QoL, and subgroup-specific overall survival (OS). The comparison of PRO values between study arms was performed using linear mixed models with adjustment for baseline PRO and other confounders.

Results: 909 patients were included in the main analysis (456 in the intervention and 453 in the control arm. Mean age at randomization was 50.7 (range 19-81) in the intervention vs. 51.1 (range 25-83) years in the control arm. 726 (79.9%) patients had HR+ disease, 366 in the intervention vs. 360 in the control arm. 183 patients presented with HR- breast cancer, 90 in the intervention vs. 93 in the control arm. 178 patients self-reported an ECOG 0 (21.5% vs. 18.5%), 496 an ECOG 1 (53.7% vs. 57.8%) and 216 ECOG 2 or worse (24.8% vs. 23.7%) at baseline. Median follow-up time was 64 (IQR 37 – 100) and 52 (IQR 26 – 91) weeks in the intervention and control arms, respectively. During the study period a total of 39,817 questionnaires were sent, 36,845 to the intervention and 2,972 to the control arm. The return rate at 6 months was 80% vs. 61%, at 12 months 72% vs. 51%. Patients in the intervention arm reported a fatigue T-score of 57.6 (SD 9.5) at baseline vs. 60.2 (SD

9.0) for the control arm. After 6 months, the fatigue T-score in the intervention arm was significantly lower than in the control arm (54.5 vs. 59.9, mean difference = -5.4 [95%CI: -6.6 to -4.1], p<0.001) after adjustment for baseline fatigue score. Similar significant differences were observed at 3, 9, and 12 months between both arms. Moreover, the PF and QoL T-scores in the intervention arm were significantly better than those in the control arm over the study period. OS in the intervention arm was better than the control arm (OS rate at 12 months 88% vs. 85%, HR 0.71 [95%CI: 0.51 to 0.99], p=0.043). Additionally, similar results were observed in patients with visceral metastases (HR 0.46 [95%CI: 0.12 to 1.82]) and patients with HR+/Her2- (HR 0.71 [95%CI: 0.48 to 1.04]).

Conclusions: Our study provides further evidence that integrating alerts based on PRO monitoring into routine care of advanced breast cancer patients leads to improved symptom control and better functioning scores. Alert-based PRO monitoring significantly decreased levels of fatigue, improved physical functioning and overall quality of life. A benefit in overall survival was observed in the intervention as well as in a subgroup-specific analysis. Based on these findings PRO monitoring should become standard of care for advanced breast cancer patients.

GS1-08: Association between risk-reducing surgeries and survival in young BRCA carriers with breast cancer: results from an international cohort study

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Background: In carriers of germline pathogenic/likely pathogenic variants (PVs) in *BRCA1* and/or *BRCA2* genes, cancer risk management strategies are widely recommended. While risk-reducing salpingo-oophorectomy (RRSO) has shown to improve overall survival (OS), bilateral risk-reducing mastectomy (RRM) reduces the risk of developing breast cancer (BC) but has no proven OS benefit. Very limited evidence exists on RRSO and/or RRM specifically in *BRCA* carriers with prior BC diagnosis at a young age.

Here we investigated the association between RRM and/or RRSO with survival outcomes in the largest global cohort of young *BRCA* carriers with BC.

Methods: The BRCA BCY Collaboration (NCT03673306) is an international, multicenter, hospital-based, retrospective cohort study including women harboring germline *BRCA1* and/or *BRCA2* PVs and diagnosed between January 2000 and December 2020 with stage I-III invasive BC at the age of 40 years or younger. Patients with no information on uptake or timing of RRM and/or RRSO, or their uptake before BC diagnosis and *BRCA* healthy carriers were excluded.

Primary endpoint was OS. Disease-free survival (DFS) and BC-free interval (BCFI) were secondary endpoints. Survival endpoints were computed from BC diagnosis.

To account for potential lead time bias, in the primary analysis Cox models were used to explore the association between RRM and RRSO both included as time-dependent covariates and survival outcomes. Stratification factors were year at BC diagnosis, region/country and nodal status; OS models were also adjusted by development of distant recurrences/second primary malignancies as time-dependent covariates. In addition, sensitivity analyses were performed including a 3-year landmark analysis and a subgroup analysis including only patients tested for *BRCA* before or within 6 months from BC diagnosis.

Results: From 109 centers in 5 continents, 5290 patients were included. Median age at BC diagnosis was 35 years (IQR 31-38). A total of 3361 (63.5%) patients were *BRCA1* carriers, 2708 (51.2%) had node-negative and 2421 (45.8%) hormone receptor-positive (HR+) BC.

A total of 2910 (55.0%) patients underwent RRM and 2782 (52.6%) RRSO. Median time from BC diagnosis to RRM was 0.8 years (IQR 0.5-2.7) and to RRSO was 3.0 years (IQR 1.3-6.8); median follow-up was 5.1 years (IQR 2.7-8.3) after RRM and 4.9 years (IQR 2.3-8.1) after RRSO.

At a median follow-up of 8.2 years (IQR, 4.7-12.8), 686 (13.0%) OS, 1923 (36.3%) DFS and 1751 (33.1%) BCFI events were observed.

RRM was associated with a significantly reduced risk of OS events (adjusted HR [aHR] 0.65, 95% CI 0.53-0.78). This association was observed irrespective of specific *BRCA* gene, age at BC diagnosis, tumor subtype, tumor size and nodal status. RRM was also associated with a significantly reduced risk of DFS (aHR 0.58, 95% CI 0.52-0.65) and BCFI (aHR 0.55, 95% CI 0.48-0.62) events.

RRSO was associated with a significantly reduced risk of OS events (aHR 0.58, 95% CI 0.48-0.71). This association was observed irrespective of age at BC diagnosis, tumor size and nodal status. A significant interaction was observed according to specific *BRCA* gene (*BRCA1* carriers: aHR 0.44, 95% CI 0.34-0.57; *BRCA2* carriers: aHR 0.85, 95% CI 0.64-1.15) and tumor subtype (triple-negative BC: aHR 0.44, 95% CI 0.33-0.58; HR+ BC: aHR 0.80, 95% CI 0.60-1.05). RRSO was also associated with a significantly reduced risk of DFS (aHR 0.68, 95% CI 0.61-0.77) and BCFI (aHR 0.65, 95% CI 0.57-0.74) events.

Sensitivity analyses provided consistent results.

Conclusions: In this unique international cohort of *BRCA* carriers with prior BC diagnosis at a young age, RRM and RRSO were both associated with a significant improvement in OS, DFS and BCFI. These findings are critical for improving the counseling of young *BRCA* carriers with BC on cancer risk management strategies.

GS1-09: OlympiA: A phase 3, multicenter, randomized, placebo-controlled trial of adjuvant olaparib after (neo)adjuvant chemotherapy in patients w/ germline BRCA1 & BRCA2 pathogenic variants & highrisk HER2-negative primary breast cancer:

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Background The OlympiA trial (NCT02032823) compared 1 year of adjuvant oral poly(adenosine diphosphateribose) polymerase inhibitor olaparib (OL) to matching placebo (PL) in a randomized trial of 1836 patients (pts) with pathogenic or likely pathogenic variants in germline BRCA1 or BRCA2 (gBRCApv) and high-risk human epidermal growth factor receptor 2 (HER2)-negative primary breast cancer (BC) who had completed all (neo)adjuvant chemotherapy, surgery and radiation. The first pre-specified interim analysis (IA) demonstrated statistically significant improvements in invasive disease-free survival (IDFS) and distant disease-free survival (DDFS). The second IA demonstrated statistically significant improvement in OS and maintained improvement in IDFS and DDFS, irrespective of hormone receptor status, prior platinum administration, timing of prior chemotherapy or type of gBRCApv. No excess acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) was observed. This updated analysis reports the results of the third pre-specified IA with median follow-up (MFU) of 6.1 years (maximum, 9.6 years). Methods Descriptive analyses are presented with longer-term follow-up comparing the primary endpoint IDFS, and key secondary endpoints DDFS and OS, across two arms: oral OL 300mg BID versus PL. Estimates of the hazard ratio (HR) based on the stratified Cox's Proportional Hazards Model and 95% confidence intervals (CI) are presented for each endpoint with event rates reported at the 6 year (yr) MFU. Safety analyses including adverse events of special interest (AESIs) and all deaths are highlighted. Results With longer follow-up, the benefit of OL in terms of IDFS, DDFS and OS was maintained with effect sizes similar to those reported in previous analyses. For IDFS, HR=0.65 (95% CI: 0.53, 0.78); 6-yr IDFS percents (OL vs PL): 79.6% vs 70.3% (diff. 9.4%; 95% CI, 5.1%, 12.7%). For DDFS, HR=0.65 (95% CI: 0.53, 0.81); 6-yr DDFS percents (OL vs PL): 83.5% vs 75.7% (diff. 7.8%; 95% CI, 3.8%, 11.5%). For OS, HR 0.72 (95% CI: 0.56, 0.93); 6-yr OS percents (OL vs PL): 87.5% vs 83.2% (diff. 4.4%; 95% CI, 0.9%, 6.7%). Total deaths/pts were 107/921 vs 143/915 in OL and PL, respectively. OL benefit was consistent across all key subgroups, including for pts with high-risk, hormonereceptor-positive disease. Fewer BRCA-associated cancers were reported with OL

vs PL: contralateral invasive BC (31 vs 40); contralateral non-invasive BC (3 vs 4), new primary ovarian cancer (3 vs 9), new primary fallopian tube cancer (1 vs 4). The percent of pts experiencing AESIs was lower with OL vs PL (6.3% vs 9.3%), comprising MDS or AML (OL, n=4; PL, n=6), pneumonitis (OL, n=9; PL, n=13), and fewer new primary cancers overall (OL, n=45; PL, n=68). Conclusions At 6.1 years MFU, one year of adjuvant OL after (neo)adjuvant chemotherapy continues to demonstrate meaningful improvements in IDFS, DDFS and OS in pts with gBRCApv and high-risk, HER2-negative primary BC, including all key subgroups, and with acceptable toxicity and no evidence of increased risk of MDS or AML. These data continue to highlight the importance of gBRCApv testing in high-risk, HER2-negative primary BC.

GS2-01: Exclusive endocrine therapy or radiation therapy in women aged 70+ years with luminal-like early breast cancer (EUROPA): preplanned interim analysis of a randomized phase 3 trial

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Background. Optimal therapy after breast conserving surgery (BCS) in older adults with low-risk early breast cancer (BC) is controversial. This study compares the effects of radiation therapy (RT) and endocrine therapy (ET) as exclusive treatments on health-related quality of life (HRQoL) and ipsilateral breast tumour recurrence (IBTR) rate in women aged ≥70 years with stage I luminal-like BC.
Methods. EUROPA (NCT04134598) is a phase 3, randomized, controlled trial. Women with early luminal-like BC (ER/PgR ≥10%, HER2 negative, Ki-67 index ≤20%, pT1ab N0/Nx, any grade or pT1c, grade 1-2), were 1:1 randomized after BCS to receive exclusive ET or exclusive RT. Central randomization was stratified by G8 health status (≤14 vs >14) and age at baseline (70–79 vs ≥80 years). The study coprimary endpoints are 2-year HRQoL as assessed by the global health status (GHS) scale of the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30 and 5-year IBTR rate. Secondary endpoints are locoregional recurrence (LRR), contralateral BC (CBC), distant metastases (DM), BC specific- (BCSS), and overall-survival (OS) rates, adverse events, individual scale scores from QLQ-C30, QLQ-BR45, QLQ-ELD14 EORTC modules up to 5 years after treatment. We present the pre-planned interim HRQoL analysis results after at least 152 patients reached the 2-year HRQoL assessment. Data were analysed by intention to treat, using repeated mixed-effects methods.

Results. Between Feb 2021 and June 2024, 734 patients were enrolled and 731 randomly assigned to receive RT (n=365) or ET (n=366); 78.9% of the planned 926 patients from 21 centres. In the current interim analysis, the RT and ET arms included 104 and 103 patients, respectively. Age distribution was similar across treatment arms (74% aged 70-79 and 26% aged 80+ years); G8 scores were comparable (40% ≤14 and 60% scoring >14). At baseline, RT arm (n=104) had a mean GHS score of 71.9 (SD 19.05), while ET arm (n=99) had a mean score of 75.5 (SD 19.34). RT arm showed mean changes of -1.1 (24-month, SD 18.80) as compared to -10.0 (24-month, SD 25.80) of ET arm. Significant factors influencing GHS score changes were treatment type (p=0.045) and baseline GHS value (p<0.0001). Concerning adjusted mean GHS score reductions, for RT arm the mean changes were -3.77 (3-month; p=0.0452), -0.59 (6-month; p=0.7420), -4.33 (12-month; p=0.0333), and -3.40 (24-month; p=0.1314). For ET arm, mean changes were -6.45 (3-month; p=0.0015), -5.38 (6-month; p=0.0043), -6.60 (12-month; p=0.0025), and -9.79 (24-month; p<0.0001). The adjusted mean differences between RT and ET arms at 24 months showed a significant difference of 6.39 favouring RT arm (95%CI 0.14 to 12.65; p=0.0453). ET resulted in a more significant decline at 24 months in most of the functional and symptom scales of the QLQ-C30 questionnaire compared to RT. No IBTR, LRR, or DM events were reported in either group. CBC events occurred in 2 RT arm patients (1.9%) and 1 ET arm patient (1%). Deaths were 4 (3.8%) in RT arm and 2 (1.9%) in ET arm, none BC-related.
Conclusions. ET patients had significantly reduced HRQoL over 24 months compared to RT patients. These findings will help shared decision-making while awaiting final study results.

GS2-02: Impact of Tamoxifen Only after Breast Conservation Surgery for "Good Risk" Duct Carcinoma in Situ: Results from the NRG Oncology/RTOG 9804 and ECOG-ACRIN E5194 Trial

Jean Wright, Jennifer Moughan, Habib Rahbar, Amit B Shah, Christopher Comstock, Lesly A Jarvis, Judy A Tjoe, Isabelle Germain, Sunil S Badve, Eric Strom, Abram Recht, Diane C Ling, Laura A Vallow, Joseph J Stephenson, Adam Currey, Eleanor M Walker, Harold Reiter, Michael L. Steinberg, Lori J Pierce, Kathryn Winter, Joseph Sparano, Nancy E Davidson, Antonio C. Wolff, Robert J Gray, and Beryl McCormick

Background The NRG/RTOG 9804 and ECOG-ACRIN E5194 studies sub-classified duct carcinoma in situ (DCIS) into different risk groups after breast conservation surgery (BCS) based on size, DCIS grade, and margin width. NRG/RTOG 9804 randomized patients with “good risk” DCIS (size ≤2.5 cm, grade 1-2, margin ≥3mm) to whole breast radiation (RT) or none, and ECOG-ACRIN E5194 had 2 cohorts, one observing patients with the same “good risk” characteristics after BCS without RT. In both trials, the use of tamoxifen) was optional but tracked. This ancillary analysis of both trials was undertaken to assess the role of tamoxifen alone on ipsilateral breast recurrence (IBR) in this “good risk” group not receiving RT. Methods A combined database from the non-RT arm of NRG/RTOG 9804 and the “good risk” cohort from ECOG-ACRIN E5194 was created and distributions of patient and DCIS characteristics by tamoxifen use (yes vs. no) were compared using the Chi-square

test. IBR, invasive IBR, DCIS IBR and contralateral breast event (CBE) were estimated by the cumulative incidence method and distributions between tamoxifen use were compared using Gray's test. A 2-sided significance level of 0.05 was used. Univariate and multivariable Fine-Gray regression was used to analyze the effects of factors, in addition to tamoxifen use, that may be associated with endpoints. Results 878 patients were analyzed, 317 patients from NRG/RTOG 9804, and 561 from ECOG-ACRIN E5194. Median age was 59, margin width was ≥3mm or negative by re-excision in 97.8%, size was ≤5mm in 48.1%, and grade was 1-2 in 87.5%. The use of tamoxifen in the combined no-RT group was 43.1% (65.6% in NRG/RTOG 9804 and 30.3% in ECOG-ACRIN E5194). Median follow up of all patients was 14.85 years. There were 117 IBR, 65 invasive and 52 DCIS. There was a statistically significant association for reduced IBR with tamoxifen use (p=0.001); estimated 15-year IBR (95% CI) with tamoxifen is 11.4% (7.9%, 15.5%) and without is 19.0% (15.3%, 22.9%). Further analysis showed the reduction to be significantly associated with tamoxifen use for invasive IBR (p=0.0048) but not for DCIS IBR (p=0.089). No associations were seen for CBE. On univariable analysis, pathologic size (≤ 5 mm vs. > 10 mm) was significantly associated with IBR (p=0.0001) as was DCIS grade (1 vs 2, p=0.042). On multivariable analysis for IBR, DCIS grade fell out of the model and after adjusting for pathologic size, tamoxifen use remained statistically significantly associated with reduced IBR. On multivariable analysis for invasive IBR, size fell out of the model and adjusting for grade, tamoxifen use remained statistically significantly associated with reduced invasive IBR. Patients who received tamoxifen were 44% less likely to have any IBR (HR =

0.56, 95% CI: 0.38, 0.84; p=0.0044), and 51% less likely to have invasive IBR (HR=0.49, 95% CI: 0.28, 0.84; p=0.0092), as compared to patients with no tamoxifen. Conclusions For women with "good risk" DCIS who opt for BCS without RT, the use of tamoxifen is significantly associated with a reduction in IBR overall and specifically invasive IBR, not DCIS IBR.

GS2-03: Does postmastectomy radiotherapy in 'intermediate-risk' breast cancer impact overall survival? 10 year results of the BIG 2-04 MRC SUPREMO randomised trial: on behalf of the SUPREMO trial investigators

Ian Kunkler, Nicola Russell, Niall Anderson, Fidelis Muturi, Geertjan van Tienhoven, David Cameron, John Bartlett, Karen Taylor, Tammy Piper, Mike Dixon, Galina Velikova, Edwin Aird, Boon Chua, Coen Hurkmans, Karen Venables, Linda Williams, Jeremy Thomas, Marjory Maclennan, Richard Sainsbury, Susan Cleator, Eldo Verghese, Yexiong Li, Shulian Wang, Peter Canney

Background The NRG/RTOG 9804 and ECOG-ACRIN E5194 studies sub-classified duct carcinoma in situ (DCIS) into different risk groups after breast conservation surgery (BCS) based on size, DCIS grade, and margin width. NRG/RTOG 9804 randomized patients with "good risk" DCIS (size ≤2.5 cm, grade 1-2, margin ≥3mm) to whole breast radiation (RT) or none, and ECOG-ACRIN E5194 had 2 cohorts, one observing patients with the same "good

risk" characteristics after BCS without RT. In both trials, the use of tamoxifen) was optional but tracked. This ancillary analysis of both trials was undertaken to assess the role of tamoxifen alone on ipsilateral breast recurrence (IBR) in this "good risk" group not receiving RT. Methods A combined database from the non-RT arm of NRG/RTOG 9804 and the "good risk" cohort from ECOG-ACRIN E5194 was created and distributions of patient and DCIS characteristics by tamoxifen use (yes vs. no) were compared using the Chi-square test. IBR, invasive IBR, DCIS IBR and contralateral breast event (CBE) were estimated by the cumulative incidence method and distributions between tamoxifen use were compared using Gray's test. A 2-sided significance level of 0.05 was used. Univariate and multivariable Fine-Gray regression was used to analyze the effects of factors, in addition to tamoxifen use, that may be associated with endpoints. Results 878 patients were analyzed, 317 patients from NRG/RTOG 9804, and 561 from ECOG-ACRIN E5194. Median age was 59, margin width was ≥3mm or negative by re-excision in 97.8%, size was ≤5mm in 48.1%, and grade was 1-2 in 87.5%. The use of tamoxifen in the combined no-RT group was 43.1% (65.6% in NRG/RTOG 9804 and 30.3% in ECOG-ACRIN E5194). Median follow up of all patients was 14.85 years. There were 117 IBR, 65 invasive and 52 DCIS. There was a statistically significant association for reduced IBR with tamoxifen use (p=0.001); estimated 15-year IBR (95% CI) with tamoxifen is 11.4% (7.9%, 15.5%) and without is 19.0% (15.3%, 22.9%). Further analysis showed the reduction to be significantly associated with tamoxifen use for invasive IBR (p=0.0048) but not for DCIS IBR (p=0.089). No associations were seen for CBE. On univariable analysis, pathologic size (≤ 5 mm vs. > 10 mm) was significantly associated with IBR (p=0.0001) as was DCIS grade (1 vs 2, p=0.042). On multivariable analysis for IBR, DCIS grade fell out of the model and after adjusting for pathologic size, tamoxifen use remained statistically significantly associated with reduced IBR. On multivariable analysis for invasive IBR, size fell out of the model and adjusting for grade, tamoxifen use remained statistically significantly associated with reduced invasive IBR. Patients who received tamoxifen were 44% less likely to have any IBR (HR =

0.56, 95% CI: 0.38, 0.84; p=0.0044), and 51% less likely to have invasive IBR (HR=0.49, 95% CI: 0.28, 0.84; p=0.0092), as compared to patients with no tamoxifen. Conclusions For women with "good risk" DCIS who opt for BCS without RT, the use of tamoxifen is significantly associated with a reduction in IBR overall and specifically invasive IBR, not DCIS IBR.

GS2-05: Early Oncologic Outcomes Following Active Monitoring or Surgery (+/- Radiation) for Low Risk DCIS: the Comparing an Operation to Monitoring, with or without Endocrine Therapy (COMET) Study (AFT-25)

Eun-Sil Hwang, Terry Hyslop, Thomas Lynch, Marc D Ryser, Anna Weiss, Anna Wolf, Kelsey Norris, Meredith Witten, Lars Grimm, Stuart Schnitt, Sunil Badve, Rachel Factor, Elizabeth Frank, Deborah Collyar, Desiree Basila, Donna Pinto, Mark A Watson, Robert West, Louise Davies, Jenny Donovan, Ayako Shimada, Yutong Li, Yan Li, Antonia V Bennett, Shoshana

Rosenberg, Jeff Marks, Eric Winer, Marc Boisvert, Armando Giuliano, Kelsey Larson, Kathleen Yost, Priscilla McAuliffe, Lisa Carey, Alastair Thompson, Ann H Partridge

Background: Over 50,000 women in the United States will be diagnosed with ductal carcinoma in situ (DCIS) this year alone. Almost all of these diagnoses will be made in completely asymptomatic individuals with a highly variable risk of progression to invasive cancer. In some low-risk malignancies, “watchful waiting,” is offered as a treatment option. Such an approach is likely reasonable for some DCIS and could reduce the harms of treatment while helping to identify those most likely to benefit from more aggressive therapy. To date, this approach has not been tested in a clinical trial setting. Methods: The COMET study (Comparing an Operation to Monitoring, with or without Endocrine Therapy for low risk DCIS; AFT-25) is a large pragmatic randomized non-inferiority trial that compares oncologic outcomes between patients randomized to guideline concordant care (GCC; surgery +/- radiation therapy) or active monitoring (AM). The study population were women seeking treatment for DCIS at one of the Alliance Clinical Trial sites. Eligible participants were age>40 with low-intermediate grade estrogen and/or progesterone receptor positive, HER2 receptor negative (if HER2 tested) DCIS on core biopsy without microinvasive or invasive cancer. The choice for endocrine therapy was offered in both groups. Participants in the AM group had surgical intervention only upon diagnosis of invasive progression. All study endpoints were collected prospectively. Results: This is the first planned interim Intention-to-Treat (ITT) analysis of the COMET trial primary endpoints at a median follow up of XX months. We will present patient characteristics for the 997 participants who enrolled in the study and were randomized to either GCC or AM. The primary endpoint to be presented is whether the ipsilateral invasive cancer rate for AM is non-inferior to that for GCC. Characteristics of invasive cancer events in the two groups will be compared. Secondary endpoints (rates of mastectomy, radiation, chemotherapy) and survival endpoints between groups will also be presented. Conclusion: These data will provide the first randomized trial evidence of whether an active monitoring strategy is a safe alternative for women with low-risk DCIS. Longer-term data could support practice changing guidance as to how DCIS is managed and treated and will have future implications for treatment guidelines for these excellent prognosis patients.

GS2-06: Patient Reported Outcomes Following Active Monitoring or Surgery (+/- Radiation) for Low Risk DCIS in the Comparing an Operation to Monitoring, with or without Endocrine Therapy (COMET) Study (AFT-25)

Ann Partridge, Terry Hyslop, Shoshana Rosenberg, Antonia Bennett, Sarah Drier, Mattias Jonsson, Ayako Shimada, Yutong Li, Yan Li, Thomas Lynch, Elizabeth Frank, Deborah Collyar, Desiree Basila, Donna Pinto, Anna Weiss, Anna Wolf, Kelsey Norris, Meredith Witten, Marc Boisvert, Armando Giuliano, Kelsey Larson, Kathleen Yost, Priscilla McAuliffe, Amy Krie, Nina Tamirisa, Sonja Darai, Lisa Carey, Alastair Thompson, Shelley Hwang

Background: Data comparing patient-reported outcomes (PROs) comparing management strategies for low-risk ductal carcinoma in situ (DCIS) are lacking. Comparing an Operation to Monitoring, with or without Endocrine Therapy (COMET), for low-risk DCIS is a prospective randomized controlled trial that evaluated the effects of active monitoring (AM) compared to breast surgery (lumpectomy followed by radiation vs. unilateral mastectomy vs. bilateral mastectomy) on PROs including quality of life (QOL), anxiety, depression and specific symptoms. Methods: We compared PROs among patients who completed questionnaires at baseline prior to randomization, at 6 months and 1 year after randomization, then annually in years 2-5. Patients completed validated measures that assessed QOL, anxiety and depression and breast cancer treatment-related symptoms using the SF-36, EQ-5D-5L, a modified 19-item version of the Breast Cancer Prevention Trial (BCPT) Symptom Checklist, the Breast-Q, four items from the Quality of Life in Adult Cancer Survivors (QLACS), the State Trait Anxiety Inventory (STAI) scale and the Center for Epidemiologic Studies Depression Scale (CES-D-10), as well as the Breast Cancer Pain and Brief Pain Inventory. Results: We will present patient characteristics for the 997 patients who enrolled in the study and PRO questionnaire completion rates overall and within relevant subgroups to assess response bias. The analysis of differences by randomized group will include differences in the SF-36, STAI and CES-D, as well as the Breast Cancer Pain, Brief Pain Inventory and BCPT symptoms. We will also assess group differences over time and within specific subgroups (e.g., by age). Conclusion: In this analysis of PROs after active monitoring or surgical +/- radiation management for low-risk DCIS, quality of life, anxiety, depression and symptom patterns (severity, recovery, decline etc.) likely differed between the two groups; how they differed will be presented and whether these differences persisted over time.

GS2-07: No axillary surgery versus axillary sentinel lymph node biopsy in patients with early invasive breast cancer and breast-conserving surgery: Final primary results of the Intergroup-Sentinel-Mamma (INSEMA) trial

Toralf Reimer, Angrit Stachs, Kristina Veselinovic, Thorsten Kuhn, Jorg Heil, Silke Polata, Frederik Marme, Thomas Muller, Guido Hildebrandt, David Krug, Beyhan Ataseven, Roland Reitsamer, Andrea Stefek, Carsten Denkert, Inga Bekes, Dirk Michael Zahm, Marc Thill, Michael Golatta, Johannes Holtschmidt, Michael Knauer, Valentina Nekljudova, Sibylle Loibl, Bernd Gerber on behalf of the INSEMA investigators

Background: Axillary nodal status is an important prognostic factor in breast cancer (BC), guiding (neo)adjuvant systemic treatment and postoperative radiotherapy. As axillary surgery does not significantly affect BC mortality itself, it is considered as a staging procedure in clinically node-negative patients. The replacement of axillary lymph node dissection (ALND) by sentinel lymph node biopsy (SLNB) two decades ago and later omitting completion ALND (cALND) according to the ACOSOG Z0011 criteria led to surgical de-escalation. The Intergroup-Sentinel-Mamma (INSEMA) trial (NCT02466737) aims to investigate whether surgical axillary staging as part of breast-conserving therapy (BCT) for

early BC can be avoided without compromising oncological safety.
Study Design:
The INSEMA trial was conducted between September 2015 and April 2019 in Germany and Austria. This prospective, randomized trial compares no axillary surgery with standard SLNB in pts with early invasive BC (tumor size ≤ 5 cm; c/iT1–2 c/iN0) scheduled for BCT, including postoperative whole-breast irradiation. The primary objective is to assess whether no axillary surgery is non-inferior to SLNB regarding invasive disease-free survival (iDFS). Clinical non-inferiority is a hazard ratio (HR) below 1.271 when comparing the non-SLNB with the SLNB group. The randomization was carried out in 4:1 allocation (SLNB vs. no SLNB) because pN1a(sn) pts in the SLNB arm underwent a second randomization to either SLNB alone or cALND (key secondary outcome). The primary analysis is based on the per-protocol (PP) set. Adjusting for 1:4 randomization, 5230 pts (PP set) are needed. Assuming a 5% exclusion rate from the PP set, about 5505 pts must be randomized.
Results:
5502 eligible pts were randomized to no SLNB (n=1101) vs. SLNB (n=4401). The drop-out rate was 6.3%, leading to an intent-to-treat (ITT) population of N=5154. After excluding 296 patients (n=252 without postoperative radiotherapy), 4858 patients (no SLNB: n=962, SLNB: n=3896) were included in the PP set. The median follow-up (FU) is 73.6 months (IQR 61.3-86.4).
Patient and tumor characteristics are well-balanced between treatment arms. The median age at diagnosis was 62.0 years (range 24.0–89.0). Most pts presented with low-risk BC (78.6% pT1 stage, 98.5% hormone receptor-positive, 3.6% HER2-positive, and 3.6% G3 tumors). Significantly more pts received adjuvant chemotherapy in the SLNB arm (13.2% vs 10.7% in the no SLNB arm).
The primary analysis in the PP established non-inferiority in iDFS between study arms with a HR=0.91 (95% CI: 0.73-1.14) for no SLNB to SLNB. The estimated 5-year iDFS rates are 91.9% (89.9%-93.5%) in the non-SLNB arm and 91.7% (90.8%-92.6%) in the SLNB arm. The first iDFS events (n=525, overall 10.8%) for no SLNB vs. SLNB consist of invasive locoregional recurrences (1.9% vs. 1.4%), including axillary recurrences (1.0% vs. 0.3%), invasive contralateral BCs (1.0% vs. 0.6%), distant metastases (2.7% vs. 2.7%), secondary malignancies (3.3% vs. 3.9%), and deaths (1.4% vs. 2.4%). The estimated 5-year overall survival (OS) rates are 98.2% (97.1%-98.9%) in the non-SLNB arm and 96.9% (96.3%-97.5%) in the SLNB arm.

Conclusion: The INSEMA trial, enrolling 5500 pts, demonstrated that the omission of SLNB in clinically node-negative BC pts undergoing BCT resulted in a statistically significant non-inferior iDFS meeting the primary endpoint. INSEMA demonstrates oncological safety in all aspects when the axillary SLNB is omitted in cN0 patients with an early BC planned for primary BCT. This practice-changing concept is suitable for patients presenting with low-grade (G1/G2), hormone receptor-positive/HER2-negative invasive BC with tumor size up to 5 cm.

GS2-09: Overweight, obesity and prognosis in 206,904 women in the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) database

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Background: Analyses of the 2014 EBCTCG database suggested that, in early-stage breast cancer, obesity was strongly independently associated with breast cancer mortality only in pre/peri-menopausal oestrogen-receptor-positive (ER+) disease (Pan et al ASCO 2014). Based on the far larger 2024 EBCTCG database, however, we can now test that unexpected finding and better characterise any relevance of patient characteristics to the association of body mass index (BMI) with distant recurrence and mortality.
Methods: We analysed patient-level data on time to distant recurrence and death from the 206,904 women with early-stage breast cancer (entered during 1978-2017 into 147 randomised trials) in the 2024 EBCTCG database who had BMI at entry (within two years of diagnosis) recorded as 15-50 kg/m2 and with complete information on age, ER status, tumour diameter, nodal status, and randomly allocated treatment. Information on menopausal status, tumour grade, and HER2 status was available for most participants. Cox regression was used to estimate the associations of BMI with rates of distant recurrence and breast cancer mortality, calculating hazard rate ratios (RRs) per 5 kg/m2 increase of BMI or comparing 3 BMI groups (obese: BMI 30-50 [mean 34.7]; overweight: BMI 25 to <30 [mean 27.3]; lean: BMI 15 to <25 [mean 22.2] kg/m2).
Results: Of the 206,904 women, 60% were postmenopausal at trial entry and 77% had ER+ disease. Their mean BMI was 27.1 (SD 5.6) kg/m2 and 26.0% (53,872) were obese (BMI ≥30 kg/m2). The prevalence of obesity increased from 19% in the early 1980s to 27% in the early 2010s. The overall adjusted rate ratio (RR) of first distant recurrence (ignoring any local or contralateral recurrences) was 1.06 (95% CI 1.05-1.07, p<0.0001) per 5 kg/m2 increase in BMI. The RR for overweight versus lean women was 1.07 (CI 1.04-1.10, p<0.0001), and that for obese versus lean women was 1.17 (95% CI 1.14-1.20, p<0.0001). This approximately log-linear association between BMI and the rate of distant recurrence was seen irrespective of patient or tumour characteristics, type of adjuvant systemic therapy, year of diagnosis, or time since diagnosis. In the 82,464 pre-menopausal women the RR per 5 kg/m2 increase of BMI was 1.08 (1.07-1.10, p<0.0001), and in the 124,440 post-menopausal women it was 1.05 (1.03-1.06, p<0.0001; heterogeneity between RRs p=0.0004). There was little heterogeneity between the RRs in ER+ and ER-poor disease. In the 159,119 women with ER+ disease the RR per 5 kg/m2 increase of BMI was 1.06 (1.05-1.08, p<0.0001), and in the 47,785 with ER-poor disease it was 1.06 (1.04-1.08, p<0.0001). The associations of BMI with breast cancer mortality mirrored those with distant recurrence.
Conclusion: Overweight and obesity are associated with increased distant recurrence and breast cancer mortality in all types of patients with early-stage breast cancer, but the risk associated with a substantial (e.g. 5 kg/m2) difference in BMI is only moderate.

Nevertheless, randomised assessment of the effects among overweight or obese women with early breast cancer of weight-loss interventions (perhaps utilising a GLP-1 receptor agonist) could usefully be added, using a factorial design, to some current and future adjuvant treatment trials addressing unrelated questions.
Reference: Pan H, Gray R, on behalf of the EBCTCG. Effect of obesity in premenopausal ER+ early breast cancer: EBCTCG data on 80,000 patients in 70 trials. J Clin Oncol 2014; 32:5s

GS2-10: A long-term image-derived AI risk model for primary prevention of breast cancer

Mikael Eriksson, Kamila Czene, Christopher Scott, Shawn Stoddard, Hugh Smith, Per Hall, Celine Vachon

Background: Image-derived artificial intelligence (AI) risk models have shown promise in short-term risk assessment for improving breast cancer screening. No image-derived long-term AI risk model for primary prevention has been developed and externally validated.
Methods: We performed a two-site case-cohort study of women aged 30-90 in a population-based screening study including two screening settings in Olmsted County, Minnesota (U.S.) and the KARMA cohort (Sweden) with women recruited between 2009-2017. Median follow-up was 10 years. An image-derived AI-risk model was developed in an independent Swedish population and we report on the validation in the Olmsted/KARMA studies. Absolute 10-year risks were calculated at study entry. Time-dependent Area Under the receiver operating characteristics Curve (AUC(t)) and the ratio of expected versus observed events (E/O) were estimated. Comparison with the clinical Tyrer-Cuzick v8 model was performed in KARMA using clinical guidelines. Analyses were performed for risk of all breast cancer and restricted to invasive cancer alone.
Results: The Olmsted/KARMA case-cohorts included 8,721 women with mean age 54.4 years (SD 10.6) in the subcohort and 1,633 incident breast cancer cases with mean age 57.0 years (SD 10.6). The image-derived AI 10-year average risk was estimated as 3.85% in Olmsted and 3.16% in KARMA. The E/O ratio was 1.01 (95% CI 0.95-1.06) in Olmsted and 0.98 (95%CI 0.90-1.07) in KARMA. The 10-year AUC(t) was 0.71 (95%CI 0.68-0.73) in Mayo and 0.72 (95%CI 0.69-0.77) in KARMA. Using the National Institute for Health and Care Excellence (NICE) guidelines, considering women at 8% as high risk, 32% of breast cancers could be subject to preventive strategies in the 9.7% of women at high 10-year risk based on the AI risk model. The corresponding numbers were 7.2% and 2.2% for Tyrer-Cuzick. Results were similar when restricted to invasive cancers only.
Conclusions: The 10-year image-derived AI-risk model showed good discriminatory performance and calibration in the two case-cohorts and, showed a significantly higher discriminatory performance than the clinical Tyrer-Cuzick v8 risk model in KARMA. The image-derived AI-risk model has the potential for clinical use in primary prevention and targets up to one third of breast cancers.

GS2-11: APOBEC3 mutagenesis induces resistance-promoting genomic alterations in breast cancer

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Background: Large-scale genomic profiling has cataloged the prevalence of single base substitution (SBS) mutational signatures associated with the activity of Apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3) cytidine deaminases in breast cancer (BC). These mutational signatures are enriched in metastatic BC (mBC) compared to early tumors indicating an association with poor prognosis and a potential function in therapy resistance and disease progression. We sought out to investigate whether APOBEC3 mutational signatures can serve as biomarkers for poor treatment outcomes and if APOBEC3 mutagenesis-driven genomic instability can induce therapy resistance in mBC.
Methods: We analyzed SBS mutational signatures in 3,880 BC samples with paired tumor-normal sequencing by the MSK-IMPACT assay using the SigMA algorithm. We utilized the detailed clinical annotation to assess the clinical characteristics of APOBEC3-dominant tumors including survival analyses on endocrine and targeted therapies. We generated cellular models of BC to investigate the molecular drivers of APOBEC3 mutagenesis and its function in promoting therapeutic resistance. We performed whole genome sequencing (WGS) of BC models and paired primary/metastatic patient samples to identify broader genomic alterations mediated by APOBEC3 activity.
Results: Building on published results, we found that APOBEC3 mutational signatures were highly prevalent in all subtypes of BC and enriched in metastatic hormone receptor-positive (HR+) and triple-negative breast cancers (TNBC) compared to unmatched primary tumors ($p < 0.0001$ for HR+/HER2-, $p < 0.01$ for HR+/HER2+ and TNBC). APOBEC3 mutational signatures were independently associated with shorter progression-free survival on antiestrogen plus CDK4/6 inhibitor combination therapy in patients with HR+ mBC (HR 1.5, 95% CI 1.2 - 1.8, $p < 0.001$). Expression of APOBEC3A (A3A) and APOBEC3B (A3B) enzymes generated APOBEC3-associated alterations including single nucleotide variants, copy number alterations (CNAs), and clustered mutations in a deamination-dependent manner, and promoted resistance acquisition of estrogen receptor-positive (ER+) BC cells to agents including an ER degrader and CDK4/6 inhibitors. In HER2+ cells, endogenous A3A-driven APOBEC3 activity was also necessary for faster resistance development against anti-HER2 therapies. WGS analyses of resistant models identified CNA events such as loss of heterozygosity in chromosome 13 exclusively in APOBEC3-positive cells. Upon exposure to the CDK4/6 inhibitor abemaciclib, these cells acquired APOBEC3-context truncating mutations in RB1 tumor suppressor gene, a well-characterized mechanism of resistance. Detailed analyses of WGS of five paired patient samples also highlighted acquired resistance-linked alterations such as PIK3CA E54XK mutations in APOBEC3-dominant

tumors, which were corroborated in paired pre/post-treatment samples in our clinical cohort. The acquisition of APOBEC3-context alterations in APOBEC3-dominant samples highlights the causality of APOBEC3 mutagenesis in driving resistance-promoting changes. Lastly, comparison of mutational signatures in the paired cohort demonstrated pre-existence of APOBEC3 signatures in 75% of pre-treatment samples that became APOBEC3-dominant post-treatment illustrating that APOBEC3 mutagenesis can be active during early stages of BC.
Conclusions: Our work reveals that APOBEC3 mutational signatures predict poor treatment outcomes of HR+ mBC. We demonstrate that APOBEC3 mutagenesis, primarily through the enzymatic activity of A3A and A3B, drives resistance to endocrine and targeted therapies by causing APOBEC3-context resistance-associated changes. We further show that the presence of APOBEC3 mutagenesis can be detected before therapy exposure and may therefore represent a valuable biomarker and therapeutic target.

GS3-01: Circulating tumor DNA surveillance in ZEST, a randomized, phase 3, double-blind study of niraparib or placebo in patients w/ triple-negative breast cancer or HER2+ BRCA-mutated breast cancer with molecular residual disease after definitive therapy

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Background: Circulating tumor DNA (ctDNA) testing in plasma offers the opportunity to identify early breast cancer (BC) recurrence in patients (pts) who, despite completion of definitive therapy, have molecular residual disease (MRD). ZEST is a randomized, phase 3, double-blind trial evaluating whether niraparib can enhance disease-free survival in pts with BC and ctDNA detection (ctDNA+) without evidence of radiographic recurrence after completion of curative intent therapy. Methods: Pts with stage I–III triple negative breast cancer (TNBC) or BRCA-mutated HER2− BC were eligible. A personalized, tumor-informed ctDNA assay (Signatera) was designed based on whole-exome sequencing of tumor tissue and matched normal blood. Testing for ctDNA-based MRD began any time after the end of definitive treatment (EODT); pts with HR+ disease were allowed concurrent endocrine therapy (stable regimen). Upon ctDNA+, radiographic staging was performed. Those without evidence of metastatic disease were randomized (1:1) to niraparib (200 or 300 mg/day, depending on weight and platelet count) or placebo (PBO). Imaging was performed every 12 weeks. The primary endpoint was safety of niraparib; disease-free survival (measured as time from randomization to recurrence or death from any cause) was also assessed.

Results: As of May 8, 2024, a total of 2746 pts were prescreened; 1901 pts had ≥1 ctDNA test result (ctDNA+, 147 [8%]). Of the 1901 pts, median age was 52 (range, 22–88) years;

1683 pts (89%) had TNBC (ctDNA+, 135), and 218 (11%) had BRCA-mutated HR+ disease (ctDNA+, 12); 620 (33%) pts received neoadjuvant therapy, 643 (34%) received adjuvant therapy, and 593 (31%) received both neoadjuvant and adjuvant therapy. Due to low rates of ctDNA+, study enrollment was terminated early. ctDNA+ was 5.2% on first test of pts with ≥1 test, and 4.4% on second or subsequent test of pts with ≥2 tests. Of pts with ctDNA+ (n=147), 66% had ctDNA+ on the first test, 91% on the first or second test, and 59% were ≤6 months from EODT. Compared with pts with ctDNA undetected, pts with ctDNA+ tended to have positive lymph nodes, T3/T4 tumor size, stage III disease, residual disease after neoadjuvant therapy, and received both neoadjuvant and adjuvant therapy. Of the 147 pts with ctDNA+, 72 (49%) had radiographic recurrence upon initial staging before randomization. The rate of radiographic recurrence was 52% (51/98) at first ctDNA+ test and 44% (21/48) at second or later ctDNA+ test. Of pts with ctDNA+, 40 were enrolled and randomized (niraparib, 18; PBO, 22); 36 pts (90%) had TNBC, and 4 pts (10%) had BRCA-mutated HR+ disease. At data cutoff, 6 pts in the niraparib arm and 4 pts in the PBO arm remained on study without radiographic recurrence. Median disease-free

survival was 11.4 (95% CI, 5.7–18.2) months for niraparib vs 5.4 (95% CI, 2.8–9.3) months for PBO (hazard ratio, 0.64; 95% CI, 0.30–1.39). No new safety signals were observed for niraparib. Conclusions: The ZEST study was terminated early because of infeasibility of completing enrollment due to a low rate of ctDNA+ and a high rate of metastatic disease at the time of ctDNA+. Although ctDNA testing began any time after EODT, ctDNA+ occurred most frequently on the first test and ≤6 months from EODT. Pts, predominantly those with TNBC, had a high rate of radiographic recurrence at time of ctDNA+, consistent with early recurrence typical of TNBC. These findings have implications for future trial design, emphasizing the importance of early ctDNA testing, and careful selection of criteria defining risk of recurrence. Updated survival data and on-treatment ctDNA dynamics will be presented.

GS3-03: Impact of Anthracyclines in High Genomic Risk Node-Negative HR+/HER2- Breast Cancer

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Background: The Anthracyclines in Early Breast Cancer (ABC) trials demonstrated no clear improvement in invasive disease-free survival with the addition of anthracycline to a taxanecontaining chemotherapy for patients with hormone receptor-positive (HR+) breast cancer. Thus, an anthracycline-free regimen is often administered for lower risk patients with HR+/HER2- disease. However, the benefit of anthracyclines for patients with high Oncotype DX recurrence score (RS) has not been studied, despite the widespread use of RS to guide the use of adjuvant therapy for HR+ patients with 0-3 positive nodes. Methods: We analyzed data from patients who received taxane + anthracycline/cyclophosphamide and similar regimens (T-AC) or taxane + cyclophosphamide (TC) chemotherapy in the TAILORx trial (NCT00310180), which enrolled patients with stage I/II, node-negative, HR+/HER2-

negative breast cancer. Patients with an RS between 11 and 25 were randomized to endocrine therapy or endocrine therapy plus chemotherapy of physicians' choice, whereas patients with an RS > 26 received chemotherapy of physician's choice. Distant recurrence-free interval (DRFI), recurrencefree interval (RFI), distant recurrence-free survival (DRFS), recurrence-free survival (RFS), and overall survival (OS) were compared using adjusted hazard ratios (aHR) controlling for age, RS, grade, tumor size, and estrogen/progesterone receptor status. Outcomes were stratified by RS > 31 and < 31. Inverse probability of treatment weighting was used to estimate adjusted 5-year event rates for these outcomes, controlling for differences in the above covariates. Restricted cubic spline regression was used to estimate aHR for receipt of TAC (versus TC) for these endpoints as a function of RS. Results: Of 2,528 cases that met eligibility, 437 were treated with T-AC and 2091 received TC. Treatment regimens in the T-AC group included anthracycline + cyclophosphamide (dose dense or standard) followed by taxane (n = 298, 68%), concurrent anthracycline, cyclophosphamide, and docetaxel (n = 59, 14%), and other anthracycline + taxane combinations (n = 80, 18%). All patients in the TC group received treatment with any taxane with cyclophosphamide. 32% had an RS >26 (n = 816) and 20% had an RS > 31 (n = 501). The mean age was 55 and median follow-up time was 7.3 years. Patients treated with T-AC had a higher RS (mean 30 vs 23), larger tumors (mean 20 mm vs 18 mm), and were more likely high grade (38% high grade vs 25%) than those treated with TC. In patients with an RS > 31 after adjusting for covariates, receipt of T-AC was associated with improved outcomes at 5 years, DRFI adjusted rate 97.5% with T-AC vs 89.4% with TC (aHR 0.27, p = 0.01), DRFS adjusted rate 96.5% with TAC vs 88.3% with TC (aHR 0.45, p = 0.03), RFI adjusted 5- year rate 95.7% with T-AC vs 87.7% with TC (aHR 0.31, p < 0.01), RFS adjusted 5-year rate 94.6% with T-AC vs 86.6% with TC; aHR 0.45, p = 0.02), and a trend towards improved OS at 5 years (adjusted rate 97.7% with T-AC vs 92.5% with TC; aHR 0.58, p = 0.22). Among cases with an RS < 31, receipt of T-AC was not associated with improved DRFI (aHR 1.12, p = 0.73), DRFS (aHR 1.09, p = 0.75), or other outcomes. Spline regression estimated the effect of T-AC over TC on DRFI at RS 20 to be aHR 0.84, (95% CI 0.30 – 1.39), at RS 30 aHR 0.63, (95% CI 0.14 – 1.12), at RS 40 aHR 0.54, (95% CI 0.18 – 0.90), and at RS 50 aHR 0.47, (95% CI 0.10 – 0.84) indicating increasing anthracycline benefit as RS increased. Conclusions: Patients with early-stage, HR+/HER2-negative breast cancer and high RS values (> 31) may benefit from adjuvant taxane and anthracycline-containing therapy more than from TC. Genomic RS testing may predict anthracycline benefit more accurately than other factors such as nodal status.

GS3-04: (Neo)adjuvant nab-PAC weekly vs sb-PAC q2w, followed by EC q2w, in genomically or clinically high-risk HR+/HER- early breast cancer according to ET-response: final survival results from the WSG ADAPT-HR+/HER2- chemotherapy-trial

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Schumacher, C; zu Eulenburg, C; Kates, R; Burmeister, S; Graeser, M; Wuerstlein, R; Baehner, R; Christgen, M; Kreipe, H; and Harbeck, NBackground
In high-risk hormone receptor-positive (HR+)/human epidermal growth factor receptor 2-negative (HER2-) early breast cancer (EBC), nanoparticle albumin-bound (nab)-paclitaxel (PAC) showed promising efficacy versus solvent-based (sb)-PAC in neoadjuvant trials. However, the optimal chemotherapy regimen and impact of pCR on survival in HR+/HER2- EBC are still unclear. In the WSG ADAPT HR+/HER2- chemotherapy trial, we saw a significantly higher pathological complete response (pCR) in the neoadjuvant cohort. Here, we present final survival results from the chemotherapy part of the ADAPT HR+/HER2- trial utilizing clinical risk or Oncotype DX® recurrence score (RS) and endocrine therapy (ET) response (by post-endocrine Ki-67 after 2-4 weeks of induction standard ET) for therapy stratification.
Methods
Within the WSG-ADAPT trial (NCT01779206), high-risk HR+/HER2- EBC patients were randomized to (neo)adjuvant 4 x sb-PAC 175 mg/m2 q2w or 8 x nab-PAC 125 mg/m2 q1w, followed by 4 x EC (90 mg/m2 / 600 mg) q2w.
Inclusion criteria for the chemotherapy trial comprised: (i) c/pN0-1, RS 12-25, and post-ET Ki-67 >10 %; (ii) cN0-1 with RS >25. Patients with cN2-3 or G3, baseline Ki-67 >40 %, and tumor size >1 cm were allowed to be included without RS and/or ET response-testing. 2,242 patients were required to test superiority of 8 x nab-PAC weekly vs. 4 x PAC q2w regarding iDFS after 317 invasive events or 5 years of completed follow-up.
Results
2,233 patients were randomized (1,129 / 1,104), median age was 51.0/ 51.0, clinical (in neoadjuvant chemotherapy-treated patients) or pathological N2-3 status was 19.5 % vs. 18.3 %, RS >25 occurred in 48.2 % vs. 50.7 % of the patients, and G3 tumors in 56.1 % vs. 57.5 %; ET-responders were 22 % in each treatment arm. Survival results: There was a trend for nab-Pac towards a better 5y-iDFS (85.7% versus 82.9%) compared to sb-Pac (p-value 0.054) (primary endpoint). DFS (5y 84.9 vs. 81.7%, p=.035) and RFS (5y 86.9% vs. 84.0%, p-value 0.041) showed a significant advantage with nab-Pac. Interestingly, preplanned subgroup analysis showed a benefit for nab-Pac in patients with a RS <=25, which mainly corresponds to ET non-responders (5y-iDFS nab-Pac 89.5% vs. sb-Pac 84.5%, p-value: 0.011), but not for RS>25 (5y 82.9 vs. 82.7%) . Current analysis showed no significant advantage with regard to the dDFS (5y 87.1 vs. 84.8%, p=0.13) and OS (5y 94.0 vs. 92.9%; p-value 0.387).
No new safety signals were observed so far. Details will be presented at the meeting.
Conclusions
WSG ADAPT HR+/HER2- is the largest prospective study so far, which compared two taxane-based dose-dense regimens in patients selected by RS and endocrine response. nab-Pac seems to be superior to sb-PAC in particular in patients with endocrine-non-responsive tumors.
These results will help to further optimize chemotherapy in the different prognostic subgroups of high-risk HR+/HER2- EBC.

GS3-05: NSABP B-59/GBG-96-GeparDouze: A randomized double-blind phase III clinical trial of neoadjuvant chemotherapy with atezolizumab or placebo followed by adjuvant atezolizumab or placebo in patients with Stage II and III triple-negative breast cancer

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Background:Sequential neoadjuvant chemotherapy (NAC) regimens of taxanes with carboplatin followed by anthracyclines with cyclophosphamide (AC/EC) result in pathological complete responses (pCR) in 55-60% of patients with Stage II/III triple-negative breast cancer (TNBC) who enjoy a favorable prognosis. However, those with residual invasive disease (RID) following NAC have an increased risk of recurrence even with adjuvant capecitabine. Immune checkpoint inhibitors have demonstrated improved outcomes in patients with PD-L1-positive metastatic breast cancer and increased pCR when added to NAC for Stage II/III TNBC, irrespective of PD-L1 status. This trial evaluated the efficacy and safety of adding atezolizumab to NAC followed by atezolizumab as adjuvant therapy to complete 1 year of therapy in Stage II/III TNBC. The required number of events was confirmed on 09/16/2024 to establish the data cut-off for conduct of the planned definitive analysis of the primary endpoint, event-free survival (EFS), along with secondary endpoints of pCR, overall survival (OS), and safety with results available for presentation at SABCS 2024.

Methods Design: This is a phase III, double-blind, placebo-controlled trial evaluating neoadjuvant atezolizumab with NAC followed by adjuvant atezolizumab in Stage II/III TNBC. Patients were stratified by region (North America; Europe), tumor size (1.1-3.0 cm; >3.0 cm), AC/EC schedule (q2w; q3w), and nodal status (positive; negative), then randomized 1:1 to receive atezolizumab/placebo 1200 mg IV every 3 wks concurrently with both sequential regimens of weekly paclitaxel 80 mg/m2 IV for 12 doses with every 3-wk carboplatin AUC of 5 IV for 4 doses followed by AC/EC every 2-3 wks (per investigator discretion) for 4 cycles. Following surgery, patients resumed atezolizumab/placebo 1200 mg IV every 3 wks as adjuvant therapy to complete 1 year. Radiotherapy based on local standards was co-administered with atezolizumab/placebo. Patients with RID were allowed to receive adjuvant/post-operative capecitabine or olaparib per investigator discretion. Atezolizumab/placebo could be co-administered with capecitabine but not with olaparib.
Eligibility criteria: Centrally confirmed ER-negative, PR-negative, HER2-negative invasive breast cancer by ASCO/CAP guidelines. The primary tumor must be stage T2 or T3 if cN0 or

cN1 with negative biopsy or T1c, T2, or T3 if cN1 with positive biopsy or cN2 or cN3. LVEF >55% and no significant cardiac history.
Statistical methods: The study was designed to detect a hazard ratio of 0.7 between atezolizumab and placebo for the primary endpoint of EFS with a 2-sided alpha of 0.05. Secondary endpoints include pCR breast/nodes, distant disease-free survival, OS, safety, and toxicity. Exploratory endpoints include brain metastases-free survival.
Results: 1,550 patients were randomized from December 2017–May 2021. Patient characteristics included age <60 in 79%, primary tumors >3 cm in 41%, clinically node-positive in 41%, and PD-L1 positive in 36%. With a median follow-up of 44 months, the pre-specified number of EFS events for definitive analysis was reached for top-level analyses to be completed by mid-November 2024 and available for presentation at SABCS 2024.
NCT #: NCT03281954
Support: Genentech/Roche

GS3-06: Neoadjuvant camrelizumab plus chemotherapy (chemo) for early or locally advanced triple-negative breast cancer (TNBC): a randomized, double-blind, phase 3 trial

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Background: For early or locally advanced TNBC, preferred neoadjuvant strategies include a four-drug chemotherapy regimen containing anthracyclines, cyclophosphamide, taxanes, and platinum. Accumulating evidence suggests that blockade of the PD-1/PD-L1 pathway may enhance the efficacy of conventional neoadjuvant chemotherapy. Camrelizumab is an anti-PD-1 antibody that has demonstrated antitumor activity in advanced or metastatic TNBC. Herein, we conducted a double-blind, randomized phase 3 trial (NCT04613674) to evaluate the efficacy and safety of neoadjuvant camrelizumab plus chemo in early or locally advanced TNBC.
Methods: Patients with previously untreated, invasive stage II (T2N0-1M0/T3N0M0) or III (T2N2-3M0/T3N1-3M0) TNBC were randomized (1:1) to receive neoadjuvant camrelizumab (200 mg, Q2W) or placebo plus chemo (nab-paclitaxel [100 mg/m2, D1, D8, D15, Q4W] + carboplatin [AUC 1.5, D1, D8, D15, Q4W] for 16 weeks, followed by dose-dense epirubicin [90 mg/m2, Q2W] + cyclophosphamide [500 mg/m2, Q2W] for 8 weeks). Randomization was stratified by tumor clinical stage (stage II vs III) and PD-L1 expression (combined positive score [CPS] <10 vs ≥10). After surgery, patients allocated to the camrelizumab group received camrelizumab (200 mg, Q2W) for up to a year (from first dose). The primary endpoint was pathological complete response (pCR; ypT0/is ypN0).

Secondary endpoints included event-free survival (EFS), disease-free survival (DFS), distant disease-free survival (DDFS), and pre-surgery objective response rate (ORR; per RECIST v1.1).
Results: As of data cutoff (Sep.30, 2023), 441 patients were randomized and treated (camrelizumab, n=222; placebo, n=219). Median follow-up was 14.4 mo. Overall, median age was 48.2 years; 35.8% of patients had stage III disease at baseline, and 70.5% presented with nodal involvement (N3 disease, 9.1%). pCR rate was 56.8% (95% CI 50.0-63.4) with camrelizumab + chemo and 44.7% (95% CI 38.0-51.6) with placebo + chemo (rate difference, 12.2% [95% CI 3.3 to 21.2]; 1-sided p=0.0038). The benefit in pCR with camrelizumab + chemo was observed regardless of PD-L1 expression, nodal status or disease stage at baseline. Specifically, among patients with poor prognostic factors, the pCR rate with camrelizumab + chemo vs placebo + chemo was 57.8% (89/154) vs 42.7% (67/157) for node-positive disease (rate difference, 15.1% [95% CI 4.1 to 26.1]), and 49.4% (39/79) vs 38.0% (30/79) for stage III disease (rate difference, 11.4% [95% CI -4.0 to 26.8]). Pre-surgery ORR reached 87.4% (95% CI 82.3 to 91.5) with camrelizumab + chemo and 82.6% (95% CI 77.0 to 87.4) with placebo + chemo. EFS (HR, 0.80 [95% CI 0.46-1.42]), DFS (HR 0.58 [95% CI 0.27 to 1.24]) and DDFS (HR 0.62 [95% CI 0.29-1.33]) were immature, with a trend favoring the camrelizumab + chemo group. Across stages, TRAEs of grade ≥3 occurred in 90.1% of patients in the camrelizumab + chemo group vs 82.6% in the placebo + chemo group; all events with incidence ≥10% were hematological toxicities.
Conclusions: Addition of camrelizumab to platinum-containing intensive neoadjuvant chemotherapy significantly improved pCR rate in early or locally advanced TNBC, with a manageable safety profile. Early survival data also favored the camrelizumab + chemo group.

GS3-08: In situ detection of individual classical MHC-I gene products in breast cancer identifies gene- and subtype-specific biased antigen presentation loss

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Background: Tumor-specific HLA-ABC expression is required for cytotoxic T-cell elimination of cancer cells expressing tumor associated- or neo-antigens. Moreover, the success of MHC-I-targeted cancer vaccines typically hinge on the carriage of specific types (e.g. HLA-A2) in the patient as well as type-specific antigen presentation in the tumor itself. In contrast to specific type carriage, the retention of protein expression of individual HLA genes (e.g. A/B/C) is not assessed in situ in tumor cells, despite the widely recognized knowledge that cancers downregulate or eliminate antigen presentation via multifaceted mechanisms to avoid adaptive immunity. Unfortunately, the highly polymorphic nature of the genes encoding these proteins coupled with quaternary-structure changes after formalin-fixation, complicate detection by immunohistochemistry. Moreover, only a few commercially available antibodies recommended for IHC have been characterized to

determine their recognition of specific HLA-ABC alleles.
Results: In this study, we determined allele and type-specificity for 15 commercially available antibodies for immunohistochemical use by staining HLA-ABC-null K562 cells transduced with 16 specific common HLA-A, B and C alleles. We identified and validated EMR8-5, EPR22172 and HC-10 as true pan-HLA-ABC antibodies and EPR1394Y, 2A11G7, EPR26121-73 as specific HLA-A, B, and C antibodies, respectively, to facilitate the investigation of HLA-ABC loss in situ. We applied this novel approach to a series of early-stage HER2-negative breast cancers (n=175) and DCIS (n=24) as a proof of utility. HLA-A, B and/or C expression on <15% of tumor cells was defined as loss, 15-75% as partial loss and >75% as fully conserved expression. Samples from hormone receptor-positive (HR+) breast cancer patients showed a higher rate of total HLA-A, B and/or C loss, any combination, than triple-negative breast cancer (TNBC) patient samples (73% vs 50% Fishers test p=0.0027, n=89 vs 77), driven by loss of HLA-A and/or B (total HLA-A loss 69% vs 30% p=0.0001 and total HLA-B loss 59% vs 34% p=0.0048). In contrast, samples from TNBC patients showed a higher rate of conserved expression for each single molecule and combined HLA-ABC (total conserved HLA-ABC 2% vs 16% in HR+HER2- vs TNBC p=0.0009). Among cases with HLA-A, B and/or C expression, most showed partial loss of at least one molecule, partial expression/loss was often observed within matching discrete spatial areas for HLA-A, B and/or C. Samples from patients with DCIS showed a pattern of HLA-A, B and/or C loss more similar to HR+HER2- invasive cancer regardless of HR status, both showing prominent AB loss and no cases with single molecule C loss. DCIS presented lower rates of HLA-C loss, any combination, than invasive carcinoma (8% vs 32% p=0.0165) regardless of subtype. Of note, the pan-HLA-ABC clones were insufficient to identify single or double molecule loss. All samples showed HLA-ABC expression on surrounding non-tumor tissue; however, we observed lack of staining on tumor and non-tumor cells in 5 samples with HLA-C specific EPR26121-73 antibody that were excluded from the analysis.
Discussion: We demonstrate the novel finding that up to 70% of early HR+ breast cancers lack any detectable HLA-A expression, compared to 30% of early triple-negative breast cancers. HLA-A and B loss are early events present in DCIS while HLA-C loss was more common in invasive carcinoma. Moreover, HLA-C expression was the most conserved across breast cancer subtypes. We also report an unprecedented level of individual gene product expression loss across breast cancers, which would not be detectable using poorly validated or pan-MHC-I antibodies in situ. These findings have clear implications on the success of checkpoint inhibitors and vaccine strategies in this setting, and hint at 'para-genomic' mechanisms of MHC-I gene-specific silencing in cancer-specific contexts, enabling the discovery of novel tumor evolution principles.

GS3-09: Multimodal integration of real world clinical and genomic data for the prediction of CDK4/6 inhibitors outcomes in patients with HR+/HER2- metastatic breast cancer.

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Introduction: Addition of CDK4/6 inhibitors (CDK4/6i) to endocrine therapy (ET) have led to marked improvement of outcomes in patients (pts) with HR+/HER2− metastatic breast cancer (MBC). However, the responses vary significantly with a subset of patients experiencing early therapeutic resistance. In view of many potential alternative therapeutic approaches for these patients, we sought to develop machine learning (ML) models based on clinical and genomic characteristics at the time of metastatic recurrence, to predict outcomes on 1st line CDK4/6i+ET in pts with HR+/HER2- MBC.

Methods: We identified 535 pts with HR+/HER2- MBC who received 1st line CDK4/6i+ ET between 12/2013 and 10/2023 from MSK translational database for whom MSK-IMPACT targeted tumor sequencing was performed on samples collected pre-treatment or within 2 months of the start of CDK4/6i. The models were trained on pts with complete clinical and genomic data (n=370) with 10-fold cross validation and tested on the hold-out set (n=165). OncoCast-MPM ML framework and Gradient Boosting Machine (GBM) algorithm was used to generate 3 models to predict CDK4/6i progression-free survival (PFS) based on clinicopathological features (CF), genomic features (GF) and integrating CF and GF (CGF). The Kaplan-Meier estimator with log-rank test and Cox proportional hazard models were used to assess differences in PFS, and to calculate hazard ratios (HRs) between risk groups.

Results: The median PFS (mPFS) of the full cohort was 17.8 m (95% CI: 16.1-19.7), with 67% of pts having an event. The mPFS of the training and hold-out set cohorts were 17.3 m (95% CI: 15.2.1-19.6), and 18.3 (95% CI: 16.1-23.5) with 68% and with 65% of pts having an event, respectively. The model trained on CF identified 2 risk groups (RGs): good RG (n=221), with a mPFS of 23.2m (95% CI: 18.3-28.5), and poor RG (n=149), with a mPFS of 11.6m (95% CI: 8.78-13.71). The model trained on GF identified 2 RGs: good RG (n=209), with a mPFS of 28.1m (95% CI: 21.9-31.7), and poor RG (n=161), with a mPFS of 9.7m (95% CI: 8.4-11.9). The CGF integrated model identified 3 RGs: good RG (n=113), with a mPFS of 31.3m (95% CI: 24.9-44.4), intermediate RG (n=156), with a mPFS of 18.5m (95% CI: 15.2-21.9), and poor RG (n= 101), with a mPFS of 7.9 m (95% CI: 5.4-10). The HRs between the good and poor RGs were 1.95 (95% CI: 1.5-2.5; p= 2.6e-7), 2.4 (95% CI: 1.9-3.1; p= 2.0e-11) and 4.2 (95% CI: 3.0-5.9; p= 2.8e-16) for the CF, GF and CGF models, respectively, highlighting the superior stratification power achieved by integrated clinico-genomic features. Interestingly the top 10 key features used by the multimodal model represent the union of the top 5 clinical and molecular features of the unimodal models. These features, in order of importance were tumor mutational burden (TMB), fraction of genome altered

(FGA), TP53 alteration, fraction of genome with loss of heterozygosity (LOH), presence of liver metastasis,

adjuvant treatment free interval < 1 year, primary tumor grade III, presence of visceral metastasis, primary tumor PR negativity and whole genome doubling. The results of the CGF model on the hold-out test set confirmed the presence of 3 RGs with similar HR to those identified in the training set, with no statistically significant difference in results between the training and tests sets.

Conclusions: The multimodal machine learning model integrating both clinical and genomic features, provided superior stratification and predictive power for outcomes on 1st line CDK4/6i combination compared to unimodal models. Precise risk stratification of HR+/HER2- MBC patients at the time of metastatic recurrence is key for devising therapeutic and monitoring strategies and trial design.

GS3-10: Paired DNA and RNA analysis of CALGB 40603 (Alliance) reveals insights into the molecular and prognostic landscape of stage II-III triple-negative breast cancer

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Background: Heterogeneity within triple-negative breast cancer (TNBC) makes it challenging to target therapeutically. Despite recent advances in the standard of care treatment of stage II-III TNBC, which now includes multiple chemotherapeutic agents plus an immune checkpoint inhibitor, the cure rate for these patients remains suboptimal. There is still a critical need for improved molecular characterization of TNBC and the development of accurate prognostic tools to assist in making treatment escalation and de-escalation decisions. Here, we present an analysis of the DNA and RNA landscape in a set of patients treated on a clinical trial and validation of molecular and prognostic features using three additional data sets.
Methods: CALGB 40603 (Alliance) is a randomized phase II trial that evaluated the impact of adding carboplatin and/or bevacizumab to standard anthracycline/taxane neoadjuvant chemotherapy in stage II-III TNBC. Targeted panel DNA sequencing of pre-treatment tumor samples from 238 patients enrolled in this study was performed and analyzed alongside matched pre-treatment RNAseq data. These results were then combined with clinical outcomes data to develop a prognostic multi-variable elastic net model of overall survival. We subsequently evaluated this model on samples from 380 stage II-III TNBC patients from three independent publicly available datasets.
Results: Similar to other TNBC data sets, DNAseq results of 40603 identified TP53 (86%) as the most frequent somatically mutated gene, followed by MT-ND5 (16%), MT-ND4 (12%), CSMD3 (8%), and PIK3CA (7%). BRCA1 (germline 8%, somatic 4%), BRCA2 (germline 2%, somatic 0.4%), and PALB2 (germline 1%) gene mutations were also detected, and when

combined into a single “homologous recombination deficiency” category totaled 15%. The DNA copy number landscape of TNBC was very similar across the four data sets, and largely mirrored the landscape seen for basal-like breast cancer characterized in TCGA. Importantly, only one somatically mutated gene (PIK3R2), and no DNA copy number altered regions, were associated with pathologic complete response or survival at a false discovery rate < 0.05. We next sought to use these multi-platform data to train a prognostic Cox elastic net regression model using CALGB 40603 data and a diverse set of features, including tumor stage, 804 RNA expression signatures, 727 genes with somatic mutations, and 534 chromosomal segment-level copy number alterations. This yielded a 31-feature prognostic model with C-index performance values (95% confidence interval) of 0.68 (0.66-0.69), 0.61 (0.59-0.63), and 0.83 (0.82-0.84) using FUSCC (PMID 30853353), METABRIC (PMID 22522925), and TCGA (PMID 23000897) test sets. The most heavily weighted features in this model were tumor stage and multiple immune signatures including an IgG signature, and this model had higher C-index values than a model trained using only tumor stage.
Conclusions: This study provides a comprehensive and integrated characterization of the DNA- and RNA-based landscape of a large stage II-III TNBC patient data set. Through the development of a multi-omic elastic net model of TNBC survival, we show that we may improve the prognostic accuracy for overall survival beyond stage alone by incorporating machine learning selected molecular features. Furthermore, the model’s ability to identify good overall survival outcome patients when given neoadjuvant chemotherapy illustrates its potential utility for informing treatment escalation/de-escalation decisions, which could be clinically valuable if further validated.
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LB1-01 - immediate breast surgery versus deferral of surgery in women aged 70+ years with operable breast cancer: patient-level meta-analysis of the three randomised trials among 1,082 women

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Background: Endocrine therapy alone, with breast surgery offered only at local progression, is still sometimes considered for older women with operable early breast cancer, but the long-term risks of deferring surgery are uncertain. We evaluated long-term outcomes in the three unconfounded randomised trials that compared immediate breast surgery plus tamoxifen versus tamoxifen alone. None of these trials scheduled radiotherapy or chemotherapy.
Methods: Individual patient data meta-analyses compared effects on breast cancer

outcomes in 3 trials, initiated in the 1980s, among 1082 women (age ≥70, all to receive tamoxifen for at least 5 years) comparing immediate surgery versus surgery only in the event of local progression. Primary outcomes were time to locoregional failure, to distant recurrence, and to breast cancer mortality. Locoregional failure was defined as any locoregional recurrence after surgery or, if no immediate surgery, ≥25% increase in tumour diameter. Age-adjusted intent-to-treat log-rank analyses, stratified by nodal status, were used to estimate first-event-rate ratios (RRs).
Results: Median age at randomisation was 76 (IQR 73-80) years, and 63% (666/1082) had clinically estimated tumour diameter >20 mm. Mean follow-up while still alive was 7.3 woman-years. Of 518 women allocated immediate surgery, 45.7% had mastectomy, 47.3% had breast-conserving surgery, and 7.0% had neither. Locoregional failure was greatly reduced by allocation to immediate surgery (RR=0.24, 95% CI 0.19-0.30, p<0.00001). This extreme RR was little affected by age, disease stage, or time period. Although the proportional reduction in the annual rate of locoregional failure was similar during years 0-1, 2-4 and 5-9, the absolute reduction in locoregional failure was mainly before year 5 (Kaplan-Meier 5-year risks 12.1% vs 45.8%). On average over the whole follow-up period the rates of distant recurrence (RR=0.72, 0.57-0.90, p=0.003), breast cancer mortality (RR=0.68, 0.54-0.86, p=0.002), and all-cause mortality (RR=0.83, 0.72-0.97, p=0.016) were also reduced, but these benefits emerged only after years 0-1. The distant recurrence rate ratio was 0.97 (0.67-1.42) during years 0-1 after randomisation, 0.73 (0.49-1.07) during years 2-4 and 0.52 (0.36-0.76) after year 5 (trend: p=0.012).
Conclusion: In early breast cancer, immediate breast surgery greatly reduces locoregional progression rates during the first 5 years and approximately halves the annual rates of distant recurrence and of breast cancer death after the first 5 years, despite having had little clinically apparent effect on distant recurrence rates during the first few years. These findings could indirectly inform the planning and interpretation of trials of less extreme de-escalation of surgery or radiotherapy.

LB1-02: MARGOT/TBCRC052: A randomized phase II trial comparing neoadjuvant paclitaxel/margetuximab/pertuzumab (TMP) vs paclitaxel/trastuzumab/pertuzumab (THP) in patients (pts) with stage II-III HER2+ breast cancer

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Background: Margetuximab combined with chemotherapy modestly improves outcomes compared to trastuzumab plus chemotherapy in pts with heavily pre-treated HER2+ metastatic breast cancer. The efficacy of margetuximab for pts with newly diagnosed HER2+

early breast cancer (EBC) is unknown.
Methods: Eligible pts had previously untreated stage II-III HER2+ EBC, any hormone receptor (HR) status, and CD16A genotype FF or FV by central testing. Pts were randomized 2:1 to neoadjuvant TMP or THP for 4 cycles (weekly T x12; q3wk MP or HP x4). ER status and baseline clinical stage were stratification factors. The primary endpoint was pathologic complete response (pCR; ypT0/isN0). With 171 pts randomized, there was 80% power to detect improvement in pCR rate from 45% with THP to 65% with TMP with one-sided alpha 0.05. pCR rates were compared between arms using Fisher's exact tests. Additional endpoints included pCR rate according to CD16A genotype (FF vs FV), safety and tolerability of neoadjuvant TMP and THP, and event-free survival (EFS).
Results: 174 pts were randomized from 10/2020-5/2024, and 171 pts started tx on trial: 117 TMP and 54 THP. One pt was found to be ineligible and excluded from efficacy analyses. Pts were 100% female, with median age 53 yrs (range 30-79 yrs); 77% White, 10% Black, and 4% Asian; 13% were Hispanic. 65% of pts had HR-positive tumors, 87% had cT1-T2 tumor size, and 35% were clinically node-positive. On the TMP vs THP arms, 86% vs 89% of pts received 12 doses of neoadjuvant taxane, 97% vs 96% of pts received 4 doses of neoadjuvant M vs H, and 95% vs 94% of pts received 4 doses of neoadjuvant P. pCR rate was 56% vs 46% in the TMP vs THP arms, respectively (difference 10%, 95% CI [-8%, 27%]; p=0.25). Additional residual cancer burden (RCB) scores for TMP vs THP, respectively, were 7% vs 13% (RCB I), 25% vs 28% (RCB 2), and 2% vs 2% (RCB 3). 9% vs 11% of pts (TMP vs THP) received additional neoadjuvant tx due to incomplete clinical response to TMP or THP, and were considered non-pCR at surgery. Two vs 0 pts (TMP vs THP) did not undergo surgery on trial (one due to death that was unrelated to study therapy; one due to withdrawal of consent prior to surgery). Among HR+ pts (n=111), pCR rate was 48% overall (54% TMP vs 35% THP); among HR- pts (n=59), pCR rate was 63% overall (60% TMP vs 71% THP). Among HER2 IHC 3+ pts (n=130), pCR rate was 62% overall (66% TMP vs 55% THP); among HER2 IHC <3+ pts (n=40), pCR rate was 23% (27% TMP vs 10% THP). Pts with >1 grade 2+ treatment-emergent adverse event (TEAE) during neoadjuvant tx were similar by arm: 82% TMP vs 78% THP; those with >1 grade 3+ TEAE were also similar: 27% TMP vs 30% THP. The most common grade 2+ TEAEs were infusion-related reaction (27% TMP [4% grade 3-4] vs 15% THP [2% grade 3-4]), diarrhea (24% TMP [5% grade 3-4] vs 26% THP [6% grade 3-4]), alopecia (20% vs 4%), and hypertension (10% vs 24%). The only grade 2+ TEAEs that were more common in the TMP arm by >10% were infusion-related reaction and alopecia. There was one grade 5 AE (death unrelated to study tx) on the TMP arm. pCR rate according to CD16A genotype (FF vs FV) will be reported; all pts continue to be followed for EFS.
Conclusions: There was no statistically significant improvement in pCR rate with neoadjuvant TMP vs THP for pts with HER2+ EBC and CD16A FF/ FV genotype. Safety and tolerability were similar between the two regimens, aside from increased rates of infusion-related reactions and alopecia in the margetuximab arm (scalp-cooling use was not recorded). Given numerical improvement in pCR rate, comparative analysis of immune activation biomarkers between the two tx arms will be performed. EFS data remain immature; pCR rates by CD16A genotype will be presented.

LB1-03: Primary results of the randomised Phase III trial comparing first-line ET plus palbociclib vs standard mono-chemotherapy in women with high risk HER2-/HR+ metastatic breast cancer and indication for chemotherapy - PADMA study

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Background: Whether chemotherapy (CT) or an endocrine therapy (ET) in combination with a CDK4/6 inhibitor in HR+/HER2-metastatic breast cancer (mBC) is the preferred option is still a matter of debate. The PADMA (NCT03355157; GBG 93) prospective, randomized, open-label, multi-center, phase III trial is the first trial to compare a standard mono-CT followed by maintenance ET with a CDK4/6 inhibitor plus ET as first-line therapy in high -risk mBC patients (pts).
Study Design: Pts previously untreated for HR+/HER2- mBC with an indication for chemotherapy were randomized to receive either palbociclib 125mg on days 1-21 q28 in combination with ET (palb/ET) or to mono-CT of physician's choice (PC) with or without following maintenance ET. Stratification factors included endocrine resistance and presence of disease symptoms as defined by the investigator. Treatment was administered until disease progression, unacceptable toxicity, withdrawal of consent, or modification to the initial treatment plan. The primary endpoint (EP) is time to treatment-failure (TTF) defined as time from randomization to discontinuation of treatment due to disease progression, treatment toxicity, patient's preference, or death. Secondary EPs included progression free survival (PFS), time to first subsequent treatment (TFST), time to first subsequent chemotherapy, time to second subsequent treatment regimen, overall survival (OS), safety, compliance, and well-being and health care utilization by daily monitoring content with quality of life, degree of bother by side-effects, number and duration of phone calls, site visits.
Results:A total of 130 pts (66 palb/ET; 64 CT) were enrolled at 28 German sites between Apr 2018 and Dec 2023 of whom 120 started treatment and were included in this analysis. 12 (10%) of the pts were pre-/perimenopausal, 90 (75%) had metastases in two or more organ systems, 52 (43.3%) had symptomatic disease, 10 (8.3%) were endocrine resistant at enrolment. The median age was 62 (range 31-85) years. CT of PC was capecitabine 40 (69.0%), paclitaxel 17 (29.3%) and vinorelbine 1 (1.7%). Out of 58 pts receiving CT, 13 pts (22.4%) switched to maintenance ET.
After a median follow-up of 36.8 (15.0-47.7) months (mo) 45 pts (73.8%) in the palb/ET arm and 55 (93.2%) in the CT arm experienced a TTF event. The median TTF was significantly longer with palb/ET 17.2 (7.2, 25.9) mo vs 6.1 (4.3, 8.8) mo with CT (HR 0.46 [80% CI 0.35-0.60], log-rank p=0.0002) meeting its primary EP. The leading cause of treatment failure was progression (52.5% with palb/ET vs 76.3% with CT). Median PFS was significantly longer with palb/ET 18.7 (10.6, 29.1) mo and 7.8 (6.0, 10.0) mo with CT (HR

0.45 [95% CI 0.29-0.70], log-rank p=0.0002). Median TFST was significantly longer with palb/ET 19.9 (11.0, 30.5) vs. 8.0 (6.8, 13.1) mo (HR 0.52 [95% CI 0.34-0.80], log rank p=0.0028). Median OS was numerically prolonged with palb/ET 46.1 (27.2, n.a.) vs. 36.8 (25.2, n.a.) mo with CT (HR 0.81 [95% CI 0.46-1.43], log rank p=0.4630).
Hematologic toxicity was significantly higher in the palb/ET arm (any grade 96.8% vs 70.7%, p<0.001; high grade 56.5% vs 10.3%, p<0.001) with non-hematologic toxicity being comparable between the arms. Serious treatment related adverse events were generally low with 4 (6.5%) in the palb/ET and 6 (10.3%) in the CT arm. One death was treatment related (palb/ET arm).
Conclusion: The phase III PADMA trial in high-risk HR+/HER2- mBC shows statistically significant and clinically meaningful improved TTF and PFS with a trend for improved OS for palbociclib plus ET over mono-CT as first-line therapy. These results support existing international guidelines advocating the use of ET + CDK4/6 inhibitors as standard in 1line treatment of HR+/HER2- mBC.

LB1-04: Efficacy and safety of trastuzumab deruxtecan (T-DXd) vs physician's choice of chemotherapy (TPC) by pace of disease progression on prior endocrine-based therapy: additional analysis from DESTINY-Breast06

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Background: The Phase 3 randomized DESTINY-Breast06 study enrolled patients (pts) with hormone receptor–positive, human epidermal growth factor receptor 2 (HER2)–low (immunohistochemistry [IHC] 1+, IHC 2+ / in situ hybridization–negative) or HER2-ultralow (IHC 0 with membrane staining) metastatic breast cancer (mBC) who had progression of disease (PD) after ≥1 endocrine-based therapy and had not received chemotherapy (CT) for mBC. In the intent-to-treat population (ITT; HER2-low + HER2-ultralow), pts had a statistically significant and clinically meaningful improvement in progression-free survival (PFS) with T-DXd vs TPC (median, 13.2 vs 8.1 months [mo]; hazard ratio [HR] 0.63, P<0.0001). A PFS benefit was observed regardless of the number of lines of prior endocrine-based therapy for mBC. Additional analyses investigated the impact of response to prior endocrine-based therapy on outcomes.
Methods: Pts were assigned 1:1 to T-DXd 5.4 mg/kg given intravenously every 3 weeks or TPC (59.8% capecitabine; 24.4% nab-paclitaxel; 15.8% paclitaxel). Pts had ≥2 lines of prior endocrine-based therapy for mBC, or 1 line if PD occurred within 24 months of adjuvant endocrine therapy (ET) or within 6 mo of first-line (1L) ET + CDK4/6 inhibitor (CDK4/6i) for mBC (the latter considered rapid progression). Subgroups were time to progression (TTP) on 1L ET + CDK4/6i, and primary vs secondary endocrine resistance (per 5th ESO-ESMO ABC criteria). Outcomes included PFS, confirmed objective response rate (ORR), and

duration of response (DOR) by blinded independent central review in the ITT, and safety.
Results
Most pts in the ITT (89.3% of n=866) received at least 1 prior line of ET + CDK4/6i. The TTP analysis included pts with PD on 1L ET + CDK4/6i (65.8% of the ITT), comprising 124 pts with <6 mo, 112 with 6–12 mo, and 334 with >12 mo TTP. Pt numbers, demographics, and baseline clinical characteristics were balanced across subgroups. A total of 58.9% of pts (<6 mo; n=73), 23.2% (6–12 mo; n=26), and 7.2% (>12 mo; n=24) received T-DXd/TPC as a second-line treatment after 1 prior line of ET + CDK4/6i. PFS on study was longer with T-DXd vs TPC in the <6-mo subgroup (median, 14.0 vs 6.5 mo; HR 0.38 [95% confidence interval {CI} 0.25, 0.59]); data were consistent in the 6–12-mo (13.2 vs 6.9 mo; HR 0.69 [95% CI 0.43, 1.12]) and >12-mo subgroups (12.9 vs 8.2 mo; HR 0.67 [95% CI 0.51, 0.88]). ORR favored T-DXd vs TPC in pts with <6 mo TTP (67.7 vs 25.4%), similar to those with longer TTP (6–12 mo: 60.0 vs 28.8%; >12 mo: 59.5 vs 33.1%). Median DOR favored T-DXd vs TPC across all TTP subgroups (<6 mo: 11.1 vs 7.3 mo; 6–12 mo: 13.7 vs 11.5 mo; >12 mo: 15.7 vs 11.1 mo). Efficacy outcomes were consistent in pts with investigator-assessed primary and secondary endocrine resistance. The incidence of Grade ≥3 treatment-emergent adverse events in pts receiving T-DXd and TPC in TTP subgroups was consistent with the overall safety population: 55.4 vs 42.4% (<6 mo; n=124); 59.3 vs 40.8% (6–12 mo; n=108); and 45.8 vs 42.9% (>12 mo; n=329), respectively.
Conclusion
T-DXd demonstrated a clinically meaningful efficacy benefit vs TPC, with a >12 mo median PFS across 1L ET + CDK4/6i TTP subgroups, notably in pts with rapid (<6 mo) progression. The safety profile in subgroups was in line with the overall safety population. Results highlight the potential role of T-DXd as an early line treatment after ≥1 line of endocrine-based therapy for pts with hormone receptor–positive, HER2-low/-ultralow mBC.

Clinical trial identification: NCT04494425

LB1-06: Primary results of SOLTI VALENTINE: neoadjuvant randomized phase II trial of HER3-DXd alone or in combination with letrozole for high-risk hormone receptor positive (HR+)/HER2-negative (neg) early breast cancer (EBC)

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Background: Despite the activity of both chemotherapy (CT) and endocrine treatment in high-risk HR+/HER2-neg EBC, the risk of recurrence persists over time, highlighting the need for additional strategies to improve outcome. HER3-DXd is a potential first-in-class HER3-directed antibody-drug conjugate with activity in multiple breast cancer subtypes. In SOLTI TOT-HER3, a single dose of HER3-DXd was associated with increased CelTIL and clinical response in patients (pts) with HR+/HER2-neg EBC (Oliveira et al, Ann Oncol 2023).
Methods: SOLTI VALENTINE (NCT05569811) is a parallel, randomized, non-comparative, open-label neoadjuvant study of HER3-DXd +/- LET or CT in pts with operable stage II-III high risk (Ki67 ≥ 20% and/or high genomic risk) HR+/HER2-neg EBC. Pts were randomized 2:2:1 to: (A) HER3-DXd 5.6 mg/kg every (Q) 21 days (D) for 6 cycles; (B) HER3-DXd plus QD LET (+/- LHRH agonist); (C) CT with 4 cycles of EC/AC (epirubicin 90 mg/m2 or doxorubicin 60 mg/m2 and cyclophosphamide 600 mg/m2 Q14 or 21 D) followed by weekly paclitaxel 80mg/m2 for 12 weeks. The primary endpoint was pathological complete response (pCR, ypT0/is ypN0) rate at surgery. Secondary endpoints included residual cancer burden (RCB), overall response rate (ORR), CelTIL score change, safety, invasive disease-free survival, and overall survival. Baseline, C2D1, and surgical tissue samples were collected for evaluation of response and translational endpoints. The sample size was not selected to formally compare treatment arms in terms of pCR rate, but to achieve a certain level of precision estimating pCR. Assuming a pCR of 20%, a total of 48 per arm would provide a precision estimation of +-11.3% using asymptotic two-sided 95%CI.
Results: From Nov-2022 to Sep-2023, 122 pts were randomized to HER3-DXd (n=50), HER3-DXd + LET (n=48) and CT (n=24). Median age was 51 (29 - 82), 99.2% were females, and 52.9% pre/perimenopausal. Most pts had T2 (54.1%), N1 (70.5%), Stage II (64.0%), G2 (69.0%) tumors; median Ki67 was 35.0% (18.0; 90.0). Proportion of pts with Stage III tumors was 36.0%, 39.5% and 29.2% in Arms A, B, and C, respectively. The pCR rate was 4.0% (95%CI 0.5-13.7) in Arm A, 2.1% (95%CI 0.1-11.1) in Arm B, and 4.2% (95%CI 0.1-21.1) in Arm C. The RCB0/1 rate was 18.4%, 12.5% and 30.4%, and ORR was 72.0%, 81.3%, and 70.8%, in Arms A, B, and C, respectively. A significant change in CelTIL score from baseline to C2D1 was observed in HER3-DXd arms, but not in Arm C: median increase in CelTIL of 8.2 ($p<0.0001$) in Arm A, 7.4 ($p=0.001$) in Arm Band 3.8 ($p=0.13$) in Arm C. CelTIL change at C2D1 associated with radiological response in Arm A ($p<0.0001$) and B ($p=0.002$), but not in Arm C ($p=0.30$). A decrease in Ki67, as well as a switch from PAM50 Risk of Recurrence (ROR)-high/medium to ROR- low score and from PAM50 Luminal B subtype to Luminal A/Normal-like subtypes were observed in all treatment arms at C2D1, and was further enhanced at surgery. Treatment-emergent adverse events (TEAEs) grade≥3 were less frequent in the HER3-DXd arms (18.0% and 16.7% in Arms A and B, respectively), compared to Arm C (54.2%). The most common related TEAEs reported in Arms A/B were nausea (56%/72.9%), alopecia (52%/68.8%), fatigue (50%/68.8%), and diarrhea (42%/54.2%).
Conclusions: In SOLTI VALENTINE, treatment with HER3-DXd, with or without LET, resulted in similar pCR rates to CT, while exhibiting a lower incidence of grade ≥3 TEAEs. CelTIL score at C2D1 correlated with response to HER3-DXd, but not to CT. The ongoing

translational analysis, as well as the survival outcomes of VALENTINE, will provide further insights into the activity of HER3-DXd in EBC and clarify its potential role as a treatment strategy for high-risk HR+/HER2-neg breast cancer.

LB1-07: Exploratory Biomarker Analysis of the Phase 3 KEYNOTE-522 Study of Neoadjuvant Pembrolizumab or Placebo Plus Chemotherapy Followed by Adjuvant Pembrolizumab or Placebo for Early-Stage TNBC

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Background: In the phase 3 KEYNOTE-522 (NCT03036488) study, neoadjuvant pembrolizumab (pembro) + chemotherapy (chemo) with adjuvant pembro significantly improved pCR, event-free survival (EFS) and OS vs neoadjuvant placebo (pbo) + chemo with adjuvant pbo in patients (pts) with high-risk, early TNBC. The key objectives of this exploratory biomarker analysis were to evaluate the association of tumor mutational burden (TMB), T-cell–inflamed 18-gene expression profile (TcellinfGEP), and a set of non-TcellinfGEP consensus signatures with pCR and EFS.
Methods: Eligible pts had newly diagnosed, previously untreated, high-risk, early TNBC (T1c N1–2 or T2–4 N0–2) and evaluable pretreatment tumor samples. TMB was assessed by whole-exome sequencing (WES) and TcellinfGEP and non-TcellinfGEP consensus signatures by RNA sequencing (RNAseq). Analyses followed a pre-specified statistical analysis plan. Associations of TMB, TcellinfGEP, and non-TcellinfGEP consensus signatures (proliferation, stromal/EMT/TGFβ, RAS, angiogenesis, gMDSC, glycolysis, hypoxia, mMDSC, MYC and WNT) with pCR were evaluated respectively by logistic regression and area under the receiver operating characteristic curve analysis with 95% CIs. Associations of these biomarkers with EFS were evaluated by Cox proportional hazards regression. No multiplicity adjustment was applied for TMB or TcellinfGEP analyses; nominal P values <0.05 (1-sided for pembro + chemo; 2-sided for pbo + chemo) were deemed sufficient to support an association. Non-TcellinfGEP consensus signatures were tested at an alpha of 0.10 adjusted by the Hochberg step-up procedure. Associations of RNAseq-based molecular subtypes, BRCA (germline and tumor)/HRD status, HER2 gene expression/signature, and PTEN loss signature with pCR and EFS were secondary objectives. Data cutoff was March 23, 2021.
Results: Of 1172 randomized and treated pts (pembro + chemo, n = 783; pbo + chemo, n = 389), 946 pts had WES data (pembro + chemo, n = 641 [81.9%]; pbo + chemo, n = 305 [78.4%]) and 904 had RNAseq data (pembro + chemo, n = 618 [78.9%]; pbo + chemo, n = 286 [73.5%]). Baseline clinical characteristics in the WES and RNAseq evaluable populations were similar to the overall population. TcellinfGEP was positively associated with pCR and EFS in both the pembro + chemo and pbo + chemo arms (P≤0.001). TMB showed a clear association with pCR and EFS in the pembro + chemo arm (P≤0.001) but not

in the pbo + chemo arm (P=0.011 pCR; P=0.422 EFS). Subgroup analyses by pre-specified cut-offs for TcellinfGEP (1st tertile) and TMB (175 mut/exome) showed EFS curves were well separated and favored pembro + chemo over pbo + chemo regardless of subgroup. Among non-TcellinfGEP consensus signatures, proliferation and glycolysis were positively prognostic for pCR but not EFS in both treatment arms. Among secondary endpoints, positive associations for PTEN loss signature and BRCA/HRD status with pCR were seen in both treatment arms. HER2 gene expression was negatively correlated (−0.32) with TcellinfGEP. HER2 levels and molecular subtypes did not show associations with pCR and EFS in the pembro + chemo arm after TcellinfGEP adjustment. Pre-specified cut-off based subgroup analyses of secondary biomarkers confirmed consistent benefit of pembro + chemo over pbo + chemo.
Conclusions: This exploratory analysis of KEYNOTE-522 found that TcellinfGEP was prognostic for improved pCR and EFS with or without addition of pembro. TMB was associated with improved EFS only in the pembro + chemo arm. Pembro + chemo had an efficacy advantage vs chemo alone regardless of subgroups defined by TMB, TcellinfGEP and other biomarkers assessed. These findings may be relevant to design of future studies looking to improve on immunotherapy + chemo as the standard of care.

P1-01-01: Mainstream Cancer Genetics Testing in Primary Care at a Federally Qualified Health Center: Preliminary Findings from the TestMiGenes Study

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Introduction: Mainstream genetic testing (MGT) has been proposed as an alternative model to scale access to cancer genetic services utilizing established care providers to provide point-of-care genetic testing at the time of risk identification. However, MGT has primarily been adopted in the oncology setting. Expanding MGT into the primary care (PC) setting leverages the trusted relationship of providers within the patient's medical home increasing the opportunity for primary prevention. In 2023, the University of Illinois Cancer Center partnered with the Mile Square Health Center, a system of federally qualified health centers, to initiate a quality improvement (QI) initiative promoting the prevention and early detection of cancer through universal hereditary cancer risk assessment (HCRA) across PC sites. Building off this QI initiative is the TestMiGenes study, which compares the effectiveness of a standard-of-care referral model enhanced with navigation support to a mainstream/point-of-care testing (MGT) model on the uptake of genetic testing among adults identified at risk for hereditary cancer syndromes in PC settings.
Methods: A digital HCRA tool, developed in English and Spanish at a 7th-grade reading level, was administered by navigators to patients aged 25 and older presenting for routine PC visits across multiple FQHC sites. The HCRA tool identified patients meeting National Comprehensive Cancer Network (NCCN) criteria for genetic testing eligibility based on reported personal and family history. Patients who met NCCN criteria for genetic testing (GT) on HCRA received either a referral to genetic counseling or were offered GT by their PC

provider (MGT). From January to December 2023, the referral model was implemented with 10 PC providers across 2 clinics. Eight PC providers from these 2 clinics transitioned to the MGT model from September 2023 to mid-May 2024. PC providers were trained to provide brief education and conduct shared decision-making around HCRA, placing GT orders, and GT result disclosure via multiple educational sessions with cancer genetic counselors and cancer risk specialists.
Results: Overall, 1555 patients received HCRA, and 300 were eligible for GT. The majority of screened patients were Black or Hispanic (84%), under the age of fifty (52%), and female (68%). Of those eligible for GT, 68 received cancer genetic services under the MGT model and 232 received services under the referral model. Uptake of genetic testing was significantly higher among patients cared for in the MGT model (35%, n=24) compared to the referral model (23%, n=53) ($p<0.05$). Time from identification of GT eligibility to completion of GT also differed significantly from 7 days in the MGT model to 93 days in the referral model ($p < 0.05$). Of note, only 40% (n=69) of those eligible for GT in the referral model completed a GC visit, pointing to additional barriers in the current standard of care process.
Conclusion: Preliminary data suggests that an MGT model is feasible in the PC setting and can potentially increase uptake and reduce wait time for GT. Notably, cancer genetic services are underutilized in both models likely due to multilevel barriers. Further research is needed to explore acceptability, sustainability, facilitators and barriers, and potential harms of both MGT and referral models in PC. The TestMiGenes study is currently conducting patient and provider interviews to explore stakeholder perceptions of GT in primary care.

P1-01-02: T cell mediated breast cancer protection following lactation and involution.

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Background:The mechanisms by which parity and breastfeeding can mediate protection against breast cancer (BC), particularly triple-negative breast cancer (TNBC), remain unclear. CD8+ T cells with a tissue-resident (TRM)-like phenotype have been shown to be present in primary TNBCs with high quantities of immune infiltrate and are associated with better clinical outcomes. We have previously shown TRM-like cells are also present in healthy, non-cancer-affected normal human breast tissue. We hypothesized that the inflammatory processes induced post pregnancy could result in increased CD8+ T cell quantity and this could facilitate protection against BC in the normal breast.

Methods:We compared immune cell quantities in healthy, cancer-unaffected normal breast tissue collected from parous versus nulliparous women using flow cytometry and from single cell RNA sequencing datasets. Using immunocompetent and deficient murine models,

we characterised mammary tissue infiltrating lymphocyte populations at early (Day 10) and late involution (Day 28) timepoints, without and with lactation respectively. We next correlated immune cell profiles and rate of tumor growth by implanting mammary tumours at the different stages of involution. We further evaluated the quantity of immune infiltration from a dataset of 736 women who had developed BC with parity and breastfeeding history.

Results:We observed significantly higher quantities of CD45+ immune cells and CD8+ TRM-like cells in the normal cancer unaffected breast tissue of parous women compared to nulliparous women using both flow cytometry (n=90) and single cell RNA sequencing datasets (n=263).

In murine models, significantly more CD8+ TRM-like cells accumulated in mammary tissue at the later D28 vs earlier D10 involution timepoint compared with virgin control mice. The increase in TRM-like cells in mammary tissue correlated with significantly decreased tumor growth compared to virgin controls, whereas the early involution timepoint had similar tumor growth rates to virgin mice. The tumors implanted at D28 compared with the D10 involution also had significantly higher intratumoral T cell content.

To specifically investigate if these tumor protective effects were mediated by T cells, we used RAG2-/-yc-/- immunodeficient mice inoculated with AT3-OVA tumors into the mammary gland at the early D10 and late D28 involution timepoints after injection of OT-I cells (transgenic CD8+ T cells that specifically recognise OVA antigen) prior to mating. We observed significantly reduced mammary tumor growth at the later D28 vs earlier D10 timepoint, corresponding to increased conversion of naive OT-I cells to a resident memory phenotype (CD69+CD103+).

We next examined a cohort of patients who were diagnosed with BC and their intratumoral immune content according to subtype and parity/breastfeeding history (n=736). Consistent with the murine data, we found significantly increased tumoral immune infiltration in parous women who had developed postpartum TNBC compared with nulliparous women.

Conclusions:Our data, from both human normal breast samples and murine models of parity, provide evidence that lactation and involution modulate local breast immunity that results in increased quantity of resident CD8+ T cells in cancer unaffected normal breast tissue. In mice, these cells could restrain mammary tumor outgrowth, suggesting a protective mechanism by which lactation and involution can attenuate long term risk of BC development. Parity was also associated with increased tumor immune infiltrate in women who developed early stage TNBC, which has important implications for prognosis.

P1-01-03: Enhancing Patient Care: A Digital Approach Improves Universal Breast Cancer Risk Stratification in Imaging Centers

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Background:Identifying individuals at increased cancer risk is crucial for prevention and early breast cancer diagnosis. Integrating a risk assessment tool into imaging centers supports proactive cancer management by combining risk evaluation with immediate diagnostic capabilities. We present over 6 years of data from 15 sites of Midstate Radiology Associates (MRA), where breast cancer risk stratification was implemented and transitioned from paper screening form to a universally accessible digital platform. Our findings demonstrate that a digital approach for breast cancer risk assessment in imaging centers significantly improves the identification of individuals at elevated risk.
Methods: We conducted a retrospective study across 15 MRA Imaging Centers from 2018 to June 2024. From 2018 to 2021, 13 MRA centers used paper family history forms to screen mammography patients for eligibility for hereditary cancer genetic testing, with Tyrer-Cuzick scores included for those genetically tested. From 2021 onward, patients at 12 MRA centers used the Ambry CARE Program® before appointments to assess lifetime breast cancer risk using the Tyrer-Cuzick (version 8.0) algorithm, determining eligibility for genetic testing based on National Comprehensive Cancer Network (NCCN®) for hereditary cancers (breast, ovarian, pancreatic, prostate), Lynch syndrome, and familial adenomatous polyposis (FAP). We compared outcomes between paper screening and the digital tool, including risk assessment completion, genetic testing criteria met, pursuit of germline testing, positive germline results, and Tyrer-Cuzick scores ≥20%.
Results: During the years paper screening forms were used, there were 168,323 mammogram appointments. Of those, 24.6% (41,424) met criteria for genetic testing based on paper documentation. Among the 12.4% (5,133) who opted for testing, 6% (332) had positive results and 22% (1,133) had a ≥20% lifetime breast cancer risk. Looking into the years a digital screening tool was utilized, 84,122 individuals were invited to complete an assessment, 75.8% (63,749) responded, with 98% (60,438) being females aged 18 or older. At the time of assessment, 26.3% (16,819) met the criteria for genetic testing, and 20.7% (3,489) of these opted for germline genetic testing. Additionally, 1431 individuals who did not meet criteria chose to undergo testing. Among the 4,920 completed genetic tests, 9.6% (470) had positive results, with 46.8% (220/470) influencing breast cancer risk management options. Furthermore, 10.6% (5,984/56,245) of those females without personal history of cancer assessed using the Tyrer-Cuzick algorithm were identified as having a ≥20% lifetime risk of breast cancer, warranting modified medical management.
ConclusionWe observed poor documentation during the period when paper screening forms were used, suggesting that universal breast risk screening was not offered to all patients. Outcomes indicate that the digital tool not only increased hereditary cancer genetic testing uptake but also expanded access to lifetime breast cancer risk assessment. This study underscores the benefits of universally implementing a digital risk stratification tool in imaging centers. The digital tool ensures efficient identification of patients eligible

for modified management of breast cancer and other hereditary syndromes, thereby enhancing prevention and early treatment.

P1-01-04: EPLIN and EPLIN responsive protein APOC3 in breast cancer

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Introduction. EPLIN (Epithelial Protein Lost in Neoplasm, also known as LIMA1) is a cytoskeletal associated element and has been shown to act as a tumour suppressive molecule in breast cancer and a few other cancer types. Little is known about the regulatory and the responsive proteins for EPLIN. The present study has explored the potential EPLIN responsive proteins in cancer cells and the possible clinical connections in prognosis and treatment in breast cancer.
Methods. EPLIN knockdown cell models were created by using lentiviral shRNA for EPLIN. The responsive proteins including phospho-proteins following EPLIN knockdown were systemically evaluated by MS based bioinformatics analysis. Clinical cohorts and tissue microarrays were used to evaluate the potential EPLIN interactive candidates at gene transcript and protein levels, respectively. The prognostic connection and therapeutic values were also explored.
Results. Knockdown EPLIN resulted in active responses of a number of proteins, of which APOC3 (apolipoprotein C3) was amongst the top ones (both total level and phosphorylated proteins). In clinical cohort, APOC3 were found to be significantly correlated with EPLIN in breast tumour tissues (r=-0.218, p=0.042) but not in normal mammary tissues (p=0.555). APOC3 expression in breast tumour tissues was found to be higher in ER negative tumour (p=0.0058) and in PGR positive tumours (p=0.039), and also in those who died of breast cancer (p=0.016, versus those who remained disease free). The expression profile of the EPLIN related molecule identified patients with high survival risk in those who had PGR negative breast tumours (p=0.014, HR=1.374 (95%CI 1.007-1.875) and in triple negative breast tumours (TNBC) (p=0.045). Multivariate analysis revealed that the expression profile is an independent prognostic factor for PR negative patients (p=0.037) against other clinical factors. Finally, we explored the value of APOC3 and EPLIN in relation to patient's response to drug treatments in a public database. It was clear that high levels of APOC3 in breast cancers rendered the patients with a significant chance of developing resistance to chemotherapies (p=0.000024) and this connection is independent on status of ER, ERBB2 and the molecular subtypes. Intriguingly, APOC3 was found to be a good indicator for sensitivity to anti-Her2 treatment (AUC=0.79, p=0.00076).
Conclusion. The present study identifies a key EPLIN response protein, APOC3 which together with EPLIN forms an important prognostic factor for patients with breast cancer particularly in those with PR negative tumours. It also forms a theranostic indicator for patient's response to drug treatment.

P1-01-05: Conducting Ancillary Studies during an Active NCTN/NCORP Screening Trial – The TMIST (ECOG-ACRIN EA1151) Experience

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Background:The primary aim of the Tomosynthesis Mammographic Imaging Screening Trial (TMIST) is to determine whether women randomly assigned to be screened through 3-5 rounds with tomosynthesis (TM) have fewer advanced cancers than the population screened with digital mammography (DM) over 3-8 years after entry. In addition, there are 15 secondary aims with data being collected in the areas of imaging assessment, medical physics, breast biology and pathology, long-term follow-up, and health care utilization. Women ages 45 to 74 are eligible to participate. The study will enroll 108,508 women. Participants may also volunteer to contribute blood and/or buccal smears to the TMIST biorepository. Approximately 70% of TMIST participants have agreed to do so. Because of the size of the TMIST study and vast amount of data to be collected, there is an opportunity for investigators to utilize TMIST data to support various research questions not covered in TMIST.

Methods:The TMIST study team developed a process where investigators who would like access to the TMIST data can submit a concept while the trial is ongoing for access to data in a protected manner. The process starts with the project investigator reaching out to the TMIST study chair. If the study chair, lead statistician, and ECOG-ACRIN (EA) co-Principal Investigator think the project has promise; a timeline for when the project could take place (either (1) during the TMIST clinical trial or (2) after the end of the trial and publication of the primary paper) is developed. The next steps involve reviews by the TMIST Data Safety and Monitoring Board and the EA Executive Review Committee. All ancillary projects proposed will require an external funding plan and budget before the project moves out of concept review inside of EA. Once the project concept clears all required EA approvals it then goes to the National Cancer Institute (NCI) Division of Cancer Prevention (DCP) for their approval. NCI Central Institutional Review Board (CIRB) approval is also required for the project to start while the trial is still active but is not sought until funding has been received.

Two projects have secured external funding, have completed the EA committees' review processes, and have received NCI approval. One is a case control study assessing short-term breast cancer risk through image-based analysis of screening mammograms (Project PI: Jon Steingrimsson, PhD, Brown University). The second is a case control study to assess the impact of breast compression pressure versus force in screening mammography on the likelihood of developing interval breast cancers (Project PIs: Etta Pisano, MD and Aili Maki, PhD, University of Toronto). Both projects involve analysis of images where software is being applied to TMIST images on computer systems controlled by EA IT personnel. Both projects are expected to be completed in the next year.

Two additional projects have been approved for grant submission through the process described above. The PreSCRiB study (PI: Elizabeth Burnside, MD MPH, U of Wisconsin) will

utilize Machine Learning applied to TMIST and All of Us data, including genetics, mammograms, social determinates of health and other data to develop individualized screening strategies for women. The second project (PI; Marc Ryser, PhD, Duke University) will utilize TMIST data to validate an algorithm the investigators have developed to assess overdiagnosis.
Another project that is in development and will likely be submitted for approval and funding in the next 6-9 months is a collaboration between TMIST and UK-based clinical trial PROSPECTS study teams to compare rates of all cancers and advanced cancers for annual, biennial, and 3-year screening, with analysis by age, race, ethnicity, breast density and other factors.
The ongoing TMIST study, as of June 24, 2024, has enrolled 101,394 women. Total enrollment is expected by late 2024 or early 2025. Follow-up on enrolled participants is expected to end in early 2028.

P1-01-06: Whole-genome landscapes of 1,364 breast cancers with clinical outcomes

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Recent advancements in genomic technologies have become critical tools for deciphering the genetic complexities of cancer tissues, enabling precision medicine strategies aimed at improving patient clinical outcomes. Here we performed a comprehensive analysis of clinically annotated whole genome and whole transcriptome sequences from 1,364 breast cancer cases. Our investigation provides the most detailed genomic landscapes of breast cancer to date, which allowed us to comprehensively correlate genomic changes with clinical characteristics. Our findings include, but not limited to, (1) whole-genome-based homologous recombination deficiency profiles effectively predict responses to adjuvant chemotherapy and first-line CDK4/6 inhibitor treatment, (2) focal ERBB2 amplifications, often arising via extrachromosomal DNA mechanisms, are strongly associated with a positive response to neoadjuvant chemotherapy. Additionally, our study identified (3) recurrent copy number amplifications linked to patient survival, and (4) whole-genome-based intratumoral heterogeneity profiles that can predict patient survival as well as response to anti-HER2 therapies. Our work underscores the power of highly annotated whole-genome and transcriptome sequencing of a population-scale cancer cohorts for uncovering clinically relevant genomic mutations and novel molecular targets, thereby advancing precision oncology strategies for breast cancer.

P1-01-07: Radiogenomics during neoadjuvant therapy with aromatase inhibitors: integrating dynamic magnetic resonance data and molecular biology

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Background: Neoadjuvant treatment of locally advanced breast cancer (LABC) is now well-established and considerable progress has been made concerning drug development for subgroups of patients. However, reliable predictive markers are still lacking for many patients. Thus, radiological measures like repeated MRI of the breast and axilla, after 3 and 6 months of therapy, are used to follow the patients during neoadjuvant therapy to confirm successful downstaging. Although not established in clinical routine, dynamic MRI contains quantitative information that can provide valuable insight into the tumour microenvironment and, when combined with clinical and molecular data, has the potential to enhance the prediction of tumor responses. Here, we analyze split-dynamic MRI data in relation to clinicopathological and molecular data to further identify disease heterogeneity that can be used to improve prognostic accuracy and inform treatment decisions in breast cancer patients.
Methods: This study reports results from eligible patients with ER pos., HER2 neg. LABC that were enrolled in the NEOLETEXE-trial (1). Clinicopathological, molecular, and advanced MRI data were acquired from this trial, collected before, during, and after neoadjuvant endocrine therapy (NET) with aromatase inhibitors given in sequence. MR images were generated using a split-dynamic technique that simultaneously acquires high spatial resolution and high temporal resolution images, providing information on tissue permeability and vascularity. The images underwent post-processing on the nordicICE software where parametric values were extracted based on whole-tumour volumes of interest. These parametric values were analysed in correlation with clinico-pathological data and with molecular findings from bulk RNA-seq and single cell data from a subset of the same patients.
Results: From the kinetic MRI data, the volume transfer constant (Ktrans), a parameter reflecting capillary permeability, was significantly reduced after NET ($p = 0.005$). Bulk RNA-seq data was analysed for gene expression, pathway involvement, and cell type abundance before, during, and after treatment. Gene expression analysis revealed significant dysregulation of genes related to cell proliferation and cell cycle regulation following treatment. Biological pathway analysis revealed significant down-regulation of hemostatic activity and upregulation of cell cycle and interleukin signaling following treatment. Cell type analysis revealed a higher abundance of CD4+ T-cells and T-helper cells following treatment. These results will be further correlated with single cell data as well as measures of treatment response and clinical outcome.
Conclusions: Our findings suggest that the addition of MRI data to clinicopathological and molecular data enhances the characterization of tumor responses during NET for locally

advanced breast cancer.

1. The NEOLETEXE trial: a neoadjuvant cross-over study exploring the lack of cross resistance between aromatase inhibitors. Bahrami N. et al., Future Oncoloy 15 (32), 3675-3682, 2019.

P1-01-08: Chemoprevention Uptake Before and After the Release of Evidence-Based Guidelines for Low-Dose Tamoxifen Among Patients with Atypical Hyperplasia, Lobular or Ductal Carcinoma In Situ

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Background: Patients with a history of atypical hyperplasia (AH), lobular or ductal carcinoma in situ (LCIS/DCIS) are at elevated risk of developing invasive breast cancer, yet chemoprevention uptake with selective estrogen receptor modulators (SERM) or aromatase inhibitors (AI) in this population remains low. Efficacy of low-dose tamoxifen, for 3 years at 5 mg/day rather than 5 years at 20 mg/day, was established in 2019, which led to evidence-based guidelines from the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN). We aimed to assess changes in chemoprevention uptake before and after availability of low-dose tamoxifen and identify sociodemographic and clinical factors associated with uptake during this period.

Methods: Women diagnosed with AH, LCIS, or DCIS from 2016-2019 and 2020-2023 at Columbia University Irving Medical Center in New York, NY were identified using electronic health records (EHR). Patients under age 35 years, with concurrent or prior invasive breast cancer, with a history of bilateral mastectomy, and patients with estrogen receptor and progesterone receptor-negative DCIS were excluded from the analysis. The primary outcome, SERM or AI use, was dichotomized (yes/no ever used), as was use of low-dose tamoxifen. Descriptive statistics were generated and differences in clinical and sociodemographic variables by diagnosis date (2016-2019 vs. 2020-2023) and by chemoprevention use (yes/no) were assessed using chi-squared and two-sample t-tests. Logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for variables associated with any chemoprevention and low-dose tamoxifen uptake. Multivariable models were adjusted a priori for breast histology, age at diagnosis of high-risk breast lesion, and race/ethnicity, with significant covariates ($p<0.05$) in univariate analysis also included.

Results: Among 2258 evaluable women, mean age was 57.94 (SD 11.88), including 36% with AH, 15% with LCIS, and 48% with DCIS, with 37% ever chemoprevention use. Comparing pre/post low-dose tamoxifen periods (2016-2019 vs. 2020-2023), uptake of chemoprevention (33.99% vs 39.31%, $p=0.009$) and low-dose tamoxifen (3.27% vs 8.57%, $p<0.001$) significantly increased. During this period, low-dose tamoxifen use significantly increased for all of the following subgroups: age <50 vs. ≥50, White vs. non-White, AH/LCIS vs. DCIS. Among 834 women who initiated chemoprevention during this timeframe, 140 (17%) took low-dose tamoxifen. In multivariable analysis, women diagnosed with high-risk breast lesions in 2020-2023 compared to 2016-2019 were more likely to take low-dose

tamoxifen (OR=2.72, 95% CI=1.75-4.22). Younger women (<50 vs. ≥50) and women with less advanced breast histology (AH/LCIS vs. DCIS) were more likely to start low-dose tamoxifen (OR=3.14, 95% CI=2.07-4.74 and OR=2.97, 95% CI=2.00-4.40, respectively). Discussion/Conclusion: Since low-dose tamoxifen was introduced in 2019 as a breast cancer chemoprevention option for women with AH, LCIS, or DCIS, we observed a significant increase in low-dose tamoxifen use and chemoprevention uptake overall. Among women who initiated chemoprevention, low-dose tamoxifen use was higher among younger women and those with less advanced breast lesions. Low-dose options of proven chemopreventive agents for shorter duration may increase acceptance of risk-reducing medications for breast cancer prevention.

P1-01-09: Clinicopathological features, treatment patterns and outcomes of germline BRCA mutation (gBRCAm)-associated breast cancer compared with sporadic tumors in young women: a matched analysis.

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Background: Up to 10% of early breast cancer (BC) occur in gBRCAm carriers. Patients with gBRCAm-associated BC are often reported to have similar survival as non-gBRCAm carriers, but data with modern treatment regimens are limited (Copson et al., Lancet Oncology 2018). We previously observed a slightly better outcome for gBRCAm-associated triple-negative breast cancer (TNBC) compared with matched sporadic TNBC in a real-world cohort of women of all ages (Morganti, ESMO 2023). With a similar approach, we compared clinicopathological features, treatment patterns and outcomes between gBRCAm carriers and matched non-carriers in a separate cohort of women age ≤40 at diagnosis.
Methods: Patients were identified from the Young Women's Breast Cancer Study (YWS), a multisite prospective cohort of 1302 women diagnosed with stage 0–IV breast cancer at age ≤40 years, enrolled from 2006 to 2016. Patients with stage I-III, HER2-negative BC with known gBRCA status were included. Clinical and germline genetic data were collected from patient surveys and medical records. gBRCAm cases were matched 1:2 with non-gBRCAm controls by age group in decades (20-30, 31-40 years old), stage, hormone receptor status and year of diagnosis. Clinicopathologic features and treatments were compared between gBRCAm carriers and non-gBRCAm controls using Fisher's exact test for categorical variables and Wilcoxon rank sum test for continuous variables. Survival outcomes were compared between the gBRCAm carrier vs. controls utilizing Cox proportional hazards model and associated Wald test for the p-value.
Results: We identified 113 gBRCAm (74 BRCA1, 39 BRCA2) carriers and 226 matched non-gBRCAm controls from the 802 patients with HER2-, stage I-III BC enrolled in the YWS. Median age was 36 years (yrs) in carriers and controls. Most patients had stage II (46.3%)

BC, 31% had stage I and 22.7% had stage III. Approximately half of patients had TNBC (51.3%), and half had hormone receptor-positive (HR+; 48.7%) BC. Only 6% of patients underwent genetic testing before BC diagnosis (14.8% of gBRCAm carriers and 1.7% of non-carriers; p<0.001).
Compared with sporadic tumors, gBRCAm-associated BC had higher tumor grade (p=0.014) and higher recurrence score by genomic testing if HR+ (median 31 vs 19, p<0.001). A similar proportion of gBRCAm carriers and non-carriers received chemotherapy (91.2% vs 86.7%, p=0.29), including anthracyclines (94.2% vs 90.8%, p=0.38) and platinum-based regimens (10.7% vs 9.7%, p=0.84). Endocrine therapy for HR+ tumors was less often administered to gBRCAm carriers than controls (80.9% vs 93.3%, p=0.041).
At a median follow up of 11.1 years (IQR 9.9-13.6), invasive disease-free survival (iDFS) did not differ between gBRCAm carriers and controls after adjusting for age, grade, and chemotherapy administration (hazard ratio [HR] 0.80, 95% CI 0.50-1.29; p=0.36), similarly for HR+ BC (HR 0.85, 95% CI 0.41-1.75; p=0.66) and TNBC (HR 0.82, 95% CI 0.44-1.53; p=0.53). A numerical trend towards lower risk of recurrence was observed for gBRCAm carriers compared to controls (5-yr iDFS 82.4% vs 78.0%). Similarly, recurrence-free survival (RFS) did not differ between carriers and controls (HR 0.71, 95% CI 0.44-1.17; p=0.18) but with a trend favoring gBRCAm over controls (5-yr RFS 84.4% vs 79.0% in the overall population; 90% vs 82% for HR+ BC; 79.3% vs 76.1% for TNBC). Overall survival was similar between carriers and controls (HR 0.71, 95% CI 0.40-1.26; p=0.24; 5-yr OS 88.1% vs 87.0%). Outcomes did not differ when comparing separately gBRCA1m carriers and gBRCA2m carriers with their controls.
Conclusions
In this multicenter cohort of young patients with BC, gBRCAm-associated BC had higher-risk pathologic features compared with matched non-gBRCAm BC. Rate of chemotherapy administration was similar between carriers and controls. After adjusting for chemotherapy treatment and clinicopathologic variables, outcomes did not differ in gBRCAm carriers vs controls.

P1-01-10: Comparison of different gene expression tests in breast cancer: poor correlation of Prosigna, EndoPredict and MammaPrint

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Background: The accurate estimation of the risk of disease recurrence in patients with early-stage estrogen receptor positive, human epidermal growth factor receptor 2 (HER2) negative breast cancer is of paramount importance in order to identify those patients in whom adjuvant chemotherapy can be avoided. In clinical practice, the implementation of this approach is becoming increasingly dependent on the results of gene expression assays. This present study aimed to assess the concordance between the 50-gene (PAM50) signature Prosigna®, the 70-gene MammaPrint® (MP) as well as the 12-gene assay EndoPredict® (EP).

Material and MethodsRepresentative tissue of 48 primary tumours was analysed by MP and EP during routine diagnostic procedures between 2008 and 2015. Corresponding formalin-fixed, paraffin-embedded tissue was thereafter analysed by the PAM50. The risk categories of the three tests were compared.
Furthermore, a decision impact analysis was conducted with the objective of a more accurate categorization of the outcomes obtained from Prosigna® in comparison to those yielded from MP and EP.
Results: 82% (39/48) of the samples showed valid results by all three tests, of which only 42% (20/48) of the cases had an identical risk classification and 40% (19/48) of the cases displayed a discrepancy between the results. 9 cases (18%) had missing data for at least one assay.
The correlation between the three tests in terms of risk classification is weak.
Comparing Prosigna® with the EPclin score shows an overall correlation of 24 cases (6 low risk, 18 high risk) with 57% concordance and a discrepancy of 10% (4/42). 14 cases (33%) were considered intermediate risk by Prosigna® with 5 (12%) low risk and 9 (21%) high risk classifications by EP.
56% (22 cases) show a correlation between MP and Prosigna® (6 low risk, 16 high risk) with a discrepancy of 11% (4 cases). An intermediate result was obtained for 33% (13 cases) of which 5 (13%) were considered low risk and 8 (21%) were considered high risk by MammaPrint.
The original therapeutic recommendation was based on the MP and would have been altered in 21% (10/48) of the patients following Prosigna® test results. Included in this group are the 14 cases classified as intermediate risk, where 5 cases (10 %) would have been treated differently than the original treatment plan. Consequently, no discernible trend was evident in this cohort, if the intermediate risk assessment results in either over- or undertreatment of the tumor.
Of the discrepant and intermediate cases, 4 have developed metastases and 1 has experienced a recurrence. 3 of the respective tumors were classified as intermediate by Prosigna®. 2 of 3 cases classified as intermediate were subsequently high risk by both MammaPrint and EndoPredict, while 1 was high risk in MP and low risk in EP. 1 tumor was classified high risk by Prosigna® and EP, but low risk by MP.
ConclusionThe results of the three tests indicated disparate recommendations for treatment in 10% of the patients. Moreover, all three tests provide distinct supplementary data regarding tumor subtype and risk of recurrence. Consequently, those tests cannot be employed interchangeably. The results emphasize the pressing necessity for further comparative analysis of multi-genomic tests to prevent misclassification of risk class and disease recurrence risk in breast cancer patients.

P1-01-11: Use of a Digital Navigator to Deliver Patient-Centered Genetic Services in Mammography

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The National Accreditation Program for Breast Centers (NAPBC) 2024 Standards require implementation of a protocol to identify and manage patients at increased risk for breast cancer due to personal risk factors or family history. In response, the University of Illinois Health partnered with Nest Genomics to implement a comprehensive digital platform to streamline and scale genetic services. The Nest Digital Patient Navigator for Hereditary Cancer Risk Assessment (HCRA) was introduced into a routine mammography setting in November 2023. To date, 4153 patients underwent HCRA with 17% meeting genetic testing (GT) criteria. Successful patient identification led to a new challenge: bottlenecks in access to genetic counseling (GC). To streamline care and empower patients to select their preferred follow-up approach, post-assessment digital navigation was implemented in January 2024 and offered to GT eligible patients.

Patients receiving screening mammograms completed HCRA in the waiting room prior to imaging. Patients who met GT criteria were asked via digital navigator to choose their preferred follow-up mechanism: digital pre-test education, virtual group GC, one-on-one GC, or not interested. The digital pre-test education module was developed by genetic counselors at Nest and UIC and utilized a teach-back approach to ensure understanding of the benefits and limitations of GT, possible GT results, how results impact care, and GT logistics. A GC reviewed completed HCRAs and follow-up preferences. Patients consenting to GT after digital pretest education received a phone call and MyChart message to confirm consent and inform them the test was ordered and a saliva kit shipped. For patients requesting GC services, appointments will be scheduled. GT eligible patients not engaging with the digital navigator received standard referral and GC scheduling.

In a pilot phase from January to early May 2024, 96 patients eligible for GT were sent a link to the digital navigator via text or email to capture GT follow-up preference. Only one third of those invited (n=31, 32%) engaged and only 38 (14%) selected a follow-up preference. To increase engagement, beginning mid-May, the workflow was updated such that patients meeting GT criteria select a follow-up preference immediately after completing the HCRA and only those selecting digital pretest education are sent a link via text or email. Additionally, a Spanish version of the HCRA was added at this time. These changes increased the percent of patients who selected a follow-up preference from 14% to 74%.

In total, from January through June 2024, 156 patients selected a follow-up preference via digital navigator, with most (n=98, 63%) opting for digital pre-test education, 4 selecting group GC, and 54 selecting one-on-one GC. Of the 73 completing digital pre-test education, 31 consented to GT, 7 were not interested in GT, and 19 were unsure about GT. Of the 31 who consented, 26 had a test order placed, 2 had prior GT, 2 had GT in progress, and 1 declined GT after GC outreach. Of the 4 patients requesting group GC, 1 completed and had GT ordered, 1 missed and has not rescheduled, and 2 are scheduled. Of the 54 patients requesting one-on-one GC, none have been scheduled yet due to an administrative backlog.

Pairing post-assessment digital navigation with HCRA allows patients to access education and GT based on individual preferences. Most patients completing digital HCRA and meeting GT criteria opted for digital pre-test education over one-on-one or group GC, allowing them to have a test ordered sooner. This digital solution has the potential to reduce bottlenecks and prioritizes limited GC availability for those who prefer one-on-one

or group counseling. Further work to optimize reminders to boost engagement with the digital navigator and to improve return of sample kits to the laboratory is underway.

P1-01-12: Impact of Preoperative BRCA Testing and National Health Insurance Coverage on Surgical and Risk-reducing Decision-making in Japanese BRCA-mutated Breast Cancer Patients

Kumiko Kida, Junko Takei, Misato Suzuki, Megumi Okawa, Risa Kasahara, Asaka Wada, Yuka Kajiura, Fumi Akitani, Kyoko Shiota, Michiko Yamanaka, Atsushi Yoshida

Introduction: BRCA1 or 2 (BRCA) germline mutations cause increased risks of breast and ovarian cancers. Therefore, preoperative BRCA testing could influence surgical strategies and preventive interventions for breast cancer patients with BRCA mutations. In Japan, BRCA genetic testing and risk-reducing surgeries became eligible for national health insurance coverage in 2020, previously conducted as costly private healthcare. When Japanese national insurance coverage is approved, patients' out-of-pocket expenses are reduced to 30% or less. This study aimed to investigate the impact of preoperative BRCA genetic testing and national health insurance coverage on surgical decision-making and risk-reducing surgeries among Japanese populations.
Methods:Japanese women with BRCA germline pathogenic mutations and early breast cancer who underwent definitive surgery from 2004 to 2024 were retrospectively identified from institutional databases at St. Luke's International Hospital in Tokyo, Japan. Factors including clinicopathologic information and surgical decisions were analyzed. We assessed whether undergoing BRCA testing before or after breast cancer surgery influenced the choice of surgical procedures and risk-reducing surgeries. Additionally, we examined whether approval for national health insurance coverage affected these decisions. The chi-square test was used for statistical comparisons.
Results: A total of 273 breast cancer patients with germline BRCA mutations were identified (BRCA1: 120, BRCA2: 153). Among them, 167 patients (61.2%) underwent BRCA testing prior to breast cancer surgery. Surgical treatments included breast-conserving surgery (BCS) for 78 patients (28.6%) and mastectomy for 195 patients (71.4%). Among the 167 patients who learned of their BRCA mutation before breast cancer surgery, while BCS was feasible for 113 patients based on preoperative imaging, 24 patients (21.2%) chose BCS, and 89 patients (78.8%) opted for mastectomy due to their mutation results. The breast-conserving rate was 14.4% for patients with preoperative BRCA testing compared to 50.9% for those tested after surgery ($p<0.0001$).
Risk-reducing mastectomy (RRM) was performed in 115 patients (52.0%, excluding cases with bilateral breast cancers). Among patients with preoperative testing, 94 (63.1%) underwent RRM, including 83 (55.7%) who had RRM simultaneously with breast cancer surgery. Risk-reducing salpingo-oophorectomy (RRSO) was performed in 98 patients (61.3%, excluding cases with previous non-preventive oophorectomy), including 67 (41.9%) who had RRSO simultaneously with breast cancer surgery. Patients who learned of their BRCA mutation after breast cancer surgery were subsequently less likely to undergo

risk-reducing surgeries compared to those with preoperative testing: 29.2% underwent RRM and 50.5% underwent RRSO (p=0.0001 and 0.097, respectively).
Before national health insurance approval, 46.6% underwent preoperative BRCA testing, 28.0% underwent RRM, and 17.5% underwent RRSO simultaneously with breast cancer surgery. After approval, these rates increased to 86.9% for preoperative BRCA testing, 51.7% for RRM, and 43.0% for RRSO (p<0.0001, 0.0004, and <0.0001, respectively, compared between before and after approval).
Conclusions: Most Japanese patients who learned of their BRCA mutation before their breast cancer surgery opted for mastectomy instead of BCS due to their mutation status. Preoperative BRCA testing and national health insurance coverage significantly increased the likelihood of undergoing risk-reducing surgeries. These findings emphasize the key role of preoperative BRCA testing and national health insurance coverage in informing surgical decisions and enhancing preventive care.

P1-01-14: Risk-reducing medications and primary breast cancer prevention: a network meta-analysis of randomized controlled trials.

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Background: Risk-reducing medications can substantially reduce the risk of breast cancer and represent a viable approach to reducing the rising breast cancer incidence. However, only a very limited number of medications, selective estrogen receptor modulators (SERMs), and aromatase inhibitors (AIs) are currently approved for breast cancer chemoprevention since these approvals are mainly based on findings from randomized controlled trials (RCTs), which are challenging to perform in the prevention setting; hence, very few studies exist for other medications. Our goal in this network meta-analysis (NMA) is to pool together estimates from various RCTs and identify medications that are associated with a reduction in breast cancer risk and can be used in primary breast cancer prevention.
Methods: We performed a comprehensive literature search using Embase.com, Ovid-Medline All, Scopus, and the Cochrane Library to identify RCTs on breast cancer prevention and various risk-reducing medications. The search, completed on November 16, 2023, identified 13,642 citations. Three researchers extracted the data and assessed the quality of the studies. After removing duplicates in Endnote, we screened 8,692 citations and included 43 studies that met our criteria. The inclusion criteria included RCTs on women without a history of invasive breast cancer, focusing on pharmacological interventions. We excluded lifestyle interventions, unspecified multivitamins, and herbal drugs without active ingredient specification. The primary outcome was the incidence of overall breast cancer. In the NMA for overall breast cancer, 35 studies evaluating 13 medications were included, as these were compared to other medications more than once. These medications included tamoxifen, raloxifene, third-generation SERMs (arzoxifene, bazedoxifene, lasofoxifene), AIs (anastrozole, exemestane), statins (pravastatin, simvastatin, lovastatin), thiazolidinediones (rosiglitazone, pioglitazone), sulfonylurea, metformin, metformin plus sulfonylurea,

fenretinide, tamoxifen plus fenretinide, calcium, and calcium plus vitamin D. For the secondary analysis, 16 studies evaluating AIs, tamoxifen, and raloxifene were included for invasive breast cancer. Efficacy was measured using risk ratios (RRs) with confidence intervals (CIs), and the number needed to treat (NNT) was calculated.
Results: Six medications were associated with reduced breast cancer risk compared to placebo: sulfonylurea (RR=0.18, 95%CI=0.03-0.90), thiazolidinediones (RR=0.26, 95%CI=0.08-0.79), third-generation SERMs (RR=0.46, 95%CI=0.33-0.66), AIs (RR=0.50, 95%CI=0.39-0.66), raloxifene (RR=0.63, 95%CI=0.47-0.84), and tamoxifen (RR=0.76, 95%CI=0.65-0.88). The NMA showed no significant inconsistency (Q=5.09, d.f.=7, p=0.65), indicating agreement between direct and indirect comparisons of medications, and between-study heterogeneity was low ($\tau 2$=0.0067, I2 = 9%). Notably, third-generation SERMs (RR=0.61, 95% CI=0.42-0.89) and AIs (RR=0.67, 95% CI=0.49-0.90) showed greater efficacy than tamoxifen. The NNTs were 43.9, 48.6, 67.3, 73.0, 96.9, and 149.7 for sulfonylurea, thiazolidinediones, third-generation SERMs, AIs, raloxifene, and tamoxifen, respectively. For invasive breast cancer prevention, AIs (RR=0.48, 95% CI=0.33-0.71), raloxifene (RR=0.63, 95% CI=0.47-0.86), and tamoxifen (RR=0.63, 95% CI=0.51-0.78) were effective compared to placebo. NMA inconsistency was significant (Q=7.48, d.f.=1, p=0.01), with moderate between-study heterogeneity ($\tau 2$=0.0323, I2 = 58.8%).
Conclusions
These results offer a comprehensive comparative analysis of risk-reducing medications in relation to breast cancer. Findings can guide future research in breast cancer prevention as well as clinical considerations and guidelines on breast cancer chemoprevention.

P1-01-15: Assessing BRCA1/2 Testing in Breast Cancer Patients: A Retrospective Analysis of Real-World Data From the IntegraConnect PrecisionQ De-Identified Database

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Background: Additional clinical guidelines have been put forth by the American Society of Clinical Oncology and Society of Surgical Oncology for BRCA 1/2 testing in patients with breast cancer.1 We sought to understand from a de-identified real-world data set of patients the current rates of BRCA1/2 testing among all breast patients who were newly diagnosed or diagnosed with metastatic disease from 2021 to 2023.
Methods: We used the Integra Connect PrecisionQ real-world de-identified database of over 3 million cancer pts across 500 sites of care. From this database, we identified breast cancer patients who had undergone manual chart curation whereby BRCA testing was evaluated. Patients included in the assessment who were metastatic required a metastatic date between 1/1/2021 and 12/31/2023 and patients who were not metastatic required a diagnosis date between 1/1/2021 and 12/31/2023. Patients were stratified by age, race, hormone receptor and HER2 expression status. Testing rates were also evaluated by year. Descriptive analyses were performed and proportions were compared using a chi-squared

test.
Results: There were 2,638 metastatic patients (mets pts) and 1,754 patients without metastasis (non-mets pts). The median age of the mets pts at metastasis was 66 [IQR 55; 75] and the median age of the non-mets pts at diagnosis was 49 [IQR 52; 71]. The percentage of metastatic patients who were HER2- was 83%, HER2+ was 15% and unknown was 2%. The percentage of non-mets pts who were HER2- was 89%, HER2+ was 10%, and unknown was 1%. The percentage of mets-pts who identified as White was 72%, Black/African American was 15 % and Other was 14%. The percentage of non-mets pts who identified as White was 76%, Black/African American was 11% and Other was 12%. Among the mets pts BRCA testing was 71%. The rate of BRCA testing was 72% in 2021, 71% in 2022, and 72% in 2023 by year of patient metastasis. The rate of BRCA testing among HR+/HER2- pts was 67%, HR-/HER2- pts was 87%, and HER2+ pts was 59% (P <0.01). The rate of BRCA testing among patients age <65 was 81% and age >=65 was 63%. (P <0.01). The rate of BRCA testing among patients who identified as White was 70% and among those who identified as Black/African American was 73% (P >0.05). Among the non-mets pts BRCA testing was 52%. The rate of BRCA testing was 53% in 2021, 50% in 2022, and 52% in 2023 by year of patient diagnosis. The rate of BRCA testing among HR+/HER2- pts was 48%, HR-/HER2- pts was 65%, and HER2+ pts was 47% (P <0.01). The rate of BRCA testing among patients age <65 was 63% and age >=65 was 39%. (P <0.01). The rate of BRCA testing among patients who identified as White was 52% and among those who identified as Black/African American was 52% (P >0.05).
Conclusions: Based on our findings using real-world data for BRCA testing and in light of the current guidelines there will need to be considerable increases in BRCA testing among patients diagnosed with breast cancer regardless of stage who are less than age <65 as only 63% of non-metastatic patients are receiving BRCA testing and 81% of metastatic patients are receiving BRCA testing.
Reference
1 Bedrosian I, Somerfield MR, Achatz MI, et al: Germline testing in patients with breast cancer: ASCO-SSO guideline. J Clin Oncol. January 4, 2024

P1-01-16: Molecular landscape of HR+/HER2- male breast cancer (MaBC) compared with female breast cancer (FeBC)

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Background: Hormone receptor–positive/human epidermal growth factor receptor 2–negative (HR+/HER2−) breast cancer (BC) is more prevalent in male patients compared to female counterparts. Gender associated differences along with molecular differences, immune system, and other factors might play a crucial role in disease management. Here, we characterized molecular and immune differences between HR+/HER2- MaBC and FeBC.
Methods: 8156 female (HR+/HER2-, n = 5232) and 121 male (HR+/HER2-, n = 97) BC

samples were analysed by next-generation sequencing (NextSeq; WES, NovaSeq) and Whole Transcriptome Sequencing (WTS; NovaSeq) (Caris Life Sciences, Phoenix, AZ). Tumor mutational burden (TMB) (high ≥10 mt/MB) was calculated. Microsatellite-instability (MSI) was tested by IHC and NGS. Statistical significance was determined using chi-square and Mann-Whitney U test with p-values adjusted for multiple comparisons ($q < 0.05$).
Results: The proportion of HR+/HER2- subtype was significantly higher in MaBC (80.17% vs 64.14%, $p<0.05$) compared with FeBC. HR+/HER2- MaBC had higher frequency of BRCA2 (10.64% vs 4.38%, $p<0.05$), GATA3 (22.68% vs 14.11%, $p<0.05$), but lower frequency of TP53 (3.45% vs 30.76%, $q<0.05$), ESR1 (4.12% vs 13.62%, $p<0.05$) and CDH1 (1.06% vs 17.43%, $q<0.05$) compared to HR+/HER2- FeBC. Compared to HR+/HER2- FeBC, MaBC had lower frequency of TMB-high (2.2% vs 9.08%, $p<0.05$), but there was no difference in dMMR/MSI-high (0% vs 0.61%, $p=1$) or PD-L1 (IHC, 22C3) positivity (21.21% vs 19.47%, $p=0.72$). HR+/HER2- MaBC had lower expression of AR-RNA (fold change (FC): 1.4, $q<0.05$), higher AR-protein (IHC) positivity (97.94% vs 84.03%, $q<0.05$), but no difference was noted in the frequency of fusion variant-AR (1.04% vs 3.19%, $p=0.37$) compared to FeBC.
HR+/HER2- MaBC had increased expression of MHC class I gene HLA-B (FC: 1.2, $p<0.05$), MHC class II gene HLA-DQB2 (FC: 1.6, $q<0.05$), but decreased expression of drug efflux gene ABCC2 (FC: 1.4, $q<0.05$), and stem cell-related genes (KLF4, SOX2, POU5F1, PROM1, ALDH1A1, FC: 1.3-1.9, $q<0.05$) compared to HR+/HER2- FeBC.
Conclusions: These data indicate that HR+/HER2- MaBC has a differential mutational spectrum and TMB-high frequency, MHC Class I and MHC class II, drug efflux and stem cell-related gene expression compared to their HR+/HER2- FeBC counterparts. These suggest important differences in tumor biology between men and women with HR+/HER2- breast cancer. A better understanding of these differences with additional research may help in design future clinical trials and treatments for men with HR+/HER2- BC.

P1-01-17: PD-L1 Upregulation in Circulating Tumor Associated Cells Predicts for clinical outcomes in a phase I/II clinical trial using SV-BR-1-GM vaccine with the check point inhibitor retifanlimab in Metastatic Breast Cancer Patients, an Interim Analysis

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Background: Circulating Tumor Cells (CTCs) are clinical indicators of poorer clinical outcomes in metastatic Breast Cancer (mBC) and may be monitored post therapy induction to identify patients not responding to treatment. However, CTCs are rare in mBC (i.e. <20% of patients), and many patients without CTCs also often progress. Recently an inflammatory pro-tumorigenic PD-L1 expressing macrophage (Cancer associated macrophage-like cell [CAML]) was identified in the blood along with CTCs which are common in mBC (i.e. >90% of patients) and also indicate tumor response to new therapies. SV-BR-1-GM, which is a mBC cell line derived vaccine with antigen presenting characteristics, developed for treatment of mBC as a monotherapy, or in combination with checkpoint inhibitors. Here we

report the results of CTC & CAML changes and their PD-L1 expression profiles monitored from the peripheral blood, pre and post inoculation with SV-BR-1-GM. We present Progression Free Survival (PFS) and Overall Survival (OS) at 24 months as part of the exploratory portion of the single arm open label roll over phase 1/2 trial (NCT03328026).
Methods: SV-BR-1-GM treatment includes low pre-dose cyclophosphamide, intradermal inoculation of ~20 million irradiated SV-BR-1-GM cells, post-dose local interferon-α with cycles and an anti-PD-1 inhibitor (retifanlimib), with cycles every 3 weeks. Blinded anonymized blood samples were taken at baseline (BL), prior to starting SV-BR-1-GM therapy (n=44), a 2nd sample (T1) taken after therapy initiation (~23 days) and if possible, a third sample (T2) taken at the standard tumor assessment (~75 days). To evaluate the predictive value of CTCs/CAMLs and their PD-L1 expressions, cells were isolated and quantified using the LifeTracDx liquid biopsy test. The quantities of CTCs & CAMLs and their respective PD-L1 were analyzed based on PFS using RECIST v1.1 and OS hazard ratios (HRs) by censored univariate analysis at 24 months.
Results: Peripheral blood was available from n=41 of 44 mBC pts at BL, prior to therapy induction. CTCs were found in 42% (n=17/41) of patients and CAMLs were found in 90% (n=37/41) at BL. Presence of CTCs at BL significantly correlated with worse PFS (HR=2.3, (CI95% 1.1-4.8, p=0.0351) and OS (HR=3.6, (CI95% 1.5-8.7, p=0.0091). T1 samples were available from 83% (n=34/41) of patients, with presence of CTCs at T1 not significantly correlating with worse PFS (HR=2.5, (CI95% 1.0-5.9, p=0.0743) nor OS (HR=1.6, (CI95% 0.6-4.5, p=0.5565). However, a drop in CAMLs or CTCs after treatment at T1 was observed in 38% of patients, correlating with a significantly improved PFS HR=2.5, (CI95% 1.2-2.5, p=0.0299), but not significantly improved OS HR=2.8, (CI95% 1.0-7.7, p=0.0883). Expression of PDL1 on CAML/CTC at BL was not correlated with improved PFS (HR=0.6, (CI95% 0.3-1.2, p=0.2355) nor OS (HR=0.8, (CI95% 0.4-1.9, p=0.7847), though an increase in PD-L1 was observed in CAMLs/CTCs at the T1 (n=7) and the T2 (n=8) time points. Further, patients with high PDL1 on CTC/CAML PDL1 at the T1 or T2 were not correlated with significantly improved PFS HR=1.9, (CI95% 0.9-3.9, p=0.1259), but were correlated with significantly improved OS HR=3.9, (CI95% 1.4-10.8, p=0.0164).
Conclusions: In an interim analysis of heavily treated mBC patient population, we observed that treatment with the SV-BR-1-GM regimen was associated with decreases in the presence of CTCs and CAMLs in 38% of patients, which significantly correlated with better PFS and trended for better OS within 2 years. Further, SV-BR-1-GM therapy appeared to upregulate PD-L1 in n=13 patients which appeared to have better responses to combination treatment with the anti-PD-1 check point inhibitor retifanlimab.

P1-01-18: Prevalence of functional HRD by the RAD51 assay: a pooled analysis with 367 patients from BRCA-associated tumor types

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Background: The functional biomarker RAD51 identifies patients with homologous recombination deficiency (HRD) tumors. Furthermore, RAD51 has been validated in different clinical trials in breast cancer (GeparSixto [NCT01426880] and GeparOla [NCT02789332]), showing both prognostic and predictive value. Here, we present a pooled analysis of RAD51 results to evaluate the prevalence of HRD status in BRCA-associated tumor types.
Methods: We conducted a pooled analysis of all patients evaluated with the RAD51 test between February 2021 and June 2024 at the Vall d'Hebron Institute of Oncology. RAD51, BRCA1 and yH2AX were quantified using an immunofluorescence assay. RAD51 was evaluated both as a continuous score (ranging from 0 to 100) and as grouped categories (low vs. high, with a pre-defined cut-off of 10%). The primary objective of this study was to evaluate the percentage of functional HRD by RAD51 (RAD51 low) across BRCA-associated tumor types and stages of the disease. Fisher's exact test was used to statistically quantify the differences between percentages.
Results: A total of 574 tumor samples were evaluated; 367 (63.4%) were successfully scored by RAD51 and included in the pooled analysis. The median age at time of the biomarker assessment was 60 years. Most patients were diagnosed with breast cancer (49.0%), followed by ovarian cancer (25.9%), prostate cancer (18.6%), and other tumor types (6.5%). Samples were collected from primary tumors (46.5%), metastases (43.4%), and local recurrences (10.0%). Overall, the prevalence of low RAD51 tumors was 25.1% (92/367) and the prevalence of BRCA1 nuclear foci low was 11.1% (41/367). The highest percentage of low RAD51 tumors was found in ovarian tumors (44.2%), while the percentages in breast and prostate tumors were 12.2% and 22.1%, respectively ($p<0.001$). The percentage of BRCA1 low was 32.1% ovarian, 5.1% in breast and 1.6% in prostate ($p<0.001$). Among patients with BRCA1 low (n=41), 75.6% of them had concomitant low RAD51 values. In the subset of ovarian tumors (n=95), a numerically larger percentage of low RAD51 tumors was found in metastatic samples compared with primary tumors (53.3% vs. 44.8%). In breast cancer (n=180), low RAD51 samples were more frequently found in primary tumors than in metastatic tumors (18.2% vs. 6.2%, $p<0.001$). No association was found between RAD51 and yH2AX values (Pearson´s correlation= 0.02).
Conclusions: Functional HRD prevalence varies by tumor type and stage. RAD51 identifies patients beyond BRCA status who could benefit from PARPi therapies. Results on the association between RAD51 and clinical outcomes will be presented at the conference.

P1-01-19: Predicting response to capivasertib in AKT1 mutant advanced breast cancer

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Introduction: Activation of the PI3K-AKT and mTOR pathways is a major feature of the biology of breast cancer (BC), with these pathways altered or dysregulated in most BC. Oncogenic mutations in AKT1, most frequently E17K, are found in ~5% of advanced BC and are targetable by the approved AKT inhibitor capivasertib. We conducted genomic and transcriptomic analysis of a cohort of AKT1 mutant metastatic BCs with the aim to identify determinants of response to capivasertib.

Methods: We identified 39 AKT1 mutant BC from the plasmaMATCH clinical trial (18/39; NCT03182634), the FAKTION clinical trial (7/39 NCT01992952), and the ABC-BIO tissue collection study (14/39; CCR3991). FFPE extracted DNA was subject to whole exome sequencing (WES), and RNA to Truseq RNA sequencing. Clonal dominance was assessed in circulating tumour DNA (ctDNA) using Guardant360. Allelic imbalance at the AKT1 locus and clonal dominance of the AKT1 mutation were associated with progression free survival (PFS) in plasmaMATCH Cohorts C (capivasertib plus fulvestrant) and D (capivasertib alone) combined.

Results:WES at median coverage 93x (range 55-169x) reconfirmed AKT1 mutations in 33/39 (85%) samples: 25/33 (76%) E17K; 3/33 (12%) L52R; 1/33 (3%) E49K; 1/33 (3%) L52R+C77F; 1/33 (3%) L52R+E375K; 1/33 (3%) S129L; 1/33 (3%) Q79K. 6/39 discordant samples principally reflected AKT1 mutations identified in ctDNA analysis which were not present in the single site tissue biopsy. Most common pathogenic alterations found in the AKT1 mutated cohort were TP53 9/33 (27%), CDH1 5/33 (15%), GATA3 3/33 (9%) and MAP3K1 2/33 (6%). Rates of mutations in PIK3CA (2/33; 6%) and ESR1 (2/33; 6%) were reduced compared to those expected in ER+HER- metastatic BC sets.

Analysis of the AKT1 locus revealed amplification of AKT1 gene in 14/33 (42%) and loss of heterozygosity (LOH) of the wild type allele in 10/33 (30%). Overall LOH and/or amplification (allelic imbalance) was observed in 18/33 (55%) of AKT1 mutant tumours. 15/33 (46%) of the patients with confirmed AKT1 mutations had received treatment with capivasertib in PlasmaMATCH. Median PFS in patients was 13.08 months (10/15 patients) with allelic imbalance and 3.19 months without allelic imbalance (5/15 patients; HR 8.798, 95% CI1.58-49.00, P=0.004, log rank test).

RNA sequencing data was obtained for 36/39 samples with median 13M reads (range 11-49M) including 31/33 of the confirmed mutant samples in WES. PAM50 subtypes for the cohort were LumA (12/31), LumB (7/31), HER2Enriched (7/31), Basal (1/31) and normal-like (2/31). RNAseq showed increased (0.87 log fold change, P=0.035) expression of AKT1 in tumours with allelic imbalance compared to those without, and high expression levels of mutant transcript.

Finally, in plasmaMATCH, clonality of the AKT1 mutation was assessed in ctDNA, with 16/24 (67%) patients having clonally dominant AKT1 mutations. Median PFS in patients with clonally dominant mutations was 10.2 months and subclonal mutations 3.2 months (HR 3.1, 95% CI0.9-10.5, P=0.014, log rank test).

Conclusions: Breast cancers with mutations in AKT1 frequently have allelic imbalance favouring the mutation, resulting in increased expression of mutant transcript. Allelic imbalance of AKT1, and/or clonally dominant AKT1 mutations, were prognostic for significantly greater PFS of patients treated with capivasertib.

P1-01-20: Spliceosome Mutations (Smut) in Metastatic Breast Cancer (MBC): An Analysis of a De-centralized Clinical Trial and Large Clinical-Genomic Dataset

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Introduction: Smut's result in aberrant mRNA transcripts increasing neoantigen diversity. The Patient Response to Immunotherapy using Spliceosome Mutational Markers (PRISMM) trial is a prospective remote-supported decentralized clinical trial designed to assess responses to immunotherapy. We also evaluated Smut in MBC using a large clinical-genomic database.

Methods: PRISMM recruited patients (pts) online and directed them to a landing website to complete a form. Eligibility was defined as pts with metastatic solid tumors harboring an SF3B1, U2A1, or SRSF2 mutation. After confirming eligibility, pts were remotely consented and underwent review by the Johns Hopkins Molecular Tumor Board (MTB). If clinically appropriate, MTB recommended PD1/PDL1 inhibitor in a report provided to the patient and treating oncologist. Research blood was collected at local laboratories. Pts were followed with serial questionnaires. Feasibility was defined as enrolling 60 pts, conducting MTB reviews within 4 weeks of consent in at least 80% of pts, and achieving 80% patient response on at least 1 follow-up questionnaire.

The CARIS real-world (RW) dataset was utilized for Smut analysis in solid tumors focusing on SRSF2, SF3B1 U2AF1, and ZRSR2. Frequency distribution across cancer types and breast cancer subtypes was assessed. In pts with MBC, correlations were made with molecular aberrations, immunohistochemical biomarkers, and immune cell populations (RNA-seq). Real-world overall survival (rwOS: from time of biopsy to last contact) was assessed by Kaplan Meier. Statistical significance was determined using Mann-Whitney U and chi-square tests and adjusted for multiple comparisons ($p<0.05$).

Results: From the PRISMM study, a total of 18 subjects completed online recruitment forms. Tumor types included breast (n=10), pancreatic (3), salivary gland (1), prostate (1), and astrocytoma (1). 6 were from the South, 5 the Northeast, 4 the West, and 1 the Midwest. 2 subjects were rural, and 16 were from urban communities. 6 participants were eligible and were consented/enrolled; 4 (67%) underwent MTB review within one month of informed consent. All pts completed at least one follow up questionnaire. 3 (50%) pts were initiated on immunotherapy. Median duration of treatment was 3 months (1-4 months) and median OS after start of immunotherapy was 4 months (1-32 months). Research blood was collected for 4 pts (67%). There was concordance of Smut between ctDNA and tissue NGS in 3 pts (75%).

In the CARIS dataset of 49,150 samples were identified with Smut; bladder (7%), lung (4%), and breast (3%) cancer had the highest prevalence of Smut. Breast cancer subtype frequency distribution of Smut was as follows: HR+/HER2- (2.3%), HR-any/HER2+ (1.5%), HR-/HER2- (0.2%). In Smut MBC, genes most frequently mutated were GATA3, ESR1, and

MAP3K1 ($p < 0.05$); amplified were FGFR1 and FLT4 ($p < 0.05$). We observed expression of PDL-1 and HER2 was lower, and AR/ER/PR was higher ($p < 0.05$) in Smut MBC vs non-mutated. Smut MBC had higher B-cells and M2 macrophages, and lower CD8+ T-cell signatures ($p < 0.05$). Improved rwOS was observed in the spliceosome non-mutated vs mutated (HR=1.251, 95% CI: 1.0-1.565, $p <0.05$).
Conclusion: While the PRISMM trial did not meet its feasibility endpoint, it does demonstrate pt engagement and feasibility of remote-laboratory collections. We did not observe significant responses to immunotherapy in pts with Smut. The existence of a large clinical-genomic database allowed us to further examine Smut. Across breast cancer subtypes, HR+/HER2-had the highest frequency of Smut. Smut's were associated with genomic aberrations of endocrine resistance (ESR1, MAP3K1, FGFR1), an immune-cold phenotype (higher M2 macrophages, lower PDL-1 expression and CD8+ T-cell infiltration), and worse OS.

P1-01-23: Oncotype DX Breast Recurrence Score® distribution and prognostic value according to prior pregnancy status in young women with breast cancer

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Background: Breast cancer (BC) diagnosed in the postpartum period has been associated with a worse prognosis compared to nulligravid patients (pts), possibly due to differences in carcinogenesis associated with prior pregnancies. Persistent changes in gene expression, structural composition, immune microenvironment, and epigenetic modifications within the mammary gland have been observed following pregnancy. Oncotype DX Breast Recurrence Score® test is a gene expression profile that has been incorporated into the management of early-stage, estrogen receptor-positive (ER+), HER2-negative (HER2-) BC as a prognostic and predictive biomarker of chemotherapy effect. However, there are limited data on the impact of previous pregnancies on the expression of the 21 genes analyzed in the Oncotype DX® test and on the prognostic accuracy of this assay. The aim of this study is to evaluate the influence of pregnancy status on the distribution of Recurrence Score® (RS) results and long-term outcomes of young pts with early-stage, ER+, HER2- BC.
Methods: Pts with stage I-III, ER+, HER2- BC were classified as "nulligravid" or "postpartum" based on the absence or presence of pregnancy history prior to BC diagnosis from the Young Women's Breast Cancer Study, a prospective cohort that enrolled women with BC diagnosed at age ≤ 40 years between 2006 and 2016. Pts whose BC was diagnosed during pregnancy were excluded. RS was obtained from banked samples when not clinically performed. RS was categorized as low (< 11), intermediate (11-25), or high (> 25). Multivariable Cox hazards models were used to assess factors associated with distant recurrence-free interval (DRFI).

Results: Among 387 included pts, 117 (30.2%) were nulligravid and 270 (69.8%) postpartum. Median time from last pregnancy was 4.72 years (range 0.96 – 21.84) for the postpartum group. Median age at diagnosis was 34 and 37 years, N+ rate was 28 (24.0%) and 118 (43.7%), and chemotherapy was administered to 74 (63.3%) and 202 (74.8%) of nulligravid and postpartum pts, respectively. The median RS was 17 (range: 3-66) for nulligravid pts and 21 (4 – 77) for postpartum pts (p=0.004). The proportion of pts with low, intermediate and high RS was 16 (13.68%), 76 (64.96%) and 25 (21.37%) in nulligravid pts; and 28 (10.37%), 157 (58.15%) and 85 (31.48%) in postpartum (p=0.11). Among pts with N0 BC, 11-year DRFI rates were 91.7 (95% CI: 53.9 - 98.8), 90.7 (79.1 - 96.0), and 83.0 (55.9 - 94.2) for pts with RS < 11, RS 11-25, and RS > 25 for nulligravid women, and 83.3 (48.2 - 95.6), 92.0 (82.8 - 96.4), and 77.6 (61.3 - 87.7) for postpartum, respectively. Among pts with N+ BC, 11-year DRFI rates were 100.0, 76.2 (48.1 - 90.4), and 57.1 (17.2 - 83.7) for pts with RS < 11, RS 11-25, and RS > 25 for nulligravid women, and 79.4 (48.8 - 92.9), 71.1 (57.6 - 80.9), and 75.6 (59.1 - 86.2) for postpartum pts, respectively. In multivariable model of pts with N0 or N1-3 nodes, adjusting for RS, T-stage, N-stage, pregnancy status, and chemotherapy use, only RS (HR 1.02 per 1 point increase, 95%CI 1.002-1.039), T-stage (HR 2.01, 95%CI 1.11-3.65), and N-stage (HR 1.83, 95%CI 1.01-3.31) were independently associated with DRFI.
Conclusion: Pts diagnosed with BC in the postpartum period had higher RS results compared to nulligravid women. After adjusting for stage and RS, previous pregnancy status was not associated with worse long-term outcomes in young women with node negative or 1-3 node positive ER+ breast cancer. While further analyses incorporating time since last pregnancy will be conducted, these data suggest inferior outcomes observed in these patients may be in part related to higher genomic risk tumors.

P1-01-24: Genomic comparison of rapid vs. typical progressors on CDK4/6 inhibitor treatment in advanced breast cancer

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Background: While response to CDK4/6 inhibitors (CDK4/6i) is often 24 months in clinical trials, a subset of patients with HR+/HER2- metastatic breast cancer (mBC) will experience rapid progression on CDK4/6 inhibitors (CDK4/6i). Determinants of rapid vs. typical responders (RP, TP) are lacking. Identifying factors that contribute to rapid progression on CDK4/6i may enable refined clinical management and decision-making. Here we describe genomic profiles of patients with rapid vs. typical progression on CDK4/6i from a real-world clinical-genomic database to inform clinical decision-making. Methods: GuardantINFORM, a clinical-genomic database with de-identified genomic results and aggregated claims was queried from 2014 through March 2024 to identify patients with mBC who had ctDNA testing completed (as part of routine clinical care) prior to CDK4/6i initiation. RPs were defined as patients who had real-world time to next treatment (rwTTNT) within 4 months of starting CDK4/6i whereas TPs had 12+

months rwTTNT. While up to 83 genes are reported clinically, 500 genes were analyzed to assess genomic and/or pathway differences between RP/TP. Progression was defined as having documentation of a new therapy, with the following therapies excluded: switch to another CDK4/6i, switch of endocrine backbone, switch to endocrine-only therapy, immunotherapy, and any anti-HER2 therapy. Between RP and TP cohorts, non-synonymous genomic variants were analyzed to assess differences using Fisher's exact test (significance: $p<0.05$) and pathways analyzed via the Reactome pathway database, which counts each mutated gene per patient at a cohort-level (genes listed). "Enriched" pathways were defined as significant presence of a pathway ($p<0.05$) for one cohort, yet not significant ($p>0.05$) for the comparative cohort. Results: 138 patients were included as RP and 165 as TP; most were treated with palbociclib (RP: 72%; TP 55%). There were no significant differences in age, co-morbidity index or smoking status between cohorts. When assessing variant frequency between RP and TP, TP53 mutations were the only genomic variant enriched in RP compared to TP ($p<0.05$). Upon pathway analysis, there were multiple enriched pathways (n=56) in the RP cohort compared to TP, of which MAPK/MAP2K or NOTCH-related genes were altered at high rates (MAPK: 64%, 36/56; NOTCH: 20%, 11/56). Fewer pathways were enriched in the TP cohort compared to the RP cohort (n=12), with VHL, CCNE1/CCND1 and/or RB1 genes altered frequently (VHL: 63%, 7/11; CCNE1/CCND1: 36%, 4/11; RB1: 27%, 3/11). There were no enriched pathway differences observed in ESR1-mediated or extra-nuclear estrogen signaling pathways between cohorts. Conclusions: This study highlights that the classification of rapid vs. typical progressors in response to CDK4/6i is likely complex genomically. While TP53 alterations are significantly more common in RP, this finding may not be unique to breast cancer or CDK4/6i exposure as TP53 can be a poor prognostic factor across many solid tumors. Additional exploration into pathways involving MAPK and NOTCH-related genes may enable future studies to better identify rapid progressors to CDK4/6i to inform treatment planning.

P1-01-25: Elacestrant vs SOC in ER+, HER2- advanced or metastatic breast cancer (mBC) with ESR1-mutated tumors: ESR1 allelic frequencies and clinical activity from the phase 3 EMERALD trial

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Background: The EMERALD trial (NCT03778931) reported significantly prolonged progression-free survival (PFS) and a manageable safety profile with single-agent elacestrant vs standard of care (SOC) endocrine therapy (ET) in patients with ER+/HER2− mBC and tumors harboring ESR1 mutation following progression on prior ET+CDK4/6i; mPFS 3.8 months with elacestrant vs 1.9 months with SOC (HR=0.55; 95% CI, 0.39-0.77; P = 0.0005) (Bidard 2022). In those patients with prior ET + CDK4/6i ≥12 months and ESR1-mutated tumors, median PFS with elacestrant was 8.6 vs 1.9 months with SOC ET (HR =

0.41; 95% CI, 0.26-0.63) (Bardia 2022). The PFS benefit associated with elacestrant was maintained across ESR1 mutation variants D538G, Y537S, and Y537N, which represent nearly 90% of ESR1-mutated tumors. Variant allele frequency in circulating tumor DNA correlates with tumor disease burden and predicts outcomes in patients with advanced breast cancer. This new analysis evaluates the clinical benefit of single-agent elacestrant in patients with high vs low ESR1 variant allele frequency (VAF).
Methods: Patients with ER+/HER2- advanced or mBC who previously had 1-2 lines of ET, mandatory CDK4/6i, and ≤1 chemotherapy were randomized 1:1 to receive oral elacestrant or SOC (investigator's choice of AI or fulvestrant). A post-hoc subgroup analysis was performed in patients with ESR1-mutated endocrine-sensitive tumors (prior exposure to ET+CDK4/6i ≥12 months) detected in plasma ctDNA using Guardant Health360 gene panel to evaluate the benefit of elacestrant vs SOC by ESR1 VAF. Median VAF was 1.2% (95% CI 0.04-56.1). High and low VAF were defined as ≥1.2% and <1.2%, respectively.
Results: In patients with low ESR1 VAF (n=79) who received prior ET+CDK4/6i ≥12 months, a clinically meaningful improvement in mPFS favoring elacestrant compared with SOC was observed, 8.6 with elacestrant vs 1.9 with SOC (HR = 0.51, 95% CI 0.26-0.99, P = 0.049). In patients with high ESR1 VAF (n=79), mPFS with elacestrant was 9.1 vs 1.9 for SOC (HR=0.36, 95% CI 0.19-0.69, P = 0.001). Baseline characteristics and additional data will be presented at the meeting.
Conclusions: Elacestrant demonstrated a significant improvement in mPFS vs SOC in patients with both high and low ESR1 VAF. The clinical benefit and activity of elacestrant vs SOC was maintained regardless of type of ESR1 mutation variant and the abundance/quantity of ESR1 mutations in patients with ER+/HER2- mBC. In all patients with ER+/HER2, ESR1-mut mBC, elacestrant may replace fulvestrant-based combinations, and delay chemotherapy or ADC-based regimens.

P1-01-26: Genomic profiling of ctDNA & assoc. w/ efficacy in patients from the postMONARCH trial of abemaciclib + fulvestrant vs placebo + fulvestrant for HR+, HER2-, advanced breast cancer following progression on a prior CDK4/6i plus endocrine therapy

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Background: postMONARCH (NCT05169567) was a randomized, placebo-controlled, Phase 3 trial that demonstrated the benefit of continued CDK4/6 inhibition with abemaciclib after progression on prior CDK4/6 inhibitor (CDK4/6i) + endocrine therapy (ET) (progression-free survival [PFS] HR 0.73; 95% CI, 0.57–0.95) in patients with HR+, HER2- advanced breast cancer (ABC). Here, we report exploratory biomarker analyses from postMONARCH, describing the frequency and outcome associations of baseline circulating tumor DNA (ctDNA) alterations in treated patients.

Methods: postMONARCH randomized 368 patients 1:1 to receive abemaciclib + fulvestrant or placebo + fulvestrant (Kalinsky et al., ASCO 2024). ctDNA from baseline plasma samples was analyzed using the Guardant Infinity assay. Associations between investigator-assessed PFS and recurrent oncogenic alterations (as defined by OncoKB) were assessed using a Cox proportional hazard model using genomic subgroups (detected vs not detected) as interaction terms.

Results: Plasma samples for ctDNA testing were available for 87% of patients (161 abemaciclib; 159 placebo); 3 did not have detectable ctDNA at baseline (1 abemaciclib; 2 placebo). The ctDNA-evaluable subgroup (n=320) was largely representative of the intention-to-treat population in baseline clinical characteristics and benefit achieved from the addition of abemaciclib to fulvestrant (HR 0.77; 95% CI, 0.59–1.00).
The most frequently altered genes in the abemaciclib + fulvestrant arm compared to placebo + fulvestrant were ESR1 (40% vs 51%), TP53 (35% vs 43%), PIK3CA (36% vs 42%), RB1 (10% vs 14%), PTEN (11% vs 10%), CDH1 (12% vs 11%) and GATA3 (11% vs 11%). The most common ESR1 hotspot mutations in the abemaciclib + fulvestrant and placebo + fulvestrant arms were D538G (25% vs 36%), Y537S (18% vs 16%) and Y537N (13% vs 15%). Rarer baseline alterations were also identified in genes implicated in CDK4/6i resistance.
The abemaciclib treatment effect was generally consistent across genomic subgroups (detected vs not detected), including ESR1 (HR 0.79 [0.54-1.15] vs HR 0.78 [0.54-1.12]), TP53 (HR 0.88 [0.59-1.32] vs HR 0.73 [0.52-1.02]), PIK3CA (HR 0.76 [0.50-1.14] vs HR 0.80 [0.57-1.12]), CDH1 (HR 0.86 [0.42-1.74] vs HR 0.75 [0.57-1.00]) and GATA3 (HR 0.58 [0.28-1.20] vs HR 0.79 [0.60-1.04]). Patients with alterations (detected vs not detected) in RB1 and PTEN had diminished abemaciclib treatment effect (RB1 HR 1.19 [0.58-2.43] vs HR 0.76 [0.57-1.00] and PTEN HR 1.55 [0.74-3.23] vs HR 0.71 [0.54-0.94]). Differences were also observed across ESR1 variants (D538G HR 0.74 [0.45-1.21]; Y537S HR 0.49 [0.27-0.88]; Y537N HR 1.38 [0.73-2.61]). Efficacy by oncogenic pathways will be further presented to evaluate biological mechanisms associated with these alterations.

Conclusions: Analysis from this prospective Phase 3 study provided insight into the baseline genomic landscape following disease progression on a CDK4/6i + ET. Abemaciclib benefit after prior CDK4/6i progression was apparent regardless of common actionable alterations in ESR1 or PIK3CA. RB1 and PTEN alterations were infrequent and associated with diminished abemaciclib treatment effect, though sample size was limited. In summary, abemaciclib + fulvestrant offers a targeted therapy option for HR+, HER2- ABC after disease progression on a CDK4/6i, including for patients with actionable genomic alterations.

P1-01-27: HER2 Loss Following Treatment with Trastuzumab Deruxtecan in Patients with Metastatic Breast Cancer

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Background: Trastuzumab deruxtecan (T-DXd) is currently approved for treatment of patients with metastatic HER2-positive (immunohistochemistry (IHC) 3+ or ISH positive) or HER2 low (IHC 1+ or IHC 2+/ISH negative) breast cancer in addition to other disease-specific and tissue-agnostic indications. Because of potential clinical implications to treatment selection after exposure to T-DXd, we aimed to evaluate the changes in HER2 status, specifically loss or decrease in HER2 expression, following treatment with T-DXd in patients with metastatic breast cancer. Methods: We retrospectively reviewed patients with metastatic breast cancer who received treatment with T-DXd at The University of Texas MD Anderson Cancer Center (MDACC). We included patients who had post-treatment biopsy (or on-treatment biopsy within the 30 days prior to treatment discontinuation) with IHC re-evaluation of HER2 status at MDACC. We excluded patients who were taken off treatment for toxicity or death after the first cycle of treatment, patients who received T-DXd in addition to another agent concomitantly, and patients who received T-DXd at multiple times interspersed through their medical history. We reviewed pre-treatment HER2 IHC status in the most recent biopsy prior to T-DXd initiation and the biopsy with the highest score of HER2 across patients' medical history. For patients with multiple testing, the biopsy closest to treatment start- and end date was used to assess the change in HER2 expression status. Treatment dates were extracted from patients' medical charts and duration on therapy was calculated as the time between cycle 1 day 1 and end of treatment. A decrease in IHC score was defined as any change in IHC score from 3+ to 2+, 1+, or 0; from 2+ amplified to 2+ non-amplified, 1+, or 0; from 2+ non-amplified to 1+ or 0; or from 1+ to 0. HER2 loss was defined as an IHC score of 0 after treatment with any degree of positivity noted in the most recent sample before treatment initiation (1+, 2+, or 3+).Results: We included 45 patients with metastatic breast cancer who started treatment with T-DXd at MDACC between June 2017 and February 2024 and had come off therapy by the time of analysis. The highest HER2 score was 3+ in 14 patients (31%), 2+ in 20 patients (44%; 12 FISH negative, 5 FISH positive, and 3 FISH undetermined), and 1+ in 11 patients (24%). The median duration between pre-treatment biopsy and treatment start date was 355 days (range, 1 to 2663) and the median duration between treatment discontinuation date and post-treatment biopsy was 25 days (range, -14 to 1159). Five patients had HER2 score of 0 in the most recent biopsy before therapy and were excluded from further analysis. In those patients, treatment decision was based on another prior biopsy showing HER2-low or HER2-positive disease and the median time on treatment was 71 days. Out of 40 patients with baseline HER2 expression (1+, 2+, or 3+), almost one third (n=12; 30%) had HER2 loss following treatment with T-DXd. In addition to those 12 patients with HER2 loss, another 11 patients (28%) had a decrease in HER2 score after treatment with T-DXd. Conclusions: HER2 loss and decrease in HER2 expression are common in patients with metastatic breast cancer

receiving treatment with T-DXd. Re-evaluation of HER2 status post-therapy should be considered prior to considering alternative HER2 targeted therapy.

P1-01-28: Molecular Residual Disease Precedes Radiographic Confirmation of Recurrence in Patients with Stage III Inflammatory Breast Cancer

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Background: Early detection of molecular residual disease (MRD) correlates with disease recurrence and survival outcomes in patients with inflammatory breast cancer (IBC). We sought to determine the lead time of circulating tumor cell (CTC) and circulating tumor DNA (ctDNA) detection prior to radiographic confirmation of disease relapse in stage III IBC.
Methods: Patients were enrolled in a prospective registry from 2015-2022 and underwent serial blood draws at baseline, throughout neoadjuvant treatment, and 6- and 12-months post-surgery. CTCs were enumerated using CellSearchTM and ctDNA quantification was performed using Oncomine Pan-Cancer Cell-Free Assay. MRD was defined as ≥ 1 CTC or ctDNA variant detected at any post-operative timepoint. Among patients with recurrence, lead time was calculated from date of first positive CTC and/or ctDNA variant after surgery to date of first imaging confirmation of disease relapse. Per standard of care guidelines, surveillance imaging was obtained based on patient symptoms and physical exam findings. For patients with recurrence but no detectable MRD, date of first negative CTC and/or ctDNA variant after surgery was used. Mann-Whitney U and Fisher's exact tests were used to compare differences between groups. Kaplan-Meier method was used to estimate time to recurrence (TTR) and Cox regression analysis was performed to assess the association with variables of interest.
Results: In total, 92 patients with stage III IBC were included. The median age was 51 years (IQR 17.8) and BMI was 28.9 (IQR 11.5). The study cohort consisted of 72.9% (70) White, 16.3% (15) Hispanic, and 6.5% (6) Black patients. Patients had predominantly node-positive (87, 94.6%), high-grade (64, 66.7%), ductal (83, 86.5%) disease. Almost all patients received trimodality therapy – neoadjuvant systemic therapy (100%), modified radical mastectomy (100%), and adjuvant radiotherapy (88, 95.7%). Baseline CTC and ctDNA detection rates were 60.7% (37/61) and 65.6% (21/32), respectively. MRD detection rate ranged from 34.5-39.4% at 6- and 12-months post-surgery. There were 6 patients who recurred who had persistently negative MRD assessments (range: 1-2 post-operative assessments). At a median follow up of 7.1 years (95% CI 4.7-9.4), overall survival was 71.7% and recurrence rate was 37.0%. Patients with negative CTC and ctDNA had significantly longer time to recurrence compared to those with a positive CTC and/or ctDNA assessment at any post-operative timepoint (estimated 6-year TTR: 93.3% vs. 58.3%, $p = 0.027$). CTC positivity was significantly associated with TTR (HR 3.8, 95% CI: 1.8-7.9, $p < 0.001$) in multivariable Cox regression analysis with time to CTC positivity as a time-varying covariate. There were no differences in age, race/ethnicity, nodal status, histology, receptor

subtype, grade, LVI, and pCR by recurrence status. However, patients with recurrence had more positive lymph nodes at resection (median 5 vs. 0, $p < 0.001$) and were more likely to have positive CTCs after surgery (71.4% vs. 43.9%, $p = 0.01$), compared to those who were relapse-free. Of the 35 patients who recurred, 80% had detectable MRD after surgery. Among these, 71.4% had MRD at a median time of 5.8 months (IQR 14.7) prior to radiographic detection of disease relapse, compared to a median recurrence time of 12.5 months (IQR 14.7) for patients with no MRD detected prior to positive imaging.
Conclusion: Among stage III IBC patients with relapse, MRD was detected with a median lead time of approximately 6 months prior to radiographic confirmation of disease recurrence. Incorporation of serial assessments of MRD may offer the opportunity for early systemic treatment intervention in IBC patients with high risk of relapse.

P1-01-29: Metabolomic signatures of fruit and vegetable consumption in relation to breast cancer

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Background. Fruit and vegetable intakes have been associated with a lower risk of breast cancer. However, few studies have explored metabolites, especially unknown peaks detected in the metabolomics platforms, related to fruit and vegetable consumption. No studies have assessed the relationship between a metabolomic signature for fruits and vegetables and breast cancer risk.
Objective. We developed metabolomic signatures that reflect usual consumption of fruits and vegetables in the Nurses' Health Study (NHS) and NHSII and investigated the relationships between these signatures and breast cancer incidence.
Methods. We used data from prior nested case-control studies in the NHS and NHSII (N=10,289) with metabolomic data. Plasma metabolomic profiling, including mainly lipids and amino acids, was conducted by liquid chromatography-tandem mass spectrometry; 206 named metabolites and 1591 unknown peaks were included. Fruit and vegetable consumption was estimated from food frequency questionnaires collected near blood draw. We identified metabolomic signatures using adjusted elastic net regression. The models were trained in all case-control studies except those for breast cancer (N=6388) and tested in the breast cancer studies (N=1948 cases; 1953 controls). We assessed associations of metabolites and the metabolomic signature with breast cancer risk overall and by estrogen receptor (ER) and menopausal status using conditional logistic regression, calculating odds ratios (ORs) and 95% confidence intervals per standard deviation (SD).
Results. At blood collection, participants were on average 55 years old and consumed 1.6 servings of fruit and 3.3 servings of vegetables per day. Metabolomic signatures of named metabolites were correlated with total fruit (N=21 metabolites; r=0.45 in the training set; r=0.42 in the testing set) and vegetable (N=26 metabolites; r=0.49 in the training set; r=0.51 in the testing set) intakes. Adding in unknown peaks did not substantially increase these

correlations. Ten metabolites were significantly associated with breast cancer risk, with inverse associations observed for eight metabolites. Two of these metabolites were included in the signatures: C20:5 cholesteryl ester (OR per 1 SD=0.93; 95%CI=0.87, 1.00) was included in the vegetable signature (β=0.003), and piperine (OR per 1 SD=0.91; 95%CI=0.85, 0.98) was included in the fruit signature (β=-0.01) and the vegetable signature (β=0.07). The signatures of named metabolites for fruit and vegetable intake were associated with lower risk of breast cancer overall (OR per 1 SD of the fruit signature=0.73; 95%CI=0.56; 0.95; OR for the vegetable signature=0.67; 95%CI=0.56; 0.82). Both signatures were associated with lower risk of postmenopausal tumors, and the signature for vegetable intake was also associated with lower risk of ER+ and premenopausal tumors.
Conclusions. Using a plasma metabolomics platform including named metabolites and unknown peaks, we identified metabolomic signatures that could help reflect fruit and vegetable consumption and the underlying biological processes that may link dietary intake with breast cancer risk.

P1-01-30: Generation and validation of primary breast cancer epigenetic classifiers of pathologic nodal stage in a multi-center breast cancer cohort

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Introduction: With increasingly effective systemic therapies and expanding indications for radiation, the role of axillary lymph node dissection (ALND) in the management of patients with node-positive breast cancer (BC) continues to evolve. However, while clinical trials have progressively demonstrated that ALND offers little to no added protection from local recurrence or death in various clinical scenarios, ALND remains the only method to fully stage the axilla and differentiate pN1 vs >pN1 disease. However, certain decisions for adjuvant therapies, such as CDK4/6 inhibitor therapy in patients with estrogen receptor (ER) positive BC, still rely on the nodal stage. In this study, we used machine learning algorithms to create epigenetic classifiers predictive of nodal stage (pN1 vs >pN1) based on DNA methylation profiling of primary BC tumors in two independent cohorts of patients (UCLA and Duke) with ER-positive, HER2-negative BC.
Methodology: Eligibility criteria included women aged 18-80 years with ER+, HER2-negative BC, clinically node-positive, who underwent ALND without NAC. Tumoral tissue was microdissected from 8 µm Formalin-Fixed Paraffin-Embedded (FFPE) slides, previously annotated by a trained pathologist, and DNA was extracted using the Quick-DNA FFPE Miniprep kit (Zymo Research). DNAm profiling was performed using the Illumina Infinium Methylation EPIC BeadChip v1. DNAm data was processed using the R/ChAMP package (v.2.34.0), and the batch effect was corrected using the “champ.runCombat” function. The cohort was split into a training cohort (60%, n = 53) and a validation cohort (40%, n = 34). R/VarSelRF (v.0.7-8) was used to identify the combination of probes with the fewest sites and the highest potential to predict the pathological nodal stage. The best-performing classifiers were tested in the validation cohort.

Results: DNAm profiling was performed on 91 primary BC samples, with four samples removed due to low data quality. The UCLA and Duke cohorts presented a significant batch effect, which was successfully corrected to avoid potential bias. We identified 1,653 differentially methylated sites between pN1 and >pN1 tumors in the training cohort, successfully stratifying both subgroups of patients. These sites were used to generate a Random Forest-based classifier with the minimum number of probes. We selected the three combinations of probes with the highest Area Under the Curve (AUC) and the fewest CpG sites. We identified three classifiers, comprising 8 to 12 genomic regions, with an AUC between 0.99 and 1 in the training cohort and between 0.81 and 0.82 in the validation cohort.
Discussion: As randomized trial data support the omission of ALND in select clinical scenarios in patients with node-positive BC, clinicians will lose the pathologic nodal data that is provided by surgical dissection that guides adjuvant therapy decision-making. This study generated three classifiers that successfully identified patients with higher nodal stage (>pN1). These classifiers use a small number of genomic regions (8 to 12) and can be optimized for low-throughput techniques such as pyrosequencing or quantitative Methylation-Specific PCR, increasing the availability of this diagnostic tool in the clinical setting.

P1-02-01: Body weight modification during adjuvant ovarian suppression for premenopausal women with luminal breast cancer.

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Introduction: Obesity is one of the major health problems in the world, directly affecting the quality of life and self-esteem of the population. While various factors contribute to its development, research indicates that ovarian hormone loss increases the likelihood of weight gain in women. Body fat mass tends to increase with ovarian function suppression (OFS) in premenopausal women, and endocrine therapy (ET) has been shown to mitigate weight gain in postmenopausal women. Methods: Retrospective analysis of all consecutive premenopausal patients diagnosed with early luminal breast cancer (eBC) and treated with adjuvant OFS + aromatase inhibitors (AI) between January 2017 and July 2024 in a single Brazilian institution (A. C. Camargo Cancer Center). The primary objective was to describe the body weight changes over the years of hormonal blockade use (baseline, six months [m], 12m, 36m, and 60m). The body mass index (BMI) variation (over-weighted: BMI ≥ 25Kg/m2; obesity: BMI ≥ 30Kg/m2) and the variations >1Kg from the baseline were considered for the analysis. Results: 318 premenopausal patients were recruited, with a median follow-up of 59 months. The median age was 40 years; the majority were white (56%) and had no comorbidities (72.3%). 23.3% had luminal HER2-positive eBC, and 93.1% underwent chemotherapy (chemo: 44.3% neoadjuvant and 48.8% adjuvant). 21.4% started ET with tamoxifen before switching to OFS + IA. Monthly OFS was the starting choice for 71.7% of patients, and 65.9% changed to an every-3-month posology during the

following years of therapy. 61.8% had a BMI >25Kg/m2 and 24.5% were obese at baseline. There was a notable trend of weight gain in this cohort. The rates of overweight in patients with available data increased to 63%, 63.3%, 65.2%, and 67.3% in 6, 12, 36, and 60 months, respectively. The obesity rates decreased in 6m to 23.4% but increased in the next years: 26%, 25.9%, and 25.9% in 12, 36, and 60m, respectively. In the first 6m of OFS + IA, most patients (41.1%) maintained weight (33.4% gained > 1 kg and 25.4% lost > 1 kg). However, in the subsequent months, weight increase prevailed: 38.9%, 45.2%, and 46.2% in 12, 36, and 60m. The percentage of patients with weight loss remained relatively stable over time. An average increase of 6kg was observed for this cohort. Among patients receiving chemo, weight gain >1 kg tended to occur later than for patients without chemo. The weight increase occurred regardless of the AI (anastrozole, letrozole, and exemestane) used. The study was not powered to evaluate associations between weight gain/BMI and oncological outcomes due to the low rates of recurrence and death events in the present cohort (5-year disease-free and overall survivals of 95.5% and 97.5%, respectively). Conclusion: These findings underscore the complex interplay between hormonal treatments and weight changes in premenopausal women with early breast cancer. Effective management strategies are essential to mitigate the risk of weight gain and its implications for patient outcomes. There were few recurrences, with a disease-free survival of 95.9% at five years. Future research should focus on optimizing treatment protocols to minimize adverse effects while maximizing therapeutic benefits in this population.

P1-02-02: Indirect comparison of sacituzumab govitecan (SG) and datopotamab deruxtecan (Dato-DXd) in advanced breast cancer (aBC): safety and efficacy analysis.

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Background: SG and Dato-DXd are antibody drug conjugates (ADCs) used in aBC. Both target humanized anti-trophoblast cell-surface antigen 2 (TROP2) and both have a similar (irinotecan-based) payload. There are few comparative data on these drugs.
Methods: We searched MEDLINE, as well as proceedings from ASCO, ESMO and SABCS. Eligible studies were trials (dose expansion phase 1, phase 2 or 3) that evaluated Dato-DXd or SG in aBC patients. Safety (adverse effects [AE]) data were pooled as the mean, weighted by individual study sample size and indirect comparisons were performed using the test of two proportions (z score). For randomized trials of ADCs compared to chemotherapy, we performed a network metanalysis (using WINBUGS within Microsoft Excel) and report the hazard ratio (HR) for efficacy (overall survival [OS], progression free survival [PFS]) and odds ratio (OR) for AEs comparing Dato-DXd to SG. Statistical significance was defined as $p<0.05$.
Results: Nine studies were included in the analysis, 3 for Dato-DXd and 6 for SG. A total of 450 patients were treated with Dato-DXd and 831 with SG. Median prior lines of treatment were 1 (range 1-6) for Dato-DXd and 4 (range 0-7) for SG. There were no significant

differences in discontinuation due to toxicity (OR 0.97; 95% confidence interval [CI] 0.38-2.48)) or in toxic deaths (OR 1.11; 95% CI 0.15-8.34). Similarly, there were no significant differences in PFS (HR 0.95; 95% CI 0.71-1.28) or in OS (HR 1.06; 95% CI 0.74-1.53). Comparison of individual AEs between Dato-DXd and SG demonstrated the following: all grade AE (94.8% vs 100%, p < 0.01), grade≥ 3 AE (20.7% vs 75%, p< 0.01) and dose reduction (19.5% vs 26%, p=0.01). Ocular AE(all grade, 25.2%; grade ≥3, 1%), infusion reaction (all grade, 11.8%) and interstitial lung disease (ILD, all grade, 2.6%; grade ≥3 1%) were reported only with Dato-DXd, whereas febrile neutropenia (5.6%) and neuropathy (all grade, 11.8%; grade ≥3, 0.7%) were reported only with SG. SG showed more frequent hematological toxicities: anemia (grade ≥3, 1.7% vs 19%, p<0.01), neutropenia (grade ≥3, 1% vs 49%, p<0.01) and thrombocytopenia (grade ≥3, 0% vs 1.6%). SG was associated with more diarrhea (16.5% vs 58%, p<0.01, grade ≥3[SG only], 9.2%) while stomatitis was seen more with Dato-DXd (60.1% vs 11.9%, p<0.01; grade ≥3, 6.9% vs 1.4%, p<0.01). Alopecia (36% vs 45.6%, p<0.01) and fatigue (27% vs 46.7%, p<0.01; grade ≥3, 2.5% vs 5%, p=0.03) were more common with SG. Risk of grade 3 nausea and vomiting, as well as skin related AEs and dyspnea were similar.
Conclusions: Both Dato-DXd and SG have similar risk of discontinuation without progression and toxic death. The side effect profile of SG is driven by hematological toxicities. AEs such as ILD, ocular and infusion reactions appear unique to Dato-DXd. Febrile neutropenia, neuropathy and fatigue were observed predominantly with SG. Among GI AEs, stomatitis is prominent for Dato-DXd and diarrhea with SG. There does not appear to be any meaningful difference in efficacy. These results may inform choice of therapy in clinical practice.

P1-02-03: Major adverse cardiovascular events in postmenopausal women vs pre-menopausal women with hormone receptor-positive breast cancer treated with endocrine therapy

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Background: In the Suppression of Ovarian Function (SOFT) and Tamoxifen Exemestane Trial (TEXT), breast cancer recurrence rates were significantly lower among premenopausal women treated with the aromatase inhibitor (AI) exemestane plus ovarian suppression to those treated with the selective estrogen receptor modulator (SERM) tamoxifen alone. The association between AIs and cardiovascular outcomes in women with breast cancer is controversial, with studies often focusing on postmenopausal women. The purpose of this study was to evaluate major adverse cardiovascular events (MACE) in women undergoing hormonal therapy for hormone receptor positive (HR+) breast cancer, specifically comparing postmenopausal women to those experiencing premature menopause or ovarian function suppression with GnRH agonists, oophorectomy, and/or ovarian irradiation.
Methods: Using the SEER-Medicare database, we identified 26, 505 women with HR+ breast

cancer diagnosed at age > 65 between 2007-2019. Using the University of Colorado Health Data Compass database, we identified 790 patients with HR + breast cancer experiencing premature menopause or undergoing OFS, diagnosed < 45 between 2005- 2022. HER2 patients were excluded due to the cardiotoxicity of HER2-targeted agents. We compared patient and clinical characteristics according to hormonal treatment regimen (AI versus SERM). Logistic regression was performed to estimate the odds ratio (OR) and 95% confidence intervals (CIs), MACE was defined as acute myocardial infarction, heart failure, potentially fatal arrhythmias, or cerebral vascular accident. Cardiac death was excluded from the analysis due to its rarity.
Results: In the SEER database cohort of postmenopausal women, AI treatment was not associated with higher odds of any MACE (OR 1.05, 95% CI [0.97, 1.13], p= 0.92), acute myocardial infarction (OR 1.02, 95% CI [0.90, 1.15], p=0.45), heart failure (OR 1.01, 95% CI [0.91, 1.13), p=0.44), potentially fatal arrhythmia (OR 1.13, 95% CI [0.91, 1.40], p=0.39), or cerebral vascular accident (OR 1.12, 95% CI [0.96, 1.27], p=0.49) compared to tamoxifen when controlling for traditional cardiac risk factors. Data from the Compass database for the premature menopausal and OFS group shows a lower proportion of MACE (3.54%, N= 28) compared to the SEER cohort (28.22%, N= 7479) when controlling for traditional risk factors, with this difference being statistically significant (p< 0.0001, Fisher's exact test).
Conclusion: Among postmenopausal women with HR+ breast cancer, AI treatment was not associated with increased risk of cardiac outcomes compared to tamoxifen. There was a lower proportion of cardiac events in young women with premature menopausal or those undergoing OFS compared to postmenopausal women. More work is needed to study the effects of premature menopause and to develop cardioprotective strategies.

P1-02-04: Impact of abemaciclib dose escalation on compliance and safety in hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) breast cancer

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Background: Abemaciclib, a cyclin dependent kinase 4/6 inhibitor (CDK4/6i), is approved for the treatment of hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) early stage (EBC) and advanced breast cancer (ABC) patients (pts) in combination with endocrine therapy (ET). Abemaciclib (150 mg twice daily), when used in combination with ET, leads to high rates of adverse events (AEs) such as diarrhea which affects over 80% of pts resulting in dose omissions, dose reductions, and discontinuation (DC). While there is data to support dose reduction in the early and advanced setting without negatively impacting efficacy, there is limited data on the impact of a dose escalation strategy on AE profile and compliance. This retrospective study aims to determine an optimal dosing strategy of abemaciclib for early and advanced HR+, HER2- BC and to assess discontinuation rate (DCR) and AE rates.
Methods: This retrospective, single-center, cohort study included pts 18 years of age and

older with HR+, HER2- breast cancer who were treated with abemaciclib in combination with ET at the Duke Cancer Institute (DCI) from October 1, 2015 to September 22, 2023. EBC and ABC pts were divided into three cohorts based on dosing strategy at initiation: Cohort A (n=36), dose escalation (50 mg), Cohort B (n=12), non-traditional (100 mg), and Cohort C (n=80), standard (150 mg). In cohorts A and B, pts were dose escalated at varying intervals dependent on pt tolerance. The primary outcome was DCR within 90 days due to AEs. Secondary outcomes included DCR due to all causes within 90 days, reason for DC, grade of AE experienced, percentage of pts on the standard regimen (cohort C) requiring a dose reduction, highest dose maintained in the dose escalation group (cohort A), and time to progression for ABC pts. Logistic regression was used for the primary outcome to examine the association between DCR and dosing strategy, and descriptive statistics were used for the remaining outcomes.
Results: A total of 128 pts were included, 43 (34%) with EBC and 85 (66%) with ABC. All pts were female and the majority were postmenopausal (80.5%). DCR at 90 days due to AEs was 5.6% in cohort A, 8.3% in cohort B, and 11.3% in cohort C (p = 0.801). Pts in cohort A had 81% lower odds of abemaciclib DC within 90 days compared to cohort C (OR: 0.19, 95% CI 0.02 to 1.04, P = 0.07). For every 5-year increase in age, there was a 67% increase in the odds of DC due to AEs (OR: 1.67; 95% CI: 1.2 to 2.49, p = 0.004). 90.6% of pts reported AEs, with diarrhea and fatigue being the most frequently reported across all cohorts. 54.5% of pts with EBC and 50.7% of patients with ABC in cohort C required a dose reduction, with 43.9% of pts reducing to 50 mg. 37.9% and 14.3% in cohort A with EBC and ABC, respectively, achieved a dose of 150 mg. In the ABC group time to progression (TTP) of 278 days (Q1, Q3 146.5, 356) in cohort A and 287 days (Q1, Q3 149, 461) in cohort C.
Conclusion: An abemaciclib dose escalation strategy may be considered in an effort to reduce the rate of AEs experienced in women with HR+, HER2- EBC and ABC prescribed abemacicib. The DCR of abemaciclib due to AEs within 90 days was lower with a dose escalation strategy demonstrating reduced rates of diarrhea, although this was not statistically significant. Many EBC pts were able to successfully escalate to full dose abemaciclib, while half of pts who started at standard dose required a dose reduction. Additionally, TTP was similar in ABC patients regardless of dosing strategy. This study was limited by small sample size and uneven distribution between study groups. Additional prospective data is needed to further assess dose escalation strategies with abemaciclib.

P1-02-05: The association between statin use and risk of cardiovascular events among breast cancer patients receiving pembrolizumab

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Background: The use of immune checkpoint inhibitors (ICIs), such as pembrolizumab, has been linked to serious immune-related cardiovascular events, including myocardial infarction, heart failure, and myocarditis. There is limited data on the prevention of these

immune-related cardiovascular events. Statins, a class of HMG-CoA reductase inhibitors, have long been used to prevent and reduce the risk of atherosclerotic disease. This study aimed to investigate if the use of statins might decrease cardiovascular adverse events associated with pembrolizumab in patients with breast cancer.
Methods: We conducted a retrospective, propensity score-matched cohort study using the TriNetX Analytics Network database, which includes de-identified electronic health records from over 70 healthcare organizations and 101 million individuals. We included adult female breast cancer patients treated with pembrolizumab and chemotherapy from November 2020 to June 2023. The inclusion criteria specified patients with hypertension, diabetes mellitus, or hyperlipidemia, as these comorbidities increase the risk of atherosclerotic disease and may indicate the use of a statin. We excluded patients who received endocrine or HER2-targeted therapies. The index date was the start of ICI therapy. We compared patients who received statins to those who had not received statins within one year prior to the index date. The primary outcome was major adverse cardiovascular events (MACE), including myocardial infarction, heart failure, and cardiac arrest. Secondary outcomes included individual MACE components, myocarditis, and pericarditis. All outcomes were captured within one year following the start of ICI therapy.
Results: We identified 747 eligible patients who received pembrolizumab and chemotherapy, of which 229 received a statin and 518 did not receive a statin within one year of starting pembrolizumab. After propensity score matching, 161 patients in each cohort were well-balanced for demographics, breast cancer-directed therapy, underlying comorbidities, and cardiovascular medication use. Over a one-year follow-up period, the incidence of MACE was lower in the statin cohort compared to the non-statin cohort (3.1 vs. 8.7 events per 100 patient-years). In a Cox proportional hazard analysis, statin use was associated with an approximately 65% lower risk of MACE (Hazard ratio (HR), 0.35 [95% CI: 0.12-0.96]; log-rank p = 0.033). Patients on statins had a lower incidence of heart failure (1.2 vs. 5.6 events per 100 patient-years) than those not on statins. Statin use was associated with a lower risk of heart failure (HR, 0.21 [95% CI: 0.05-0.99]; log-rank p = 0.030). There were no statistically significant differences in the rates of myocardial infarction (HR, 0.66 [95% CI: 0.19-2.35]; log-rank p = 0.521), myocarditis (no cases for statin vs. 1 case for non-statin), and pericarditis (HR, 0.97 [95% CI: 0.20-4.82]; log-rank p = 0.974) between the statin and non-statin cohorts.
Conclusion: The use of statins is associated with a lower risk of MACE, principally heart failure, among breast cancer patients receiving pembrolizumab.

P1-02-06: Efficacy analysis & updated safety from the phase 2 PRIMED study of prophylactic granulocyte-colony stimulating factor (G-CSF) & loperamide for patients (pts) with HER2-negative advanced breast cancer (ABC) treated w/ sacituzumab govitecan (SG)

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Cortez, Zuzanna Piwowarska, Eileen Shimizu, José Antonio Guerrero, Miguel Sampayo-Cordero, Alejandro Martínez-Bueno, Javier Cortés, Antonio Llombart-Cussac

Background SG is a Trop-2 directed antibody drug conjugate that has shown a statistically significant and clinically meaningful overall survival benefit for pts with HER2- ABC in two phase III trials. The most common SG-related adverse events (AEs) were neutropenia and diarrhea, which frequently led to treatment modifications. The PRIMED trial previously demonstrated that primary prophylactic administration of G-CSF and loperamide resulted in a clinically meaningful reduction of neutropenia and diarrhea during the first two treatment cycles. Herein we report the extended safety follow-up and secondary efficacy endpoints.

Methods PRIMED (NCT05520723) is an open-label, single-arm, phase II trial. Pts with HER2- ABC previously treated with 1-2 lines of chemotherapy in the advanced setting were eligible. Pts with hormone receptor-positive (HR+) tumors had to be refractory to at least one prior endocrine therapy-based regimen and have received a CDK4/6 inhibitor for ABC. SG (10 mg/kg intravenously, days 1 and 8, every 21 days) was administered until disease progression or unacceptable toxicity. G-CSF (0.5 MU/kg/day subcutaneously, days 3, 4, 10, and 11) and loperamide (2 mg orally, twice a day or 4 mg daily, days 2, 3, 4, 9, 10, and 11) were given during the first two cycles and could continue based on physician's discretion. Secondary efficacy endpoints included progression-free survival (PFS), objective response rate (ORR), clinical benefit rate (CBR), and overall survival (OS).

Results Between February 2023 and September 2023, 50 pts were enrolled (triple-negative breast cancer [TNBC], n=32; HR+/HER2-, n=18). At data cut-off (May 5, 2024), with a median follow-up of 9.0 months (range; 0.2-13.5), 9 pts remained on SG treatment. The median PFS for TNBC pts was 6.4 months (95%CI; 6.1-10.3) and for pts with HR+/HER2- tumors, it was 5.8 months (95%CI; 2.2-NA). The ORR and CBR were 34.4% and 71.9% for TNBC, and 16.7% and 44.4% for HR+/HER2- pts, respectively. OS data was immature at the time of analysis. During the first two cycles, the incidence of any grade (G) neutropenia and diarrhea were 28.0% and 34.0%, respectively. A total of 8 pts (16.0%) had ≥ G 3 neutropenia (12.0% G3; 4.0% G4) and 8 pts (16.0%) had ≥ G 2 diarrhea (4.0% G3, with no G4). With the extended follow up, the incidence of any G neutropenia and diarrhea were 42.0% and 44.0%, respectively. A total of 12 pts (24.0%) had ≥ G 3 neutropenia (18.0% G3; 6.0% G4; with no febrile neutropenia) and 9 pts (18.0%) had ≥ G 2 diarrhea (4.0% G3, with no G4). The overall rate of all AEs associated with dose reductions and treatment interruptions was 22.0% and 50.0%, respectively. Four pts discontinued due to AEs, two of which were SG-related (G2 enteritis and G3 diarrhea). Conclusions The efficacy results of SG in the PRIMED study are consistent with previously reported data, with lower rates of all grade and ≥ G 3 neutropenia and diarrhea. G-CSF and loperamide should be considered as prophylactic treatment for pts receiving SG.

P1-02-07: Topical modulation of nociceptor epidermal terminals delays and ameliorates CIPN improving the quality of life of breast cancer patients

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Background & Aims:There is strong evidence that >50% of patients undergoing chemotherapy are affected by peripheral polyneuropathy referred to as CIPN. Notably, up to 40% of these patients may develop a chronic neuropathy. A recent meta-analysis revealed that CIPN-associated neuropathic symptoms may persist in >80% of early breast cancer patients for 1-3 years after treatment. Considering the high survival prognosis of these patients, it appears essential to develop strategies that attenuate CIPN to increase their quality of life (QoL). Chemotherapeutic drugs such as paclitaxel and oxaliplatin produce neuropathic symptoms during or immediately after treatment. These drugs have been shown to potentiate thermosensitive receptors, sensitizing epidermal nociceptive terminals. Topical control of this sensitization may improve the sensory symptoms of CIPN and increase patients' quality of life. A previous pilot study in a cohort of 27 patients with CIPN grades I/II showed that topical application of a formulation containing a thermosensitive receptor modulator (Oncapsisens®) relieved sensory symptoms and increased patients' dermatological QoL. Therefore, preventive modulation of nociceptive epidermal endings with Oncapsisens® may help delay the onset of CINP and decrease the intensity of sensitive symptoms.
Methods: A randomized, double-blind study (hydrating cream (A) and nociceptive formulation Oncapsisens® (B) was approved by the Ethics Committees of participating hospitals. A cohort of 121 patients diagnosed with stage I-III breast cancer were included. Participants started a daily application of the assigned cream in hands. Upon appearance of sensory symptoms in hands or/and feet, participants applied the cream twice daily in hands and feet. Follow up of CIPN grade and adverse effects was conducted by oncologists, and quality of life questionnaires by sponsor. Differences between groups of qualitative variables were analyzed with the Fisher or Chi-square tests, and of quantitative variables with the non-parametric Wilcoxon signed-rank or Mann-Whitney rank tests.
Results: 60% of participants with a variety of chemotherapeutic treatments containing taxanes and/or platins developed distal CIPN. Withdrawals were similar in both groups. Application of cream B significantly delayed the appearance of sensory neuropathic symptoms (66.7% cream B vs. 49% cream A), attenuated the incidence of CIPN in hands, and improve sensory symptoms according to the Leonard scale. We also observed a lower incidence trend of CIPN in patients using cream B. Both creams were satisfying to patients. Only 2 patients in both groups complained of pruritus and were withdrawn from the study.

Conclusions: These findings suggest that topical modulation with a cream of nociceptor thermosensory sensitization delays the onset and improves CIPN symptoms.

P1-02-08: Incidence of cardiotoxicity in patients with breast cancer receiving human epidermal growth factor 2 (HER2) targeted monoclonal antibodies and antibody-drug conjugates: A Systematic review and meta-analysis.

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Background: Trastuzumab and other HER2-directed agents have changed the natural history of HER2-positive breast cancer; these agents have also been associated with cardiac dysfunction. Current guidelines recommend monitoring left ventricular ejection fraction (LVEF) every 3 months while patients are receiving selected HER2-directed agents. However, the incidence of symptomatic or clinically significant cardiotoxicity appears to be low, especially with ADCs, and the high frequency of cardiac monitoring may not be cost-effective. This study aimed to define the incidence of clinically relevant cardiotoxicity in patients receiving trastuzumab, trastuzumab emtansine (T-DM1), or trastuzumab deruxtecan (T-DXd).
Methods: A literature search of the Ovid Medline, Elsevier Embase, and Ovid Cochrane databases was conducted. The results were then uploaded to Covidence online software for the formal review by two independent reviewers. Statistical analysis was carried out using R software. This was a single-arm cumulative incidence meta-analysis investigating the cardiac safety of HER2-directed agents. A random-effects model was used for analysis, assuming the data come from varied populations with different distributions. The results were presented as pooled analysis in forest plots. We used the Cochrane Q chi-square test and I2 statistic to examine heterogeneity across studies; P values <0.10 and I2>50% were considered significant for heterogeneity. PROSPERO database ID: CRD42024513386.
Results: A total of 55 publications (n=39,335) were included: 37 for trastuzumab (n=28,808), 6 for T-DXd (n=2,331), 13 for T-DM1 (n=8,196), one used T-DM1 and T-DXd. We included one phase 1, two phase 1/2, 22 phase 2, and 30 phase 3 studies. A total of 25 studies included patients with early breast cancer and 30 with metastatic disease. A random-effects single-arm meta-analysis was completed to determine the incidence of cardiotoxicity associated with these drugs; the overall incidence of cardiotoxicity was 8% (95% CI 6; 10.4) with trastuzumab, 1.4% (95% CI 0.8; 2.6) with TDM-1, and 2% (95% CI 1.4; 2.9) with T-DXd. The incidence of symptomatic cardiotoxicity was 1.6% with trastuzumab, 0.4% with T-DM1 and 0.3% with T-DXd. There was a numerically higher incidence of decreased LVEF in those with metastatic disease relative to those with early breast cancer (trastuzumab: 9.8% vs. 7% and T-DM1: 1.7% vs. 0.9%, respectively). Patients treated with concurrent pertuzumab had a numerically slightly lower risk of decreased LVEF (trastuzumab 6.2 vs. 9% and T-DM1 1.3% vs. 2.5%).

Conclusions: In this meta-analysis, we concluded that the incidence of cardiotoxicity was higher with trastuzumab when compared to T-DM1 and T-DXd. Moreover, the risk of symptomatic cardiotoxicity is low across all these agents, ranging from 0.3-1.6%. The incidence of cardiotoxicity was lower with ADCs than with trastuzumab. Our findings suggest that the incidence of clinically relevant cardiotoxicity is low and low and therefore, monitoring LVEF every 3 months may not be necessary. Less frequent monitoring could decrease the number of visits for patients as well as financial toxicity. Future steps include developing a cost-effectiveness analysis to determine if cardiac monitoring every 3 months is the most appropriate for patients at standard cardiovascular risk.

P1-02-09: Evolution of Breast Cancer Management after Hodgkin Lymphoma: Towards a breast- Conserving Approach

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Background:The treatment of Hodgkin lymphoma (HL) has significantly evolved over the past few decades, combining less toxic chemotherapy and radiotherapy approaches. These advancements have improved relapse-free survival, particularly in the early stages of the disease. However, improved survival has led to an increase in secondary cancers, with breast cancer being the most frequent. Furthermore, despite transitioning to less extensive radiotherapy regimens like involved-node (INRT) and involved-site (ISRT) radiation, the risk of developing secondary breast cancer persists.
Purpose: The aim of this study was to analyze the clinical and histological characteristics of breast cancers occurring after Hodgkin lymphoma, as well as their outcome. Particular attention was given to the effectiveness and safety of breast-conservative surgery in this population.
Materials and Methods: We conducted a retrospective study on the records of 218 patients who developed stage 0 to III breast cancer (in situ or invasive) after treatment for HL at the Institut Curie between 1951 and 2022. Comprehensive demographic, clinical, and therapeutic data were collected, including treatment modalities for HL and breast cancer, as well as survival and locoregional control outcomes. Statistical analyses were performed using R software version 4.1.1.
Results: The median age at HL diagnosis was 24 years [7-79 years]. The median radiotherapy dose received by the mediastinum was 40 Gy, mainly using the mantle technique (79.8%). Breast cancer appeared at a median age of 47 years [22-86 years], with a median interval of 21 years [5-51 years] after Hodgkin lymphoma. A total of 12 patients (5.5%) were diagnosed with synchronous bilateral breast cancer. Locoregional treatment included mastectomy in 117 patients (56.0%) and lumpectomy in 92 patients (44.0%), with adjuvant radiotherapy in 99 patients (47.6%). Isocentric lateral decubitus irradiation (ILD) was performed for 48 patients treated by tumorectomy (63.2%).
With a median follow-up of 29.7 years after HL and 7.7 years after breast cancer, the 5-year overall survival and locoregional control rates were 89.2% and 86.4% for invasive cancers, and 100% for in situ cancers. There was no significant difference in terms of 5-year local

control between patients who underwent lumpectomy and those who underwent mastectomy: 95.1% and 88.1%, respectively (p=0.36). The 5-year survival rate without controlateral cancer was 93.8% [95% CI: 90.3–97.5%]. The 5-year metastasis-free survival rate was 87.4% [95% CI: 82.7–92.4%], with no difference observed between patients treated conservatively compared to those treated with mastectomy (96.5% versus 80.7%, p = 0.19). Long-term effects and aesthetic outcomes were evaluated in all patients, respectively. No late sequelae were reported, only slight decreases in glandular volume were observed.
Conclusion: Breast-conserving surgery, combined with appropriate radiotherapy, can be considered in the treatment of breast cancers after HL despite prior thoracic irradiation. This approach provides comparable outcomes in terms of local control and survival while reducing the risk of long-term complications associated with mastectomy. Incorporating techniques such as the lateral decubitus isocentric radiotherapy approach further enhances the efficacy and safety of breast-conserving therapy in this patient population.

P1-02-10: Real-world analysis of interstitial lung disease in patients with HER2-positive unresectable or recurrent breast cancer treated with trastuzumab deruxtecan: all-patient post-marketing surveillance study in Japan

Junji Tsurutani, Kengo Noguchi, Ayumi Tanabe

Background: Trastuzumab deruxtecan (T-DXd) has been initially approved for the treatment of HER2-positive unresectable or recurrent breast cancer after prior chemotherapy in Japan. While there is accumulating evidence demonstrating the efficacy of T-DXd in various types of cancers, interstitial lung disease and pneumonitis (ILD/p) are recognized as important identified risks associated with the use of T-DXd. Understanding the risk of ILD/p is crucial for optimizing ILD risk management and promoting the safe use of T-DXd. We conducted an all-patient post-marketing surveillance (PMS) study in patients with HER2-positive unresectable or recurrent breast cancer in Japan to investigate the incidence of ILD/p and factors associated with its development.
Methods: This study was conducted as an observational, multicenter, PMS (jRCT1080225197) with an observation period of 18-months that enrolled all patients treated with T-DXd for breast cancer in Japan. Patients who initiated T-DXd treatment between May 2020 and November 2021 were enrolled. Physician-assessed ILD/p events were retrospectively reviewed by an independent adjudication committee; events adjudicated as drug-related ILD/p were summarized. The factors associated with the development of adjudicated drug-related ILD/p were investigated using a Cox proportional hazards model.
Results: A total of 1731 patients (99.5% females) with a median age of 60 years (range: 27-87) were included in the safety analysis set. The majority of patients (92.1%) had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 1 or less. The

median duration of T-DXd treatment was 9.4 months (range: 0.7-17.9), and the discontinuation rate during the 18-month observation period was 74.9% with the most common reason for discontinuation being progression of primary disease (46.0%). The incidence of any grade, grade ≥3, and grade 5 adjudicated drug-related ILD/p were 16.1%, 3.0%, and 1.0%, respectively. More than 80% of adjudicated drug-related ILD/p had resolved, were resolving, or resolved with sequelae within 24 weeks from onset. A multivariate analysis indicated that medical history and/or comorbidity of ILD/p [hazard ratio (HR) = 2.237, 95% confidence interval (95% CI): 1.210, 4.134], baseline renal impairment [mild impairment (creatinine clearance, 60≤ to <90 mL/min; HR = 1.719, 95% CI: 1.272, 2.322), moderate impairment to end stage (creatinine clearance, <60 mL/min; HR = 1.850, 95% CI: 1.240, 2.761)], higher body mass index (BMI ≥21.3 kg/m2 [median], HR = 1.649, 95% CI: 1.275, 2.133), and male gender (HR = 3.634, 95% CI: 1.299, 10.163) were associated with the development of ILD/p.
Conclusions: This study provides the first real-world experience with T-DXd, including over 1700 patients with breast cancer in Japan. The findings indicate that the risk of ILD/p in the real-world setting does not differ from that observed in clinical trials, suggesting that no new safety concerns were identified by this PMS. Medical history and/or comorbidity of ILD/p, baseline renal impairment, higher BMI, and male gender were identified as factors of interest associated with the development of ILD/p in patients with breast cancer. Further investigation into the identified factors of interest may offer insights into the development of ILD/p among T-DXd treated breast cancer patients in Japan.

P1-02-12: Prospective phase II study on the efficacy and safety of hetrombopag for the treatment of cancer therapy-induced thrombocytopenia in patients with breast cancer

Hanfang Jiang, Anjie Zhu, Huiping Li, Yaxin Liu, Ran Ran, Guohong Song, Bin Shao, Jiayang Zhang, Nan Wang

Background: Cancer therapy-induced thrombocytopenia (CTIT) is a common hematologic toxicity for patients with breast cancer (BC) causing anti-cancer therapy delays, dose reductions, and discontinuation. As a novel oral thrombopoietin-receptor agonist (TPO-RA), hetrombopag might raise platelets in patients with CTIT. We carried out a prospective, single-arm trial to assess the efficacy and safety of hetrombopag monotherapy in CTIT among patients with BC (ChiCTR2200062811). Methods: Thrombocytopenic patients with BC (Age ≥18 years, platelet counts<75×109/L) caused by anti-tumor treatments were eligible. The enrolled patients received hetrombopag monotherapy (5.0mg, qd) until reaching a recovery in PLT ≥ 80×10 9 /L or an increase of ≥50×109/L compared to the baseline. The treatment would stop when patients accepted 14 consecutive days of treatment or reached the discontinuation criteria. Platelet examination was taken every 3 days during the study period. The daily blood test was required if the baseline platelet counts <50×109 /L. The primary endpoint was platelet response within 14 days, denoted by a recovery in PLT ≥75×109/L compared to the baseline. The secondary endpoints included

the proportion of patients with platelets recovered to ≥ 100×10 9 /L, the proportion of patients with platelet transfusion, the incidence of dose reduction, delay, or discontinuation of consecutive cancer therapy cycles, and the safety. Results： From January 1, 2024, to June 30, 2024, 19 patients were screened for eligibility. The baseline characteristics were as follows: The median age was 51, with the majority (89.5%,17/19) at clinical stage IV. All patients with BC were treated with different anti-tumor regimens (31.6% (6/19) with chemotherapy therapy only, 21.1% (4/19) with antibody-drug conjugate (ADC) therapy, 15.8% (3/19) with chemotherapy plus targeted therapy, 15.8% (3/19) with endocrine therapy plus targeted therapy, 5.3% (1/19) with chemotherapy plus PD-1 inhibitors, 5.3% (1/19) with chemotherapy plus PD-1 inhibitor and antiangiogenic agent, 5.3% (1/19) with ADC plus antiangiogenic agent). Among them, 42.1% (8/19) of patients have received ≥3 lines of therapy. The median time from the last anti-tumor treatment to meet the inclusion requirement was 7 days (range, 1-22 days). The median value of baseline platelet counts was 65×10 9 /L (range, 28-74×10 9 /L). All patients experiencing ≥ grade 2 thrombocytopenia (PLT<75×10 9/L) after anti-tumor received hetrombopag monotherapy 5.0mg/day. Among them, 10.5% (2/19) experienced grade 3 thrombocytopenia. Treatment response was 89.5% (17/19), with a median time of 3 days (range, 3-9 days) to respond. The proportion of patients with platelets recovered to ≥100×109/L was 68.4% (13/19), with a median time of 6 days (range, 3-6 days) to respond. The incidence of dose reduction and delay of consecutive planned chemotherapy cycles were 18.8% (3/16, 3 patients had changed treatments due to disease progression or turning into maintenance therapy) and 42.1% (8/19), respectively. Especially, the platelet counts of 2 patients with severe thrombocytopenia (PLTs were 28×109/L and 39×109/L at baseline, respectively) increased to ≥100 × 109/L in 6 days after hetrombopag treatment. For safety, during the treatment of herombopag, there were 15.8% (3/19), 10.5% (2/19), and 5.3% (1/19) experienced grade 3-4 white blood cell count decreasing, neutrophil count decreasing, and anemia, respectively. No thrombosis was observed. There were no ≥ grade 3 increased AST and/or ALT. Conclusion: In this study, hetrombopag monotherapy is efficacious and well tolerated, substantiating its potential role as a novel treatment strategy for CTIT patients with breast cancer.

P1-02-13: Longitudinal analysis of cancer-related cognitive impairment in patients with luminal-like breast cancer undergoing chemotherapy and/or endocrine therapy - the Champalimaud-Helsinki study

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Background: Cancer-related cognitive impairment (CRCI) negatively impacts the quality of life (QoL) of patients with breast cancer (BC) and their ability to return to work. CRCI is a complex and multifactorial condition, with consensus that longitudinal studies are

necessary to study this phenomenon. This study aimed to investigate differences in the trajectory of cognitive function in patients with BC following exposure to chemotherapy (CT) or endocrine therapy (ET).
Patients and Methods: The Champalimaud-Helsinki study is a prospective cohort study developed as part of the BOUNCE project (H2020 Grant Agreement 777167), to assess cognition in patients with BC undergoing systemic treatment. Women aged 18-70 years, with Stage I-III Luminal-like BC treated with curative intent, and with no neurological or major psychiatric diseases, were eligible. All patients received local treatment with surgery and some with radiation therapy, according to standard practice. Two longitudinal cohorts were defined: 1) High-risk disease, where patients received neo (adjuvant) CT with taxanes with or without anthracyclines, and sequential ET; 2) Low risk disease, where patients received ET only. Cognitive complaints (Cognitive Function domain of the EORTC QLQ-C30 questionnaire), and battery of objective neuropsychological tests covering the domains of memory, attention, and executive function were assessed before starting systemic treatment, and at 6 months and 1 year after the baseline assessment Baseline comparisons were made between the CT and ET groups. Longitudinal analysis was conducted using a Linear Mixed-Effects Models.
Results: A total of 207 patients were recruited between February 2020 and October 2021, 102 in the CT group and 105 in the ET group, and 19.5% and 18.1% attrition rates, respectively. Women in the CT group were younger [mean age 51.4 years (SD 9.1) vs 55.7, (SD 8.8)], were more frequently in pre- or perimenopausal status (51% vs 31.4%), and had a more advanced disease (stage II/III: 76.4% 20.1%). There were no differences in education or other relevant sociodemographics known to affect cognition. At baseline, the CT group had higher rates of clinically significant anxiety (24.7% vs. 4.1%) and depression (3.7% vs. 0%; $p<.001$), but no differences were observed regarding cognitive complaints or neuropsychological test performance. In the first 6 months, both groups reported a decline in self-reported cognitive function, indicating more cognitive complaints ($p<0.001$), with partial recovery at 1 year. A time-group effect was not found ($p=0.40$). No decline or time-group effect was seen in objectively assessed memory, attention, or executive function.
Conclusions: Six months after initiating systemic treatment, patients with luminal-like BC experienced increased cognitive complaints, regardless of whether they received chemotherapy or endocrine therapy only, with no decline observed in objective neuropsychological performance. This underscores the impact of endocrine therapy on self-reported cognitive function. A better understading of CRCI is essential for developing interventions in the early-phase of BC systemic treatments, aiming to lessen CRCI severity and duration and improve QoL.

P1-02-14: Rate of Fracture After Discontinuation of Bone Modifying Agents in Patients with Early-Stage Breast or Prostate Cancer on Hormonal Therapy

Mara Hofherr, Alisar Aljundi

Background: Breast cancer patients on adjuvant aromatase inhibitor (AI) therapy or ovarian failure secondary to treatment are at high risk of decreased bone mineral density (BMD). NCCN[VP1] (National Comprehensive Cancer Network) currently recommends non-hormonal agents to prevent the loss of BMD, i.e. bisphosphonates and denosumab. The duration of denosumab treatment can vary, however will typically be discontinued after AI treatment is stopped. When discontinued, the decreased osteoclast activity can increase significantly, causing a rebound effect. It is becoming common practice to administer a dose of zoledronic acid, which does not carry the rebound effect, after discontinuing denosumab. There is minimal evidence that this practice is clinically necessary in the oncology population.
Methods: In this retrospective, single center study we examined patients with early-stage breast cancer who received and discontinued denosumab or zoledronic acid. IRB approval was obtained. Number and length of treatments, fractures of any kind, and delay in BMA were collected from the electronic medical record using diagnosis codes.
Results: Of the 587 patients with cancer treated from July 1, 2018 – July 1, 2023 who received hormonal therapy (AI) and received more than one dose of zoledronic acid or denosumab, 258 did not have bone metastases, alternative indication for bone modifying agents, or other primary cancers. Baseline characteristics were well balanced. 185 patients received zoledronic acid. 11 patients (5.9%) had a fracture after stopping zoledronic acid, with an average of 240 days after treatment. 73 patients received denosumab. 32 patients (44%) had a dose of zoledronic acid after denosumab, 41 patients (56%) did not receive zoledronic acid. After stopping denosumab and receiving a dose of zoledronic acid, 4 (13%) patients had a fracture an average 656 days after the last dose. Patients who [VP2] did not receive a dose of zoledronic acid after stopping denosumab, 3 patients (7%) had a fracture at an average of 627 days. 5 patients had a significant delay in treatment (gap of ≥1 month or greater) with either denosumab or zoledronic acid and no patients had a fracture during or after treatment.
Conclusions: The authors saw similar rates of fracture after discontinuing zoledronic acid or denosumab. If patients had discontinued denosumab, there were similar rates of fracture whether they received a dose of zoledronic acid to prevent the rebound effect or not. Further research needs to be completed to continue to investigate if the rebound effect of denosumab is clinically relevant in early-stage breast cancer patients for risk of fracture.

P1-02-15: Natural history including the incidence, severity and management of diarrhea in patients with breast cancer receiving abemaciclib and pertuzumab-based therapies with or without standard chemotherapy: Data from placebo arm of the OnTarget study

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Background: Cancer therapy-related diarrhea (CTD) is a common adverse effect of targeted therapies (e.g., TKIs, CDK4/6 inhibitors), resulting in treatment modifications and poor

clinical outcomes. Yet, longitudinal patterns of CTD's impacts on cancer therapy (e.g., dose reductions, delays, and/or discontinuation) remains poorly characterized. Here we present patient reported outcomes (PRO) data on the incidence and severity of diarrhea in adult patients with breast cancer receiving abemaciclib and pertuzumab-based therapies in the placebo arm of the OnTarget study (NCT04538625).

Methods: OnTarget was a randomized, multicenter, double-blind, placebo-controlled prophylactic trial evaluating crofelemer versus placebo in adults with solid tumors receiving targeted therapies with or without standard chemotherapy. Patients were randomized to crofelemer or placebo given orally twice daily for 12 weeks. PRO-based diarrhea events were captured real-time using a mobile device. Key exclusions included immunotherapy, neratinib, irinotecan, colitis/ostomy/abdominal surgery ≤3 months, and antidiarrheal or antibiotic use ≤7 days. PRO grading used The National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 for the number of loose/watery stools (LWS)/day using the Briston Stool Form Scale (6 or 7) for grades 1-4 of diarrhea. The incidence of diarrhea was evaluated as the proportion of days with LWS for each 21-day period of the 84-day treatment period. The proportion of patients requiring targeted therapy or chemotherapy dose modifications, including reductions, interruptions or discontinuations, were evaluated over each 21-day period. The proportion of patients using antimotility drugs as rescue medications for each cycle were also evaluated.

Results: The OnTarget study enrolled 287 adults with 10 solid tumor types and 24 unique targeted therapies. Patients were randomized 1:1 to placebo (n=142) or crofelemer (n=145) to prevent diarrhea. The safety population of patients with solid tumors randomized to the placebo arm was 135. The median age was 59 yrs [IQR: 51-57 yrs], 18.5% non-White). PRO data on diarrhea were available for 126 patients with different solid tumors over the 12-week period. Specifically, 81 (57%) were patients with breast, 18 (12.7%) lung, 14 (9.9%) renal, 9 (6.3%) liver, and 4 (2.8%) gastrointestinal cancers in the placebo group. Kinase inhibitors were administered to 49 (38.9%) patients with lung, renal, liver, breast and gastrointestinal cancers; two cohorts of patients with breast cancer received abemaciclib [n=44; 34.9%] and pertuzumab-based therapies with chemotherapy [n=29; 23%]. Abemaciclib patients experienced their maximum grade diarrhea during days 22-42 of their treatment, with a median of 7.1 LWS/week (IQR: 3.1-10.4). Over the 12-week, abemaciclib treatment period, a total of 18 (43.9%) patients with breast cancer required a dose modification due to diarrhea, and 24 (58.5%) reported rescue loperamide use. In contrast, those receiving pertuzumab/trastuzumab/docetaxel and carboplatin [n=17] had maximum median grade diarrhea during cycle 1 with 7.1 LWS/week (IQR: 4.6-12.3), and 75% of these patients reported rescue loperamide use. For the 4-cycle period for this pertuzumab cohort, the median LWS/week was 6.19 (IQR: 3.3-8.4), with all patients requiring a dose modification and 88.2% reporting rescue loperamide use.

Conclusion: This PRO-based prophylactic study on the incidence of diarrhea in patients with breast cancer receiving abemaciclib or pertuzumab-based therapies with standard chemotherapy over a 12-week period showed that most patients with breast cancer required dose modifications due to diarrhea and/or used rescue medications to remain on

their cancer therapy. The use of real-time PRO data on CTD in adult patients with breast cancer shows that diarrhea remains a neglected and under-reported comorbidity of cancer treatment.

P1-02-16: Matched pair single-cell RNA-sequencing highlights the impact of abemaciclib and endocrine therapy on different myeloid cell populations in advanced ER+/HER2- breast cancer

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Background: The integration of cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors with endocrine therapy (ET) has revolutionized the management of estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-negative (HER2-) breast cancer. The ability of CDK4/6 inhibitors to induce cell cycle arrest is well documented, however, their potential to modulate the tumor immune microenvironment (TIME) has recently been appreciated. Pre-clinical studies suggest that CDK4/6 inhibitors elicit an anti-tumoral immune response through increased antigen presentation, activation of T- cells, and suppression of T regulatory cells. However, these data lack the granularity to characterize the impact of CDK4/6 inhibitors on the diverse immune cell populations including the myeloid compartment within the TIME. Here, we use single-cell RNA-sequencing (scRNA-seq) of isolated CD45+ cells to characterize the baseline immune landscape and the immunomodulatory effects of abemaciclib and ET in matched advanced ER+/HER2- breast tumor biopsies.
Methods: Sixteen patients with ER+/HER2- advanced breast cancer with lesions amenable to biopsy and beginning 1st-line abemaciclib and ET were enrolled (NCT04352777) and underwent a biopsy at baseline and 4 weeks post-treatment. Biopsies were immediately dissociated into single-cell suspensions and viably cryopreserved. Prior to scRNA-seq, cryopreserved single-cell suspensions were thawed and CD45+ cells were isolated. scRNA-seq libraries were prepared using 10X Genomics 3' Gene Expression kit and raw data were processed using Cell Ranger (10X Genomics). Data integration, clustering, and differential expression analysis were performed using Seurat. Clusters were annotated using canonical cell type markers. Responders were defined as patients on treatment for greater than 6 months without progression as assessed by RECIST 1.1. Changes in relative cell type proportion were assessed per patient, timepoint and lineage (myeloid or lymphoid). Survival analyses were performed with gene expression data from the METABRIC cohort.
Results: In total, 13 matched pairs were evaluable with an average patient age 60.8 years. Of these patients, 8 were classified as responders, 3 as non-responders, and 2 were not evaluable. From the 170,798 cells analyzed, we identified T-cells, NK cells, B-cells, plasmablasts, myeloid cells, and a cluster of proliferating immune cells. Responders had an expansion of activated, GZMA+/IFNG+ CD8+ T-cells and NK cells ($p < 0.05$) and a

concordant decrease in exhausted TIGIT+/PD-1+ T-cells. Additionally, our data reveal a complex and functionally heterogeneous myeloid subpopulation. We identified 14 diverse myeloid populations. Within the myeloid compartment, we identify a population of TREM2+ lipid-associated macrophages (LAMs), associated with a highly suppressive phenotype in multiple solid tumor types including breast, that decrease in relative proportion following treatment with abemaciclib and ET in treatment-responsive patients. Differential expression analysis was used to generate a gene signature defining this population. Lower expression of our TREM2+ LAM signature is significantly associated with improved overall survival in ER+/HER2- breast cancer patients from the METABRIC cohort.
Conclusions: Using scRNA-seq analysis, we show that response to abemaciclib and ET is associated with an increase in cytotoxic lymphoid populations and a decrease in exhausted T-cells. For the first time, we show an immunosuppressive population of TREM2+ LAMs decreases following treatment with abemaciclib and ET in responsive patients. In a separate dataset, lower expression of a TREM2+ LAMs gene signature is associated with improved overall survival. Further translational work is ongoing to assess the promise of targeting TREM2+ LAMS in ER+/HER2- MBC.

P1-02-17: Rediscovering Denosumab: Immunomodulation of the Tumor Microenvironment in Early-Stage Breast Cancer through RANK Pathway Inhibition

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Background: The RANK signaling pathway has emerged as a potential therapeutic target in breast cancer (BC). Preclinical and clinical data support that RANK inhibitors enhance the anti-tumor immune response. Recently, we have presented the primary outcomes of our window-of-opportunity clinical trial D-BIOMARK (NCT03691311), although denosumab did not reduce tumor cell proliferation (Ki67) or survival (Cleaved Caspase 3), an increase in stromal tumor-infiltrating lymphocytes (TILs) was observed. The present report focuses on the immunomodulatory effects (IME) of denosumab in the tumor microenvironment (TME).

Methods: A total of 58 evaluable patients were randomized 2:1 to receive two doses of 120 mg of denosumab before surgery or no treatment. Paired biopsies at surgery and baseline from each patient were compared using paired sample t-tests. The expression of RANK and RANKL was analyzed by immunohistochemistry (IHC). Serial tissue sections were stained for CD3, CD4, CD8, CD20, CD68, MUM1, CD4/FoxP3, PD1, and PDL1 by IHC. Quantification was performed using QuPath software, with some parameters confirmed by manual quantification. RNA sequencing was performed in 47 of the 58 included cases (31 in the experimental group and 16 in the control group). Differentially expressed genes (DEGs) and

gene set enrichment analysis (GSEA) were performed, and finally CIBERSORT analysis was used to infer immune cell content from gene expression data.

Results: An increase in CD8+ cytotoxic T lymphocytes was observed in patients treated with denosumab by QuPath analysis (paired t-test 0.002 in the experimental group vs. 0.052 in the control group). Manual quantification showed a significant increase in CD3+, CD8+ and CD68+ macrophages in both groups, but only the control group showed an increase in immunosuppressive regulatory T cells (Treg). Transcriptomic analysis revealed that the increase in DEGs between diagnostic and surgical specimens was higher in the experimental group (adjusted p-value <0.05). GSEA confirmed that denosumab regulates immune activation, metabolism and oxidative phosphorylation pathways. CIBERSORT analysis showed an increase in global TILs in the experimental (p=0.01) and control (p=0.04) arms, consistent with manual TILs quantification. Importantly, changes exclusive to the experimental group included a significant decrease in immunosuppressive cells: M2 macrophages (experimental p=0.008; control p=0.230) and regulatory T cells (experimental p=0.016; control p=0.314), with a similar trend for neutrophils (experimental p=0.065; control p=0.372) and conversely an increase in monocytes (experimental p=0.014; control p=0.436). Tumor and stromal RANK basal expression were associated with highly proliferative tumors, as indicated by Pearson correlation with Ki67 (tumor RANK: 0.290, p=0.032; stromal RANK: 0.418, p=0.001). Basal expression of RANK or RANKL in both tumor and stroma did not function as a response biomarker for the increase in TILs, and potential biomarkers are still under investigation.

Conclusion: Denosumab exhibited IME effects in early BC particularly a reduction of M2 macrophages and immunosuppressive cells. These results highlight the importance of the RANK pathway in modulating the TME and encourage further investigation into the potential targeting of the RANK pathway as an IME treatment in breast cancer.

P1-02-18: Cancer-Associated Fibroblast-Mediated Suppression of T Cell Activity as a mechanism of Resistance to Immunoradiotherapy in Breast Cancer

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BACKGROUND: Neoadjuvant pembrolizumab (Pembro) is now a standard of care for triple negative breast cancer (TNBC), but not all patients respond and that is also the case even when radiation (RT) is added. To this end, elucidation of the mechanisms of treatment resistance is in urgent need. Cancer-associated fibroblasts (CAFs), consist of inflammatory CAFs (iCAFs, which secrete cytokines and suppress immune cells) and myofibroblastic CAFs (myCAFs, which remodel the extracellular matrix), have been shown in in Vitro system that they promote tumor growth and metastasis while suppressing immune reactions by interacting with the other cells. This study aims to elucidate the mechanisms how CAFs play a role in resistance to Pembro or Pembro with RT in breast cancer patients using single-cell

analyses in NCT03366844 clinical trial.
METHODS: Phase Ib/II trial, Breast Cancer Study of Preoperative Pembrolizumab + Radiation (NCT03366844) collected biopsy samples longitudinally from the primary TNBC tumor in 34 patients; at baseline prior to any intervention, after a single dose of Pembro, and after RT while on Pembro. 23 patients achieved pathologic complete response (R) and 11 did not (NR) when treated with chemotherapy after biopsies prior to surgery. Among R group, R1 was the patients who continued to have strong immune cell infiltrates from baseline, and R2 was the patients that immune cell infiltration gradually increased while on treatments. In total of over 510,000 cells were collected and analyzed using Single-cell RNA sequencing,
RESULTS: The proportion of CAFs to all cells was higher in the NR group (18.6%) compared to the R group (7.3%). However, the proportions of iCAFs and myCAFs within CAFs were constantly similar at any point during the treatments in both R and NR groups (iCAFs: 60-70%, myCAFs: 30-40%). The proportion of CAFs to total stromal cells was about 80% at baseline, but it decreased by treatments, with an increase in the endothelial cell fraction (0-25%) regardless of R or NR. In R1, the myCAF fraction increased while the iCAF fraction decreased by treatments. In R2, the myCAF fraction was initially high but decreased, with the iCAF fraction increasing, reversing their ratios by the end of Pembro and RT. Additionally, almost no stromal cells other than CAFs were detected in R1, whereas endothelial cells increased from 38% to 65% in R2 after a single dose of Pembro. We next analyzed which cell express what as a ligand to which target cell in cell-cell communication. In the R group, T cells expressed TGF-beta1 and Interferon-Gamma, and B cells expressed IL-1beta as ligands to CAFs. Meanwhile, in the NR group, T cells expressed TGF-beta1, while B cells expressed IL-1alpha and IL-1beta when compared after first Pembro treatment to baseline. However, the ligand response from immune cells disappeared after the first Pembro treatment. CAFs expressed CXCL12 as ligands to T cells after RT+Pembro treatment in the NR group. The ligand response from CAFs to cancer cells was smaller in the R group. In contrast, CAFs expressed CXCL12 and Insulin like growth factor 1(IGF1) as ligands to cancer cells in the NR group.
CONCLUSION: Cell-cell interaction analysis using clinical specimens suggests that CAFs suppress T cell immune activity, contributing to immunoradiotherapy resistance in breast cancer.

P1-02-19: Spatially defining T-cell clonal evolution in post-neoadjuvant breast cancer and association with molecular residual disease.

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Background: Tumor-infiltrating lymphocyte (TIL) enrichment in primary breast cancers is predictive of treatment response, and specific CD4 and CD8 subsets are associated with neoadjuvant chemotherapy (NAC) and immunotherapy response. Spatially defined T-cell receptor (TCR) evolution patterns in resection tissues associated with NAC response and

survival have not been described.
Methods: To define such patterns, we applied high-throughput TCR profiling to pre- and post-NAC tissues and associated immune dynamics with tumor evolution and clinical outcomes. We generated TCR repertoires of 29 primary breast cancers and 768 spatially catalogued, post-NAC specimens from matched cases (median 24 post-treatment samples/case) using Sequence Affinity capture & analysis By Enumeration of cell-free Receptors (SABER) applied to tissue-based Cancer Personalized Profiling by Deep Sequencing (t-CAPP-Seq). To profile each primary tumor's genomic and immunologic response to NAC, we designed personalized oligonucleotide panels using pretreatment whole exome sequencing and a fixed panel of recurrently mutated genes to capture emergent alterations, as well as a comprehensive panel of TCR-β regions. Leveraging information from fragmented TCRs in FFPE tissues, we spatially enumerated TCR clonotype count and composition in each post-NAC sample and compared the relative abundance of clones shared with each pretreatment tumor. For a subset of cases, we enumerated TILs and tumor cellularity in post-NAC samples (N = 119). To interrogate clonotype composition, we computed Jaccard similarity indices between each pre- and post-NAC sample pair. We associated post-NAC clonal abundance and clonotype composition with tMRD burden and genomic composition. Finally, we associated TCR repertoire dynamics with quantitative tMRD assessment and survival.
Results: TIL density was strongly correlated with the presence of post-NAC residual disease ($R=0.94$, $p<0.001$ for histologic invasive carcinoma cellularity; $R=0.6$, $p<0.001$ for mean tMRD allele frequency (AF)). Because TILs are defined in the presence of histologic residual disease, we defined tMRD-TILs by TCRs sequenced in tMRD positive tissues, which demonstrated greater repertoire diversity than non-tMRD TCRs (greater ShannonE and inverse Simpson diversity indices, $p=.007$ and $p=1.3e-5$, respectively), suggesting a spatially defined tumor neoantigen response. Within cases with low tMRD burden (mean AF ≤3%), which experienced longer progression-free survival than those with high tMRD burden ($p=0.034$), we observed greater TCR clone count in the post-NAC tissue ($p=.012$), greater shared clone count with the pretreatment tumor ($p=5.7e-8$), and greater similarity of the post-NAC clonotypes to the pretreatment tumor ($p=5.1e-6$, Wilcoxon test of Jaccard indices). Presence of a pretreatment CSPP1 lesion was associated with decreased TCR repertoire diversity in chemotherapy-treated resection tissues (ShannonE index, $p=0.00056$), and CSPP1-mutated tMRD was associated with inferior PFS (HR 23.45, $p=.0007$)
Conclusions: By spatially defining changes in the genomic and immunologic microenvironment following NAC, we found superior molecular response is correlated with preservation of the clonal relationship between tMRD-TILs and the primary tumor with simultaneous generation of a rich TCR repertoire. Ongoing investigations involve computationally predicting neoantigen targets of clonally dominant tMRD-TILs through NAC for in vivo validation, representing an avenue for refining selection of molecularly targeted adjuvant therapies.

P1-02-20: Visceral adiposity is associated with survival and distinct gene expression patterns in HER2-enriched subtype tumors

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Background: Greater patient adiposity is thought to promote tumor progression and increase mortality after a breast cancer diagnosis, but data are lacking in less common subtypes such as HER2-enriched. Most prior studies relied on body mass index and associations have been inconsistent. Here we directly measured visceral adiposity on clinically acquired images and examined differences in cancer-specific survival and the breast tumor microenvironment (TME) within and across a diversity of molecular intrinsic subtypes of breast cancer.
Methods: We included women with a primary, stage 2 or 3 invasive breast cancer diagnosed and treated in the community setting at Kaiser Permanente Northern California between 2005 and 2015. Using computed tomography scans collected as part of routine clinical care, we measured visceral adipose tissue (VAT) areas in cm2 at the third lumbar vertebra. We isolated RNA from n=1378 breast tumors collected at biopsy or excision; these cases were a random sample within each immunohistochemical subtype group. We verified RNA quality prior to performing NanoString BC 360™ assays to calculate the PAM50 molecular intrinsic subtype and measured the expression levels of genes associated with the TME. Using Cox proportional hazards models, we examined the associations of VAT scaled to height (m2) with cancer-specific survival within PAM50 subtype adjusting for age, stage at diagnosis and race/ethnicity. We additionally examined the mean change in gene expression associated with high vs. low VAT (>=42.5 cm2/m2).
Results: Mean (SD) age at diagnosis was 56 (13) years; a majority of women were either overweight (BMI 25-<30-kg/m2: 30%) or obese (BMI>30-kg/m2: 38%), and most were diagnosed with stage 2 (58%) vs. stage 3 (42%) breast cancer with representation from each PAM50 subtype: n (%) HER2-enriched 342 (25%), Basal-like 423 (31%), Luminal B, 312 (22%) and Luminal A 301 (22%). Overall, higher VAT (present in 533 [39%] of patients) was not associated with cancer-specific mortality (Hazard Ratio [HR]; 95% Confidence Interval [CI]: 0.98; 0.78, 1.24). By contrast, in the HER2-enriched subtype, patients with higher VAT had twice the risk of cancer-specific mortality (HR= 2.00; 1.19, 3.34), which was not observed for other tumor types (Basal-like HR=0.82; 0.57, 1.20, Luminal B HR=1.01; 0.63, 1.62; and Luminal A HR=0.68; 0.37, 1.23). Within patients with HER2-enriched breast cancer, we found genes associated with stroma (FAP, COL6A3, ADAM12) and mammary stemness (VIM, DDR2, HEG1, FHL1) were differentially expressed and enriched in tumors with high VAT (FDR<0.1).
Conclusion: Excess visceral adiposity was associated with shorter survival and increased expression of stroma and mammary stemness genes in tumors of patients with HER2-enriched breast cancer, suggesting adiposity's contribution to breast cancer progression differs by molecular intrinsic subtype. This translational study provides additional evidence

for the importance of excess adiposity as is a major modifiable risk factor for patient survival.

P1-02-21: Dissecting the tumor immune microenvironment by single cell transcriptomics and immune repertoire analysis in breast tumors treated with the aromatase inhibitor letrozole and the CDK4/6 inhibitor ribociclib.

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Background: Combining aromatase inhibitors with CDK4/6 inhibitors is regarded as a significant advancement in the treatment of estrogen receptor (ER) positive breast cancers. Increasing evidence suggests an immunomodulatory role for CDK4/6 inhibitors in cancer. The impact of CDK4/6 inhibitors on the breast cancer immune microenvironment is still incompletely understood.

Clinical trial: The NEOLETRIB-study is a multicenter, single-arm, open-label phase II clinical trial. Patients with locally advanced, ER positive, human epidermal growth factor receptor 2 (HER2) negative breast cancer, characterized by cT3-cT4 tumors and/or cN2-3 lymph node involvement, receive six cycles of ribociclib (600 mg daily, 21 days on and 7 days off) in combination with letrozole (2.5 mg daily) as neoadjuvant therapy. Patients with large T2 cancers could also be enrolled.

Aim of the study: We aim at studying the immune microenvironment as well as the T and B cells repertoires of 30 breast cancer patients along the course of the neoadjuvant therapy by analyzing longitudinal single-cell data from the NEOLETRIB-trial.

Methods: Single-cell RNA (scRNA), T cell receptor (scTCR), and B cell receptor (scBCR) sequencing were performed on the tumor biopsies collected (i) before treatment, (ii) at day 21, the end of the first ribociclib cycle, and (iii) day 21 of the 6th ribociclib cycle. The transcriptomic data was analyzed using cellRanger and the Seurat package. Analysis of scTCR and scBCR data was conducted with MiXCR and scRepertoire packages.

Results: Through unsupervised clustering of scRNA-seq data, we were able to identify the major cell types present in breast tumors, including T/NK cells, B cells, myeloid cells, epithelial cells, endothelial cells, and cancer-associated fibroblasts. Additionally, single cell TCR and BCR sequencing allowed us to determine the pairing of α/β chains and heavy/light chains on individual immune cells, and to identify T and B cell clones.
By analyzing the expansion and contraction of these clones during therapy, we were able to further characterize the states of T and B cells involved in expansion, as well as understand the dynamics of the tumor environment associated with T and B cell activation.

Furthermore, through network analysis, we investigated the connectivity of the immune repertoire in the tumors.

Conclusions: Our use of single cell transcriptomics and immune repertoire analyses provided a comprehensive characterization of the tumor microenvironment in breast cancer before and during neoadjuvant endocrine therapy. Our longitudinal analysis of biopsies from the NEOLETRIB trial also allowed us to identify clonal evolution and the adaptive immune response during therapy.

P1-02-22: The role of tumor-infiltrating lymphocytes (TILs) in the progression of human high-grade ductal carcinoma in situ (DCIS) to invasive breast carcinoma (IBC)

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Background: We investigated the distribution of tumor-infiltrating lymphocytes (TILs) in the extracellular matrix (ECM) of high-grade breast ductal carcinoma in situ (DCIS) among various subtypes.
Methods: This study included formalin-fixed, paraffin-embedded specimens from 494 female patients diagnosed with DCIS between 1996 and 2018. Due to reservations received from 10 patients, we excluded these cases from further analysis. Using the Van Nuys classification system, experienced breast pathologists actively graded histopathological specimens. The extent of DCIS was recorded when this information was available. Routine diagnostic immunohistochemical (IHC) staining was performed. ER and PR IHC positivity was defined as > 1% positive tumor cells, in accordance with the updated guidelines of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP). HER2 IHC was scored based on the ASCO and CAP guidelines, as in the routine diagnostic procedures for invasive breast carcinoma (IBC). HER2 silver in situ hybridization was performed when the IHC score was 2+. We calculated the Ki67 ratio by counting 200 intraductal epithelial cells in two separate hotspot foci. DCIS cases were classified as Luminal A (LumA), LumB $HER2^-$, LumB HER2+, HER2-enriched, or triple-negative subtypes according to the 2013 St. Gallen guidelines, which is used for molecular subtyping of IBC. Each subtype was divided into three categories: "Pure", which indicates no invasive component, "W/invasive", which indicates an invasive component, and "All", which represents the entire group of the given subtype.
Results: Of the 484 DCIS cases, 422 were identified as high-grade, of which 60 (14%) presented with invasive lesions. A significant number (31 out of 60; 52%) of the “W/invasive” cases contained TILs, compared to those classified as “Pure” (118 out of 362; 32%), ($p < 0.0055$, Fisher’s exact test). We found a significant portion of TILs in the ECM in 149 of 484 (31 %) cases. All these cases (100%) were classified as high-grade DCIS. We were able to subtype 140 out of 149 of these cases. We found that 66% (n = 92) of high-grade DCIS cases with TILs showed strong membrane positive HER2 overexpression (IHC

score 3+). Of these, 27% (n = 37) were LumB HER2+, and 39% (n = 55) were HER2-enriched subtypes ($p < 0.0001$, Fisher's exact test). The proportions of LumA was 12% (n = 17), LumB $HER2^-$ was 13% (n = 18), and finally TPN was 9% (n = 13), respectively. DCIS extent differed significantly between "Pure" cases and "W/invasive" cases with a median of 20 mm and 30 mm, respectively, ($p = 0.0197$, Mann-Whitney test) and between those with TILs and those without TILs, with a median of 28 mm and 20 mm, respectively ($p = 0.0040$, Mann-Whitney test). Differences in age between patients with TILs and those without TILs were not significant, with median ages of 55 and 57 years, respectively ($p = 0.2534$, Mann-Whitney test).
Conclusion: We found that high-grade DCIS cases with an invasive component exclusively showed the presence of TILs in the ECM, highlighting their potential as a tumor progression marker. Notably, a substantial proportion of high-grade DCIS simultaneously exhibited TILs in the ECM and strong HER2 overexpression (IHC score 3+). We found this association to be particularly pronounced in the HER2-enriched subtype ($p = 0.0035$, Fisher's exact test).

P1-02-23: Deciphering VISTA immunoreceptor mechanisms to target VISTA+ triple-negative breast cancers

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Introduction: Current FDA-approved immunotherapeutics for triple-negative breast cancer (TNBC) are only available for a subset of patients, highlighting the need to develop improved immune-targeting strategies. VISTA (V-domain Ig suppressor of T cell activation) is a single-pass transmembrane immune checkpoint receptor that is an emerging clinical target for cancer immunotherapy. It is expressed widely on lymphoid and myeloid cells in healthy individuals, is commonly expressed on cells of the tumor microenvironment and can be abnormally upregulated on tumor cells in a wide variety of tumors. Although VISTA confers quiescence to naïve T cells, molecular mechanisms responsible for this effect remain unclear. Also, the effects of VISTA expression on tumor cells remains poorly defined.
Results: Through cell line multi-omics profiling and histologic examination of 250 human TNBC clinical specimens we identify a subtype of TNBC with extremely high VISTA expression (~10% of all primary tumors). These tumors had undetectable PDL1 expression, very low infiltrating tumor lymphocytes and diminished proliferative rates (Ki67 ~50%, compared to >75% for VISTA-low tumors). We characterized VISTA-high tumor biology by expressing VISTA in TNBC cell lines and monitoring tumor growth in vivo after mammary fat pad injections. In all tested cell lines (HCC1806, MDA-MB-231, 4T1, EO771) enforced expression of VISTA significantly diminished tumor growth, which was evident in both immunocompetent and immunosuppressed hosts. Molecular investigations revealed that VISTA could decrease tumor growth through its intracellular domain, which repressed EGFR trafficking and decreased EGF-dependent cell growth. We purified proteins bound to the VISTA intracellular domain, which identified PTB-domain-containing proteins and

Rab11 effector proteins as functional VISTA interactors responsible for suppressing EGFR activity. These effects were mapped to a four amino acid (NPGF) motif in the VISTA intracellular domain, which could directly bind and recruit NUMB, a PTB-domain containing protein. Mutation of the VISTA NPGF motif completely reverted binding to NUMB, repression of EGFR activity and the slow tumor growth phenotype of VISTA+ tumors in vivo. To further demonstrate the functional significance of the VISTA NPGF motif we generated mice with VISTA NPGF mutations (homozygous NP□AA) and studied their T cells, which naturally express high VISTA levels. T cells from NP□AA mutant mice evinced increased proliferation in response to T cell receptor stimulation compared to control, demonstrating that the NPGF motif is a bona fide repressor of cell division in both tumors and T cells. Mice bearing VISTA NP□AA mutations in their immune system also had diminished tumor growth compared to WT mice when injected with TNBC cell lines.
Conclusions: We report a distinct subtype of TNBC characterized by high VISTA expression, low PDL1 expression and decreased proliferative index. This work identifies mechanisms by which VISTA can diminish cell division of tumors or T cells through recruitment of effector proteins to an NPGF motif in its intracellular domain. Clinical trials of VISTA-blocking monoclonal antibodies are ongoing and may have relevance for VISTA+ TNBCs that are unlikely to respond to PD1-targeted agents. In addition, therapeutic strategies to target the VISTA intracellular domain in T cells are predicted to improve immunotherapy responses and could be considered for VISTA-negative TNBCs.

P1-02-24: Leveraging bulk and single-cell RNA sequencing to identify features predicting response and survival to abemaciclib in six mouse models of breast cancer.

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Introduction: Combining CDK4/6 inhibitors (CDK4/6i) with endocrine therapy (ET) is a standard-of-care treatment modality for hormone receptor-positive/HER2-negative breast cancer in the metastatic and adjuvant settings. Identifying biomarkers of response to CDK4/6i in breast cancer to improve their application is a subject of great clinical interest. We present a therapeutic and transcriptomic analysis of six Genetically Engineered Mouse Models (GEMMs) of breast cancer treated with abemaciclib to shed further light on tumor cell and microenvironment features correlating with response that may mirror similar patterns in human breast cancer.
Methods: Mice from six GEMMs –TP53null-2153 (luminal), TP53null-2208L (luminal), MMTV-PyMT (luminal), MMTV-Neu (luminal), C3-Tag (basal), and RBnull/TP53null-626 (claudin-low) - were used to evaluate tumor response to abemaciclib vs. no treatment (control). Acute changes in tumor volume with treatment were measured, and survival monitored. Tumor specimens from each GEMM were obtained before treatment and at day 7, whereupon they were evaluated with bulk mRNA-sequencing (mRNAseq) using 873 published gene expression signatures, and with single-cell RNASeq (scRNAseq).
Results: Tumors from 3 GEMMs demonstrated statistically significantly less growth in

tumor volume ($p<0.01$) on abemaciclib vs. control – 2153 ($p<0.0001$), MMTV-PyMT ($p=0.003$), and MMTV-Neu ($p=0.001$). Only these same 3 models also demonstrated significantly improved survival with abemaciclib vs. control as assessed by the log-rank test ($p <0.0001$, $p=0.009$, and $p<0.0001$, respectively). Notably, two non-responder GEMMs (C3-Tag and 626) are known RB1-deficient models.
Differential expression analysis of gene expression signatures using supervised learning (FDR 0%) and hierarchical clustering revealed that for the 3 responder GEMMs, abemaciclib-treated tumors had lower expression of proliferation signatures and higher expression of innate immunity-related signatures at day 7 compared to untreated tumors. The 3 non-responder GEMMs showed no significantly differentially expressed signatures between treated and untreated tumors at day 7. We next compared the pretreatment tumor profiles of the responder vs. non-responder GEMMs and identified higher expression of multiple luminal-related signatures and lower expression of innate and adaptive immunity-related signatures in responder GEMMs. All six models were also assayed using scRNAseq, which is currently being analyzed and will be presented.
Conclusions: Tumors from GEMMs with known RB1 loss were insensitive to abemaciclib, and tumors from responder GEMMs had greater luminal gene expression features, despite being ER-negative. Responder GEMMs had lower tumor immune-related gene expression than those that did not respond, and adaptive immunity-related signatures did not change significantly with abemaciclib vs. control in responders, whereas innate immunity-related signatures showed increased expression with treatment. These findings add to a growing body of evidence, including correlative analyses from human clinical trials, which support RB1 loss as a biomarker of resistance and luminal intrinsic subtype and low tumor immune features as biomarkers of response to CDK4/6i in breast cancer.
Funding: Supported by the Breast Cancer Research Foundation, Grant DRC-20-004.

P1-02-25: New Murine Mammary Tumor Cell Lines for Syngeneic Studies Including Immunotherapy

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Breast cancer (BC) is the most common cancer diagnosis in women globally, accounting for more than 1 in 10 new cancer diagnoses. While BC has historically been a poor candidate for immune checkpoint blockade (ICB), recent studies in triple-negative BC (TNBC) have demonstrated anti-PD-1 treatment efficacy when employed in conjunction with chemotherapy1. APOBEC3 (A3) enzymes, such as A3A and A3B, catalyze cytosine-to-uracil deamination in single-stranded DNA, leading to signature single base substitution mutations (SBS2 and SBS13). A3 expression and mutagenesis have been associated with poor clinical outcomes, including drug resistance2,3. Murine models for studying ICB and mutagenesis by human A3A and A3B are underdeveloped. Here, we address this gap by generating two independent mammary tumor cell lines and studying responsiveness to ICB. Two novel mammary cancer cell lines, MM001 and MM008, were isolated from

spontaneously arising tumors in C57BL/6 MMTV-Cre+;p53fl/+ mice. Each tumor line was passaged serially in the mammary fat pads of C57BL/6 (WT) mice to obtain conditioned cell lines MM001i and MM008i, which formed consistent and reproducible tumors. Tumor immune infiltrates were investigated by immunohistochemistry for CD45 (pan-immune marker), CD3 (pan-T cell), CD8 (cytotoxic T cells), B220 (B cells), Mac-1 or CD11b (macrophages), and PD-L1. Cells were also assessed via flow cytometry for surface expression and interferon inducibility of immune markers PD-L1, MHC-I component H2-Kb, and CD155.
Reproducibly growing tumors for each of MM001i and MM008i were achieved, with 50% of the tumors reaching the endpoint of 1000 mm3 around day 18 and day 21, respectively. Interestingly, despite a shared ancestral genetic background, ICB treatment of WT mice injected with MM001i and MM008i revealed differential responses. MM001i was poorly responsive, whereas MM008i showed complete tumor regression following treatment with anti-PD-1. In contrast, both MM001i and MM008i responded rapidly to anti-CTLA-4. These differential responses were not explained by surface expression of PD-L1 and MHC-1 component H2-Kb, which appeared to be basally and inducibly (IFN-β or IFN-γ) expressed at similar levels. Experiments are ongoing to deduce the underlying molecular mechanism, test additional ICB therapies, and study the impact of A3 mutagenesis.
Thus, two novel murine mammary breast cancer cell lines have been established to evaluate immunotherapies in fully immune-competent C57BL/6 mice.
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P1-02-26: Biomarkers in metastatic breast cancer with brain metastases – a subanalysis of matched tumor samples of the BMBC registry

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Background: The incidence of brain metastases (BM) from primary breast cancer (BC) increased over the past decades. Despite the increasing incidence of BM in BC, the biology and development of BM are still poorly understood. Therefore, it is crucial to understand the pathogenesis of BM development in order to find prophylactic and therapeutic

approaches. The Brain Metastases in Breast Cancer Registry (BMBC) has collected clinical data of more than 4000 BC pts. with BM and tumor tissue samples (n= 400).

Methods: For this study, gene expression data (HTG EdgeSeq Oncology Biomarker Panel) was generated from BM and the corresponding primary BC tissue samples (n= 76), followed by differential gene expression analysis. Molecular subtypes were classified according to classical IHC (ultralow, 0, 1+, 2+, 3+) based on HER2 protein overexpression and additionally Absolute Intrinsic Molecular Subtyping (AIMS) was assessed using HTG data.

Results: In total 76 corresponding BM and primary BC tissue samples were included in this analysis. Median age at BM diagnosis was 55 years. 50% of the patients had singular BM, 27.6% 2-3 BM and 22.4% >=4 BM. 46.7% were HER2-pos., 17.3% triple-negative and 36.0% ER-positive. Subtypes according to AIMS in BC were 35.5% HER2-pos., 27.6% lum. A, 17.1% lum. B, 14.5% basal-like and 5.3% normal-like. Distribution of AIMS subtypes in the corresponding BM were 43.4% HER2-pos., 11.8% lum. A, 22.4% lum. B, 21.1% basal-like and 1.3% normal-like. Following AIMS switches could be identified from BC to BM: Lum. A BC switches to lum. B and HER2-pos. BM; primary normal-like BC switch to lum. A, HER2-pos. and basal-like BM; primary lum. B BC switch to HER2-pos., normal-like, lum. A BM; primary HER2-pos. BC switch to lum. B and basal-like BM. There were no AIMS switches detected in basal-like BC to BM. AIMS subtypes in BC and BM were not statistically associated with overall survival in patients with metastatic BC and BM (primary BC p= 0.13; BM p= 0.19). HER2 IHC in primary BC were: 7.1% ultralow, 44.6% IHC 0, 16.1% 1+, 32.1% 3+. HER2-IHC in BM were: 12.5% ultralow, 37.5%, IHC 0, 14.3% 1+, 5.4% 2+ and 30.4 % 3+. HER2 switches from primary BC to BM were detected as following: ultralow to IHC 1+, 0, ultralow; IHC 0 to ultralow, 1+ and 0; IHC 1+ to ultralow, 0, 1+, 2+ and 3+; IHC 3+ to 1+, 2+ and 3+. HER2 status was not statistically associated with OS in BC and BM (Primary BC p= 0.42 and BM p= 0.46).
Among the significantly upregulated genes (logFC > 0.58) in BMs in comparison to primary tumors heat shock proteins (HSPA1B), mitochondria-related genes (COX5 and MRPL13) as well as cell cycle-related factors (CDC20, CCNF and PTTG1) were detected.

Conclusions:Our analysis identified AIMS subtype and HER2 IHC classification switches from primary BC to BM in matched pairs that could be relevant for prognosis and treatment decisions. In addition, our preliminary differential gene expression analyses showed clear differences in various cellular processes between BC and BM tissues. Whether these differences reflect a selection process or rather an adaptation mechanism to a new environment remains to be investigated.

P1-02-27: A novel 1st-in-class RAS/β-catenin inhibitor concurrently targets cancer cells and MDSC to reverse the immunosuppressive tumor microenvironment: antitumor activity in mouse models of breast and other cancers

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While RAS mutations rarely occur in breast cancers, RAS signaling is well-known to be involved in breast cancer development resulting from constitutive activation of receptor tyrosine kinases. The Wnt/β-catenin pathway is also activated and associated with more aggressive forms of breast cancer. We found that the cyclic nucleotide degrading enzyme, phosphodiesterase 10A (PDE10), is overexpressed in breast cancer cell lines and tumors compared with normal human mammary epithelial cells (HMEC) or tissues, respectively. PDE10 selective inhibitors and gene silencing inhibited the growth of cancer cell lines expressing PDE10, while normal cells lacking PDE10 were relatively insensitive. We identified a novel PDE10 inhibitor, ADT-030, that potently and selectively inhibited breast cancer cell growth by synthesizing and screening a focused library of indenes, followed by chemical optimization for oral bioavailability and metabolic stability. ADT-030 binding to recombinant PDE10 was confirmed by enzymatic assays measuring cyclic nucleotide hydrolysis and in intact cells by cellular thermal stability assays. The growth inhibitory activity of ADT-030 was associated with cGMP/PKG activation, blockage of RAS-MAPK/AKT signaling, and suppression of Wnt/β-catenin transcriptional activity, all of which occurred within the same concentration range. ADT-030 strongly inhibited primary tumor growth, blocked lung metastasis, and increased survival in the orthotopic 4T1 mouse model of breast cancer. The antitumor activity was observed at dosages causing no discernable toxicity. ADT-030 also inhibited tumor growth in mouse lung, colon, and pancreatic cancer models. Notably, ADT-030 caused tumor regression in patient-derived xenograft mouse models of pancreatic cancer, increased survival in orthotopic mouse models of lung cancer with “cures,” and synergized with anti-PD1 in the CT26 mouse model of colon cancer to enhance survival. Other studies revealed a significant impact of ADT-030 on the tumor immune microenvironment by inducing apoptosis of myeloid-derived suppressor cells (MDSC), accompanied by increased tumor infiltration of effector CD8+ T cells. Furthermore, ADT-030 treatment of cancer cells led to immunogenic cell death and enhanced dendritic cell activation. These results support further development of ADT-030, a novel PDE10 inhibitor capable of suppressing oncogenic RAS and β-catenin signaling, as a monotherapy or combined with immunotherapy or standard-of-care drugs for treating breast or other cancers with activated RAS and β-catenin signaling.

P1-02-28: PDGFRa+/FAP- fibroblasts at multiplex in situ resolution are associated with resistance to HER2 blockade in neoadjuvant treated HER2+ breast cancer: results from the Swedish PREDIX HER2 trial

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Introduction: The predictive role of cancer associated fibroblasts (CAFs) to neoadjuvant therapy in breast cancer is still under investigation. The aim of this study was to identify predictive biomarkers of response to HER2 blockade at multiplex in situ level for HER2+ breast cancer.
Methods: The PREDIX HER2 (NCT02568839) is a randomized phase II trial that compared neoadjuvant docetaxel, trastuzumab, and pertuzumab (THP) with trastuzumab emtansine (T-DM1) for HER2-positive breast cancer. Pathologic complete response (pCR) rates did not differ between the THP (45.5%) and T-DM1 (43.9%) groups (Hatschek et al, JAMA Oncology 2021). A core biopsy was obtained at baseline, after two cycles of treatment and at surgery. By using FFPE biopsies from all timepoints we performed multiplex in situ fluorescent immunohistochemistry. Two panels, one immune (CD4, CD8, CD20, FOXP3, CD68, CD163, CK and DAPI) and one stromal (FAP, aSMA, PDGFRa, PDGFRb, vimentin, pSMAD2, ki67, CK and DAPI) were selected. Each biopsy was processed by the PhenoImager HT (Akoya Biosciences, Marlborough, Massachusetts, United States) and the inForm software (version 3.0) was trained for tissue and cell segmentation. The invasive cancer area was manually drawn in QuPath.
Results: Two sub cohorts of PREDIX HER2 consisting of 57 patients for the stromal (24 pCR cases) and 54 patients for the immune (19 pCR cases) panels were used. Cancer associated fibroblasts (CAFs) were isolated from the stromal compartment (tissue segmentation from InForm) of the invasive cancer area (defined in QuPath). The different combinations of the aSMA, PDGFRa, PDGFRb and vimentin identified 15 fibroblast subsets. Tumor infiltrating immune cells were identified at the epithelial and stromal compartments of the invasive cancer area. The total populations of CAFs and TILs were not associated with pCR at baseline (adjusted p=0.4). We then looked at the specific subpopulations. Higher densities of PDGFRa+/FAP- CAFs were associated with residual disease at baseline (adjusted p=0.011). The prognostic effect remained significant for T-DM1 treated (adjusted p<0.001) and ER- patients (adjusted p= 0.061), while it was lost for THP treated patients (adjusted p>0.9). From the immune panel, higher densities of CD20+ B-cells and CD4+/FoxP3+ Tregs were associated with pCR (adjusted p=0.012). The prognostic effect of B cells remained significant for T-DM1 arm (adjusted p=0.015). Bulk RNA-sequencing from 185 patients in PREDIX HER2 at baseline verified the correlation between higher PDGFRa gene expression and residual disease at baseline. (p<0.05) Similarly, digitally enumerated TILs and CAFs in H&E slides of 171 patients in the cohort didn't correlate with pCR at baseline (adjusted p=0.14 and 0.2 respectively), highlighting the importance of subpopulations.
Conclusion: Higher densities of PDGFRa+/FAP- CAFs and lower densities of B cells are associated with residual disease at baseline to HER2 blockade. Further validation studies,

longitudinal assessment of the densities' changes under pressure of treatment as well as analysis at a spatial level are ongoing.

P1-02-29: Targeting RANK pathway in combination with CDK4/6 inhibitors decreases the growth of palbociclib-resistant breast tumors via cancer cells and tumor microenvironment-mediated effects

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The Receptor Activator of NFkB (RANK) signaling pathway has emerged as a relevant drug target in the context of breast cancer prevention and treatment, due to its role in progesterone-induced breast carcinogenesis and breast cancer progression. We have previously reported that, in Luminal breast cancer, a decreased proliferation rate and resistance to therapy accompany the RANK-induced stem cell-like and mesenchymal-like characteristics, suggesting that RANK-expressing cells may constitute a reservoir of slow-cycling therapy-tolerant cells. Recently, we have disclosed a link between RANK signaling and intrinsic or acquired resistance to CDK4/6 inhibitors (CDK4/6i) plus endocrine therapy in luminal breast cancer; and demonstrated that pharmacological inhibition of RANK pathway improves response to CDK4/6i-based therapy. Since it has been shown that loss of RANK signaling induces anti-tumoral immunomodulatory effects; and that CDK4/6i themselves can boost anti-tumor immune responses, we aimed to assess if therapy-induced immunomodulation improves the efficacy of co-targeting CDK4/6 and RANK ligand (RANKL) in breast cancer.
With this in mind, we conducted a drug-response in vivo assay, using two Rb-proficient/RANK-positive syngeneic models of breast cancer, TS/A-E1 (ER+HER2-) and 4T1 (ER-HER2-), which were characterized by having poor response to CDK4/6i in vitro (1μM Palbociclib: 41.8% and 8.2% decreased cell viability from control, respectively; 1μM Ribociclib: 13.2% and 3.4% decreased cell viability from control, respectively; assessed by clonogenic assay, mean of n=4 experiments). Cells were inoculated in the flank of 6 weeks-old Balb/C female mice (n=4, TS/AE-1; n=6-8, 4T1), and tumor-harboring mice were treated with 30mg/Kg/day p.o. Palbociclib, 10mg i.p. OPG-Fc (RANKL inhibitor) 3x/week or both. Tumor growth was monitored externally and experimental endpoint was set at Tvol ≥1000mm3 or bigger axis ≥1cm in any group. At sacrifice, tumor tissue was processed for RNAseq and immunohistochemistry (IHC) using standard methods.
Unlike monotherapy, the combination of OPG-Fc and Palbociclib was able to restrain tumor growth in both models ($p=0.0644$ and $p=0.0099$, TS/A-E1 and 4T1, respectively), and led to significantly decreased tumor volume, tumor weight and Ki67 proliferation index (assessed by IHC) at sacrifice ($p<0.05$). Accordingly, gene set enrichment analysis (GSEA-mh, FDR Q-value<0.05) has highlighted hallmarks associated with cell cycle, proliferation and senescence in Palbociclib and combination-treated tumors.
The immune cells' infiltrate was characterized using two complementary approaches. First, the mouse immune cell abundance was predicted from RNAseq data obtained from the 4T1 bulk tumors (n=3) using the ImmuCellAI-mouse software. The infiltration score was

significantly higher in the combination group ($p<0.05$), where M1 macrophages, cDC2 cells, eosinophils and basophils were the more abundant immune cells. OPG-Fc-treated tumors were characterized by increased cDC2 cells; a feature also observed in Palbociclib-treated tumors that also had decreased M2 macrophages and neutrophils. Next, the immune cells specifically infiltrating the non-necrotic tumor parenchyma were quantified by IHC in both models. Significant therapy-induced immune alterations included increased CD4+ and CD8+ T cells in all treated groups, and a decrease in tumor-associated macrophages (F4/80+, TAMs) in OPG-Fc and combination groups. M1 TAMs (iNOS+) were particularly increased in the combination group, whereas M2 TAMs (CD163+) were particularly decreased in these tumors.
Overall, our data supports that RANKLi can be an important add-on to CDK4/6i, in both luminal and TNBC, warranting future clinical studies. The benefit from this combination therapy spans from the direct anti-tumoral effect to the immunomodulatory roles of both drugs, towards an anti-tumoral microenvironment.

P1-02-30: A Stromal Proteomic Investigation of the RAHBT Cohort: Collagen Molecular Patterns Link to Recurrence in Ductal Carcinoma in Situ

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Introduction: While ductal carcinoma in situ (DCIS) is a not lethal breast pathology, its progression to invasive breast cancer (IBC) significantly increases a patient's mortality risk. It remains currently unknown which DCIS patients will experience a later breast cancer event following their primary DCIS diagnosis. Genomic and clinicopathological features have been explored for their predictive value, but stromal protein regulation, particularly of collagens, has yet to be understood in DCIS progression to IBC. We recently reported that collagen stromal signatures differentiate DCIS pathologies and distinguish DCIS from IBC. Here, we hypothesize that specific stromal molecular signatures provide a means to predict DCIS progression to IBC.
Methods: Tissue microarrays of the clinically annotated Resource of Archival Human Breast Tissue (RAHBT) cohort from 232 DCIS patients (White women; n=176, Black women; n=55, Pacific Islander women; n=1) were used under Exemption 4 of the Medical University of South Carolina IRB. The median age of diagnosis was 53 years old with a 6-year median time to recurrence. Stroma-targeting mass spectrometry imaging was used to report collagen stromal molecular patterns linked to DCIS clinical outcomes. The spatial distribution of collagen peptides was further evaluated on 20 lumpectomies (White; n=10, Black; n=10) followed by proteomic sequencing to match to the mass spectrometry imaging data.
Results: We report that the stromal microenvironment is linked to recurrence outcomes and DCIS pathologies. From the RAHBT cohort, DCIS-annotated regions and their adjacent

microenvironments were linked to 479 stromal peptides by spatial mass spectrometry imaging studies. Comparing DCIS specimens (n=232) to normal breast controls (n=36), 265 peptides were found to have significantly different intensity patterns (two-sample t-test; $p<0.01$). A comparison of the primary DCIS event from non-progressors (n=151) and patients (n=40) with ipsilateral recurrence revealed 4 peptides with differential intensity patterns (two-sample t-test; $p<0.05$). In a subset of DCIS patients who experienced ipsilateral recurrence and with matched primary and recurrence specimens (n=17), a comparison of the primary DCIS event to the recurrence revealed 120 stromal peptides to have differential expression between the primary tumor and later breast event (two-tailed t-test; $p<0.05$). Interestingly, most peptides decreased in intensity in recurrence compared to the primary DCIS event. This trend of decreased intensities with recurrence was maintained in the comparison of the contralateral recurrence cases to the primary DCIS event (n=13). Notably, 41 stromal peptides were differentially expressed between non-progressors (n=151) and patients with contralateral recurrence (n=29; two-sample t-test; $p<0.025$). Two peptides were found to be differentially expressed between Black (n=55) and White (n=176) patients in the cohort (two-sample t-test; $p<0.05$). As comedo necrosis has been associated with increased recurrence risks, stromal molecular profiles were investigated per architectural pattern (solid; n=75, cribriform; n=65, papillary; n=6, comedo necrosis; n=47). Between comedo necrosis and cribriform patterns, 21 peptides were reported to be significantly increased in comedo necrosis (two-sample t-test; $p<0.025$). Between comedo necrosis and solid patterns, 32 peptides were reported to be significantly increased in comedo necrosis (two-sample t-test; $p<0.025$).
Conclusions: The collagen matrix appears to undergo distinct spatial alterations with recurrence as well as vary by architectural pattern and ancestry. These extracellular matrix differences could contribute to observed disparities in recurrence associated with these clinical features. Stromal collagen patterns linked to recurrence could lead to novel prognostic development in DCIS.

P1-03-01: Towards Universal Germline Screening for Breast Cancer, Planning for a Sustainable Future: A Single-Center Retrospective Analysis

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Background: The implementation of germline genetic testing is complex due to emerging therapeutic indications such as adjuvant Olaparib for BRCA-mutated breast cancer (BC). Understanding the clinical-pathological characteristics of individuals meeting testing criteria for genetic testing can provide insights into the factors associated with identifying pathogenic germline variants (PVs) and likely pathogenic germline variants (LPVs). This knowledge can enhance the precision of genetic counseling and testing strategies. In this study, we reviewed our single-center experience of testing selected patients meeting clinical

and pathological features, to develop a progressive and more sustainable model on the way towards universal germline screening.
Methods: We evaluated 1,060 consecutive individuals with a personal history of breast cancer (BC) who met regional criteria for germline testing at the Hospital Clinic of Barcelona between 2016 and 2022. We excluded individuals diagnosed before 2000 (n=70), those women with DCIS (n=60), and those with missing clinical information (n=18). Clinical-pathological and molecular characteristics between carriers of PV/LPV and non-carriers. Chi-square tests or Student's t-tests were used to compare the distribution of variables between two groups. Logistic regression analysis was then employed to evaluate the association of each variable with PV/LPV. The significance level for all statistical analyses was set at a two-sided alpha of 0.05.
Results: A total of 912 individuals were evaluated. Most cases were women (n=898, 98.5%) with a median age of 49 (range 24-88) and had a family history (n=706, 78.3%). The main reasons for testing were family aggregation (n=357, 39.1%), BC onset ≤40 yrs-old (n=199, 21.8%), and triple-negative breast cancer (TNBC) onset ≤60 yrs-old (n=162, 17.7%). The rate of PV/LPV was 14.9% (n=136), and 151 individuals (16.6%) had variants of unknown significance (VUS). Overall, 776 (85.1%) had no PV/LPV identified. A higher number of PV/LPVs were identified in the BRCA2 gene (n = 41, 30.1%), followed by BRCA1 (n=31, 22.8%), CHEK2 (n=17, 12.5%), ATM (n=15, 11.0%), PALB2 (n=13, 9.6%), BRIP1 (n=5, 3.7%), TP53 (n=5, 3.7%), BARD1 (n=2, 1.5%), MSH2 (n=2, 1.5%), PTEN (n=2, 1.5%), BAP1 (n=1, 0.7%), CDKN2A (n=1, 0.7%), and RAD51C (n=1, 0.7%). Notably, 2 individuals with a PV in BRCA2 had another PV (i.e., MSH6 and CDK2NA), and 2 individuals with a PV in PALB2 had another PV (i.e., both in CHEK2). Most VUS (n=151) were identified in ATM (n=38, 25.2%), BRCA2 (n=23, 15.2%), PALB2 (n=15, 9.9%), MSH6 (n=12, 7.9%), and BRCA1 (n=10, 6.6%). The BC subtype distribution was 62.4% (n=563) HR+/HER2-, 15.0% (n=141) HER2+, and 22.0% (n=198) TNBC. The clinical-pathological variables significantly associated in univariate analyses with the identification of PV/LPV were sex, age, personal history of other cancer types, advanced TNM stage, TNBC, and bilateral BC. In a multivariable analysis, all the previously mentioned variables remained significantly associated with the identification of PV/LPV. The highest odds ratios (OR) were found for the following 2-group variables: male (OR=4.8), stage IV versus stage I (OR=3.1), bilateral BC (OR=2.7), other personal histories of cancer (OR=2.2), and TNBC (OR=1.7). Age was considered a continuous variable, and for every 10-year increase, the odds of detecting a PV/LPV decreased by approximately 34%.
Conclusions: Based on established historical testing criteria, specific clinicopathological features were significantly and independently associated with the detection of clinically significant variants. As we move towards universal germline screening for breast cancer, well-established information continues to help prioritize individuals who will benefit most from early detection of breast cancer susceptibility.

P1-03-02: Unveiling Genetic Predispositions: Germline Genetic Testing in Arab Male Breast Cancer Patients

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Introduction: Breast cancer predominantly affects women, but it can also occur in men, accounting for less than 1% of all breast cancer cases. Male breast cancer presents unique challenges in diagnosis, treatment, and management due to limited awareness and research compared to female breast cancer. Understanding the differences in pathology, genetics, and treatment responses between male and female breast cancer is crucial for improving outcomes. This study aims to investigate the baseline characteristics, genetic predispositions, treatment approaches, and outcomes specific to male breast cancer patients.
Methods: This retrospective study analyzed data from male breast cancer patients diagnosed between 2003 and 2022 at the King Hussein Cancer Center (KHCC) in Jordan. The study included all male patients with a confirmed breast cancer diagnosis within this period. Data collected encompassed demographic and clinical characteristics, including age at diagnosis, family history, tumor stage and grade, receptor status, and treatment modalities (surgery, chemotherapy, radiotherapy, and hormonal therapy). Disease-Free Survival (DFS) and Overall Survival (OS) were estimated using the Kaplan-Meier method to evaluate patient outcomes.
Results: The cohort consisted of 102 male breast cancer patients, mean age at diagnosis 58.4 (range, 24- 90) years. Invasive ductal carcinoma (IDC) was the predominant histological type (n= 97, 95.1%) of cases. The tumors were primarily grade 2 (n= 58, 56.9%) or grade 3 (n=33, 32.4%). Hormone receptors were positive in 98 patients (96.1%), while 12 (11.8%) were HER2-positive, and notably, none of the patients had triple-negative disease. Eleven (10.8%) patients had de- novo metastatic disease. For early-stage breast cancer, 61 patients (59.8%) were clinically staged as T2, with positive axillary lymph nodes in 56 patients (54.9%). Pathologically, 43 patients (42.2%) were staged as T2, with 27 patients (26.5%) had N1 and 35 patients (34.3%) had N0 axillary lymph node involvement. A family history of cancer, mostly breast, was present in 58 patients (56.9%), and almost 50% in first-degree relative. Genetic testing was performed on 47 patients (46.1%) and 7 patients (14.9%) were tested positive for pathogenic/likely pathogenic variants; mostly in BRCA2 (n=6, 12.8%), while the other patient had CDH1. None of the patients had BRCA1 mutation. Additionally, one patient (2.1%) was a carrier for MUTYH and another patient had an APC increased risk allele mutation. Variants of uncertain significance (VUS) were seen in 20 patients (42.6%). At the time of analysis, 73 (71.6%) of the patients were alive with a median disease-free survival (DFS) of 106 months (95% CI, 76 - NA), and a median overall survival (OS) of 105 months (95% CI, 96.7 -NA).
Conclusion: This study highlights the unique characteristics and challenges associated with male breast cancer. The predominance of invasive ductal carcinoma, high hormone receptor positivity, and the significant presence of familial cancer history and genetic mutations, particularly in BRCA2, underscore the need for targeted awareness, early detection, and

personalized treatment strategies. Despite a relatively high survival rate, the data emphasizes the necessity for increased research and tailored therapeutic approaches to improve outcomes for male breast cancer patients.

P1-03-03: Impact of Mainstreaming Protocol in Clinical Practice of Breast Cancer Patients

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Background: The most commonly affected genes in hereditary breast and ovarian cancer are the breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) genes. For patients carrying BRCA pathogenic variants (PV), lifetime risk for ovarian, breast and prostate cancer is substantially higher. Therefore, identifying them is important to tailor treatment decisions, acknowledge a patient's risk for other cancers and identify healthy relatives with the variant. The traditional model of genetic counseling involves visiting twice a geneticist: once to assess risk and obtain informed consent for testing (pretest counseling) and a second time to present test results and discuss future implications considering risk management for the patient and relatives. This multivisit model often delays testing, increases waiting times, costs and cannot attend to the high volume of patients requiring it. To deal with shortfall of resources and increased demand for testing, “Mainstreaming Cancer Genetics” program was developed in the United Kingdom Royal Marsden Hospital in 2013 with very successful and cost-effective results. In this initiative, the oncologists are responsible and trained for counseling, obtaining informed consent and ordering genetic testing for selected cancer patients, bypassing the need for pre-test counseling by a geneticist. If the test is positive the patient is referred to Genetics. In our center the Mainstreaming protocol (MP) was implemented in September/2018 through collaboration between the Departments of Genetics and Medical Oncology testing a number of high-risk mutations including BRCA1/2. Aims and Methods: To ascertain patient comprehension about genetic testing aims and results obtained in the MP, often in busy first appointments. Retrospective and descriptive analysis was made of all patients with breast cancer diagnosis followed at the Department of Oncology of our center that have been submitted to MP (BRCA1/2 genetic testing) between January/2018 and December/2021. Telephone questionnaires (TQ) regarding perception of results of the genetic test (9 questions) were made up to June/2024. Results: Of 193 breast cancer patients referred to MP, 160 had a test result available and were considered for analysis. Criteria referrals were as follows: 75 (46,9%) had age 31 to 39; 20 (12,5%) had multiple breast tumors (bilateral/synchronous/metachronous before age 60); 50 (31,3%) were triple negative; 8 (5,0%) had male breast cancer; 21 (13,1%) were tested due to potential targeted therapy. A total of 13 (8,1%) had PV and 10 (6,3%) had a variant of uncertain significance (VUS). TQ were obtained from 108 (67,5%) patients of which 97 (89,8%) remembered taking the test. The latter only were considered for questioning as follows: 83 (85,6%) were given written information regarding the test; 93 (95,9%) understood why the test was asked; 88 (90,7%) had been informed by their

doctors about the test results; 84 (86,6%) understood the result of the genetic testing; 68 (70,1%) understood that result could impact their treatment and 89 (91,8%) that could impact their relatives; 85 (87,6%) of patients were satisfied for having done the test. Also, 18 (78,3%) of patients with reported variants (either pathogenic or VUS) mentioned having had a Genetics appointment following their result.
Conclusions: MP is a way of fast-tracking BRCA PV diagnosis in clinical setting with a vast number of geneticist appointments being obviated. In fact only the 22 (14,3%) patients with a found variant had the need for a formal Genetics appointment. This led to communication challenges as medical oncologists took charge of counseling regarding genetic testing. In fact, a relevant 10,2% of patients did not remember being tested, showing great need for improvement in objectives and risk communication.

Breast cancer related lymphedema rates following sentinel lymph node biopsy: how concerned should patients and clinicians be?

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Background: Breast cancer-related lymphedema (BCRL) is a feared side effect following nodal surgery and breast cancer treatments. While long-term rates of BCRL after axillary lymph node dissection are known, little and highly variable historical data exists on rates following sentinel lymph node biopsy (SLNB). Since our institution has been prospectively utilizing bioimpedance spectroscopy (BIS) for screening and early-detection of lymphedema, we sought to examinate the incidence of BCRL following SLNB to help guide patient discussions regarding surgical risks, adjuvant treatment decisions, and the optimal method for long-term lymphedema surveillance.
Methods: A retrospective review of a prospectively maintained, single institution, breast cancer database was performed. All patients underwent SLNB between 11/2014-11/2017. BIS measurements were obtained on all patients preoperatively, postoperatively, and then every 3 months for 1 year, every 6 months for 1 year, then annually. BIS > 3 standard deviations above preoperative baseline was considered abnormal. Any abnormal reading was followed up with a repeat measurement one month later. Patients who received adjuvant radiotherapy and/or chemotherapy, and those who had a recent surgical procedure, were only diagnosed with BCRL if a repeat BIS four weeks later was still abnormal. Additionally, BCRL diagnosis resulted in clinical evaluation and initiation of self-directed home interventions with subsequent reassessment for resolution versus persistent BCRL (pBCRL). Groups were compared using t-tests or Wilcoxon rank sum tests for continuous variables and chi-square or Fisher's exact tests for categorical variables. Analyses used R (version 4.2.1) and $p<0.05$ was statistically significant.
Results: A total of 324 patients underwent SLNB and had both pre-operative and post-operative BIS measurements. A single abnormal BIS occurred in 58 (18%) patients. Of these, 41 (71%) returned to baseline on BIS reassessment without intervention and were therefore not diagnosed with lymphedema. Of the 17 patients (5%) with confirmed BCRL,

15 (88%) had stage 0 lymphedema and only two had stage 1 lymphedema at diagnosis. After self-directed home interventions, only two patients had pBCRL for a pBCRL rate of 0.6% for all patients following SLNB. Development of pBCRL was not associated with age (p=0.39), BMI (p=1.00), receipt of radiotherapy (p=0.52), receipt of taxane chemotherapy (p=1.00), or the number of nodes examined (p=0.40). However, the average number of positive nodes was statistically significant for the development of pBCRL (p=0.01). Conclusions: The incidence of BCRL among patients undergoing SLNB is low. When identified early, at-home interventions can be employed and pBCRL is extremely uncommon. The only risk factor associated with the development of pBCRL following SLNB is the average number of positive nodes. We speculate that increasing nodal disease burden is associated with more frequent use of adjuvant nodal radiotherapy and systemic therapy, both of which are known to contribute to risk of BCRL. It is important to recognize that isolated abnormal BIS measurements are not uncommon, and that the majority of these instances reflect intermittent fluctuations in BIS that do not reflect BCRL. Repeat BIS should be performed before BCRL is diagnosed. Clinicians should be aware of the extremely low incidence of pBCRL when determining surveillance models and counseling patients on the risks and benefits of SLNB, especially because foregoing SLNB may impact adjuvant treatment recommendations.

P1-03-05: RNA-sequencing data in diverse women diagnosed with ductal carcinoma in situ

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Black women have a higher risk of subsequent breast cancer events (SBEs) but are underrepresented in ductal in situ carcinoma (DCIS) biomarker research compared to Non-Hispanic White women. We performed RNA sequencing using the Illumina NovaSeq 6000 technology on formalin-fixed paraffin embedded (FFPE) DCIS specimens. We integrated transcript data with clinical and social factors that drive SBEs in the Resource of Archival Breast Tissue population, including 35% Black women. Cases were diagnosed with DCIS from 1999-2019 with SBEs, including invasive breast cancer or ipsilateral or contralateral local DCIS. Controls matched 1:1 were diagnosed with DCIS from 1999-2019 without SBEs during similar follow-up times. The mean age at DCIS diagnosis was 56.3. The mean follow-up was 147.8 months. Principal Component Analyses (PCA) of 19,821 protein-coding genes were conducted on 141 DCIS samples, with >20 million total reads. PC1, representing 9.0% of the variation, was significantly correlated with race. PC3, representing 4.03% of the variance, correlated with follow-up time. We detected enrichment for interleukin and PD-1 pathways but no significant differentially expressed genes (DEGs) by case-control status. We identified significant DEGs with age, follow-up time, grade, race, and invasive SBEs. Generating high-quality RNA-seq data is feasible and elucidates novel insights on DEGs in diverse DCIS samples.

P1-03-06: Abrupt Involution Leads to Long-Term Mitochondrial Dysfunction and Metabolic Shift – Increasing Risk of Breast Cancer

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Epidemiological data links higher parity and lack of breastfeeding with increased risk of breast cancer, specifically triple negative breast cancer (TNBC). TNBC is the aggressive hormone receptor and HER2 negative subtype associated with higher mortality rate1. Following pregnancy and lactation, breast remodels to near pre-pregnancy stage through apoptotic cell death and adipocyte repopulation process2. Long-term breastfeeding and gradual weaning of an infant leads to gradual involution (GI) of the breast, while lack of or abrupt discontinuation of breastfeeding after birth leads to abrupt involution (AI), when rapid and massive cell death takes place2. Our studies comparing GI vs. AI in a mouse model have shown several precancerous changes, such as increased collagen deposition, inflammation, and hyperplasia in the mammary gland of mice after AI2. While our preliminary data indicates metabolic shifts in the AI glands, the impact of AI on mammary gland metabolism and how this increases risk of breast cancer is yet to be elucidated.

Objectives: Determine the impact of AI on metabolic changes within the mammary gland and decipher the underlying mechanism that could link AI to increased breast cancer risk. Methods: Eight-week-old FVB/n mice were paired for breeding. At partum (day 0), dams were randomized to AI or GI cohort, standardized to 6 pups per dam and housed individually. AI mice had pups removed on day 7 postpartum (ppm) to mimic short-term breastfeeding. For GI mice 3 pups each were removed on day 28 and 31 ppm to mimic gradual weaning. Mammary glands were harvested on day 28, 56, and 120 ppm to assess short-term, intermediate, and long-term effects of AI vs. GI. Total mammary gland RNA was subjected to global gene expression analysis using Affymetrix and analyzed Ingenuity pathway analysis software. Differentially expressed genes were validated by qPCR and western blot. Oxidative stress was measured via MitoSox and H2CDFDA using Flow Cytometry. Whole mammary glands were subjected to untargeted metabolomics and lipidomics.

Results: On day 28 ppm, AI glands had marked upregulation of oxidative phosphorylation, ATP synthesis, and mitochondrial fatty acid b-oxidation compared to GI glands. AI glands had significantly higher levels of mitochondrial oxidative stress and enrichment of oxidized glycerophospholipids. On day 56 ppm, AI glands were metabolically comparable to GI glands. However, AI glands had an upregulation of genes related to fatty acid synthesis (PPARg, ACLY, Chrebp, GLUT4 and SLC25A1) and mitochondrial biogenesis (PGC1a). Interestingly, on day 120 ppm, AI glands showed significant downregulation of oxidative phosphorylation, ATP synthesis, glucose metabolism, and marked upregulation of mitochondria dysfunction. Amyloid precursor protein (APP) associated with mitochondria

dysfunction and downregulation of energy metabolism, was found to be elevated in AI vs. GI glands on day 120 ppm.

Conclusion: AI of the mammary gland leads to metabolic changes over time that disrupt mitochondrial function. Multiple studies have associated high levels of APP in human breast tumors and breast cancer cell lines with disruption of mitochondria function, enhanced cell proliferation, metastasis and invasion. Increased expression of APP in AI mammary glands suggests a key role of this protein in mitochondrial and metabolic dysfunction induced by AI. Further investigation is underway to decipher the role of APP in AI induced changes.
Significance: For the first time, this study demonstrates a metabolic shift in the mammary gland caused by AI. Targeting one or more key players in this metabolic deregulation could provide options for lowering breast cancer risk in women who are unable to breast feed.

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P1-03-07: Identifying risk factors for Benign Breast Disease in Donors to the Komen Tissue Bank

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Background: An estimated 1.6 million breast biopsies are performed annually in the United States. Most of these tissue samples are noncancerous and classified as benign breast disease (BBD). Women diagnosed with BBD are 2 times more likely to be diagnosed with breast cancer (BC) than women without BBD. Studies have shown that the increased risk of BC remains elevated for at least 20 years after a BBD diagnosis. Currently, there is no standard surveillance algorithm or society-guided treatment guidelines for these patients. Furthermore, scant literature guides BBD etiology and strategies for risk-stratification for future BC development. Variables such as hormone use, physical activity, diet, breast density, and genetics are associated with BBD and risk calculators (Gail and Tyrer-Cuzick scores) use some of these variables to help predict a patient's risk of BC. However, neither of these models include smoking history in their assessment.
Evidence supports that smoking is associated or causal with a wide range of cancers, but there is inconsistent data to prove its connection to BBD and/or BC. To further understand smoking and its relation to BBD and/or BC, breast tissue repositories, such as the Komen Tissue Bank (KTB) (established in 2007 at the Indiana University and is the only resource of normal, healthy breast tissue that is representative of the US population) can be utilized to evaluate modifiable behaviors, such as smoking. The aim of our study was to evaluate demographic, personal health, and modifying behaviors in females diagnosed with BBD versus those who have never had the need for a clinically indicated biopsy.
Methods: Data from 5,118 individuals who donated to the KTB (2007–2023) were compiled into an analytic dataset. Men (n=37) and those with a baseline history of BC (n=206) were excluded. The final study population consisted of 4,875 women, those with a

self-reported history of BBD (n = 807) versus those without BBD (n = 4,061). Characteristics including demographics, personal health, reproductive histories, and modifiable behaviors, were analyzed and compared using chi-square tests, and p-values of <0.05 were considered to be statistically significantly different between the groups. The BC risk assessment scores (Tyrer-Cuzick and Gail Scores) were calculated for 3,447 participants (aged 35 to 85) between those with and without BBD, recognizing the women with BBD would likely be at higher risk.
Results: Donors with BBD had increased frequencies of family histories (FH) of BC, being menopausal/older age, having prior hormone (HRT) use, and having mammograms (MMG) performed ($p<0.0001$). Modifiable risk factors such as smoking were more common in individuals with BBD ($p<0.0001$). However, alcohol and body mass index (BMI) did not differ between groups. Tyrer-Cuzick Scores in BBD patients had a 10% higher frequency of being intermediate risk for BC compared to those without BBD. Gail Scores in BBD patients had a 50% higher frequency of being intermediate risk for BC ($p<0.0001$).
Conclusions: In the KTB donors, there is an increased frequency of smoking in patients with BBD. Smoking is not a variable used in standard breast cancer risk calculators and data are inconsistent regarding smoking and BC. However, smoking is known to decrease circulating estrogen and result in women becoming menopausal two years earlier than their non-smoking counterparts. Dysregulation of estrogen levels and menopausal status are two critical variables known to increase one's risk of BC. Clinicians should be aware of this potential risk factor and subsequently better educate patients who smoke about their increased risk of BBD and possible BC. Further research needs to be done to elucidate etiology, pathophysiology and management of BBD.

P1-03-08: Clinico-pathological Characteristics of Breast Invasive Lobular Carcinoma in non-CDH1 genetic predisposition. Experience from the Institut Curie.

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Background: Invasive lobular carcinomas (ILC) represent 15% of breast cancers and are characterized by inactivation of E-cadherin (encoded by the CDH1 gene). Hereditary ILCs are rare and may be linked to germline mutations in CDH1, or to other genes (including BRCA2 and PALB2). The clinical and histopathological features of hereditary ILC unrelated to CDH1 mutation remain poorly described in the literature to date.
Materials and Methods: A retrospective series of ILC diagnosed in a context of constitutional genetic predisposition unrelated to CDH1 was retrospectively identified within Institut Curie's department of Genetics database. The selection criteria were patients with ILC and a pathogenic or likely pathogenic germline mutation in genes other than CDH1 over the period 2020 to 2023. The following data were collected from the electronic patient record:

mutated gene associated with hereditary breast cancer predisposition, patient age at ILC diagnosis, bilaterality, occurrence of other cancers, histological type, grade, hormone receptor (HR), and HER2 status.
Results: Seventeen patients with BRCA1 mutations, 80 with BRCA2 mutations and 12 with PALB2 mutations were identified. ILC was the first cancer diagnosed in almost all patients (13/14 [93%] for BRCA1, 55/59 [93.2%] for BRCA2 and 8/10 [80%] for PALB2). Patients were index cases in 75% (9/12) for BRCA1, 63.6% (35/55) for BRCA2 and 57.1% (4/7) for PALB2. The mean age at diagnosis was 42.1, 45.9 and 49.3 years, respectively for patients with BRCA1, BRCA2 and PALB2 mutation. The occurrence of bilateral disease at diagnosis was not common (0/9 [0%] for BRCA1, 6/57 [10.5%] for BRCA2, and 1/8 [12.5%] for PALB2). BRCA2- and PALB2-related cases exhibited mainly grade 1-2 (46/62 [74.1%] for BRCA2, and 8/10 [80%] for PALB2), and HR-positive ILCs (55/59 [93.2%] for BRCA2, and 8/8 [100%] for PALB2). In BRCA1-mutated patients, ILCs were enriched in grade 3 (5/13 [38.4%]) and HR-negative phenotype (4/12 [33.3%]). The prevalence of HER2-positive status was found to be slightly higher among patients with BRCA1 and PALB2 mutations (1/10 [10%] and 1/9 [11.1%], respectively) in comparison to those with a BRCA2 mutation (3/51 [5.8%]).
Conclusion: ILCs diagnosed in the context of a germline BRCA1, BRCA2 or PALB2 mutation occured at a younger age than sporadic ILCs. Hereditary BRCA2- and PALB2-related ILCs exhibit histo-phenotypical features comparable to sporadic tumors (predominantly grade 1-2, HR-negative, HER2-negative). Interestingly, ILCs associated with germline BRCA1 mutations appear to have a distinct phenotype in our series (enriched for grade 3 and HR-negative status), although this gene has not yet been identified as a predisposing factor for ILC.

P1-03-09: Predicted Probability of Germline Genetic Testing and Counseling of Breast Cancer Patients in Eastern North Carolina

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Background: Germline genetic testing is recommended for all patients diagnosed with breast cancer under 65 years old and for other select patients. Referrals for these patients to undergo genetic testing are often placed but are not always finished for various reasons. Following the Covid-19 pandemic, the travel barrier was mitigated by offering genetic counseling online to patients. Information on who remained at risk was needed to determine the patients most likely to have incomplete genetic counseling.
Methods: Records of all the patients referred for a genetics consultation at the ECU Health Medical Center were tracked and recorded from 2020-2023. Information from these patients was identified and stored in a REDCap database. The information included demographic information provided in the patient's chart including their race, marital status, employment status, and religion. Visit information including the care at the time of genetics referral, and cancer that each of the patients or their family members had as well as

whether they proceeded with germline or somatic testing. Logistic regression was used to estimate the effects of race, type of insurance, employment, marital status, religion, and timing of genetics referral relative to cancer treatment on the probability of starting germline genetic testing and post-test counseling (PTC). Predictive probabilities were derived through logistical regression using SPSS software deriving odds ratios (OR) and 95% confidence intervals (CI) while controlling for the other measured variables.
Results: Out of the 618 patients referred for genetic counseling, 312 were referred for a personal or family history of breast cancer. Of these patients, 114 (36.5%) were white, 187 (59.9%) were black, 259 completed genetic counseling with testing, and 122 were positive for a germline variant with 23 (18.9%) being pathogenic. Of patients referred to genetic risk assessment for breast cancer, those referred for personal history before undergoing treatment were more likely to complete testing than those referred for family history (OR: 3.52; 95% CI 1.42-8.76). Among patients who underwent genetic testing, those with private insurance were more likely to test positive for a germline variant than those with Medicare (OR:2.85; 95% CI 1.39-5.85) or Medicaid (OR: 2.75; 95% CI 1.22-6.25). These patients who completed genetic testing and were referred for screening were also more likely to undergo PTC (OR: 2.05; 95% CI 1.01-4.17) than those referred before treatment. Additionally, those unemployed were more likely to have follow-up after the genetic testing than those with current employment (OR: 9.70; 95% CI 1.83-51.29). Of the patients who were positive for a variant, black patients were more likely to test positive for a variant of undetermined significance (VUS) than white patients (OR: 4.77; 95% CI 1.44-15.87). Those referred for family history were more likely than those referred after treatment to undergo post-test counseling (OR: 3.08; 95% CI 1.00-9.43).
Conclusion: Patients in Eastern North Carolina referred for genetic risk assessment for a personal or family history of breast cancer, were likely to start genetic testing if they were referred before treatment began. Of those who were positive for a variant, Black patients were more likely to test positive for a VUS. Of those who completed genetic testing, having private insurance increased their likelihood of testing positive for a variant. Patients who started genetic testing who were referred for screening were more likely to get post-test counseling than those referred before treatment and those who were not employed were more likely to get follow-up. Additionally, those referred for screening were more likely to get PTC than those referred after treatment.

P1-03-10: Prevalence of BRCA mutations in patients with HER2-negative breast cancer and high-grade serous ovary in the Dominican Republic.

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Introduction: Breast cancer (BC) worldwide is the most common cancer in women; in the Dominican Republic, it is the second in frequency (3,244) and mortality (1,457) (1). Hereditary breast cancer is 5-10%, the BRCA mutation (mBRCA) is the most frequently identified (45-68%) (2). mBRCA is more frequent in African and Latino populations (3) (4),

and in the Dominican Republic, a multiracial country (5), they have not been studied. It is important to study them due to their prognostic and predictive value of innovative, effective and safe treatments; In healthy carriers, prevention would contribute to lower mortality.
Objective: Determine the prevalence of mBRCA in patients with (BC)-HER2 negative and high-grade serous ovarian cancer (OC) in the Dominican Republic, in the years 2020 - 2022 and mBRCA 1/2, in patients with BC- HER2 negative, in early and metastatic stage, and high-grade serous OC in first and second line (1L and 2L).
Method: It is a non-experimental, retrospective, cross-sectional, multicenter investigation in patients with BC- HER2 negative, high-grade serous OC, who underwent BRCA 1/2 testing, in 15 centers in the Dominican Republic, data obtained from the program "AstraZeneca BRCA 1/2 Testing", for the Dominican Republic.
The Medgenome laboratory received 881 tests, 82.5% with BC and 17.5% with OC, to determine sequence variants by NGS on the Illumina platform and analyze large deletions or duplications by MLPA, in the BRCA1/2 genes. The sample, composed of 856 valid tests to be processed, 25 invalid tests (2.9%) were excluded from the prevalence analysis. In the clinic, patients with negative BC-HER2 were stratified into two groups: hormone receptor positive BC (HR+) and triple negative breast cancer (TNBC), early stage or metastatic.
Inclusion criteria: TNBC IIA- IIIB; BCHR+ 1L neoadjuvant or adjuvant chemotherapy, under 45 years of age or family history of 1st or 2nd degree cancer; patients with advanced high-grade serous or endometroid OC, stage III or IV.
Results: 720 BC-HER2 negative tests were analyzed, 13.2% were mBRCA, 60% mBRCA1 and 40% mBRCA2. 52% were BCHR+, 11% mBRCA, 4% VUS and the rest non-pathogenic. 6% was mBRCA1 and 5% was mBRCA2. TNBC 48%, with 16% mBRCA, mBRCA1 was observed in 10% and 6% mBRCA2 and 0.3% mBRCA1/m BRCA2. VUS at 5%. In BCRH+ 52.5% were staged, 67.7% in early stage (EE) and 32.3% in metastatic (ME), PV 9% and 12% respectively. mBRCA1 5% in EE and 4% mBRCA2; in ME 6% mBRCA1/2; VUS in 3% EE and 4% ME. TNBC was staged in 47.5%, in EE 69.3% and ME 30.7%, VP mBRCA in 15% and 21% respectively. In EE 7% mBRCA1 and 8% mBRCA2; in ME 16% in BRCA1 and 5% mBRCA2. 5% VUS in EE and 3% in ME. 77% of BCRH+ were between 31 and 60 years old, with 102 patients (29%) predominating in the group between 41 and 50 years old. In TNBC aged 31 to 40 years the mBRCA1 VP was 25%.
136 OC tests were analyzed, 41.2% germline (G) and 58.8% somatic (S). VP 16.2%, VPG 18% and VPS 15%; mBRCA1 in 12.5% VPG and VPS; mBRCA2 5% in VPG and 2.5% VPS; VUSS 5% and VUSG 1.7%. In (1LG) 80% and 86% (1LS); in (2LG) 20% and 16% (2LS); 20% VP mBRCA in (1LG) and 14% (1LS). VP mBRCA in 9% in (2LG) there was no VP in (2LS). mBRCA was observed over 41 to 50 years of age, but 67% of patients were 51 to over 60 years of age.
Conclusion
The Prevalence in the Dominican Republic of BRCA mutations in patients with BC-HER2 negative is 13.2% and in OC is 16.2%. BRCA1 was the most frequent mutation, TNBC is the molecular subtype that most frequently presented the mutation; the BRCA mutation was observed more frequently in the metastatic stage. In OC, IPV was more frequent in (1L) and the most affected age group was 41 to 50 years, at a younger age for BC.

P1-03-11: Unique double BRCA2 mutations c.2254_2257del/c.5351dup in Patients with Breast Cancer from Jordan

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Background: Given the increased co-occurrence of pathogenic and likely pathogenic variants (P/LP) in hereditary cancer syndromes as a result of multigene panel genetic testing, this study focuses on the prevalence and characteristics of double mutations in patients with breast cancer.
Methods: We reviewed all patients undergoing genetic testing for breast cancer from Jan 2016- Jun 2024. Data on patient demographics, cancer characteristics, and genetic testing results were compiled and analyzed. The statistical tests used were Linear Model ANOVA and Pearson's Chi-squared test. STATA software was used.
Results: A total of 5896 breast cancer patients underwent genetic testing. Among these, 36 (0.61%) patients had two different P/LP variants. Of these patients, 35 were female, with a median age at diagnosis of 40 years (range: 20-76 years). Four patients had two primary breast cancers, one had ovarian cancer, and one had sarcoma. At diagnosis, 10 patients had metastatic disease, three had triple-negative breast cancer, and histopathology revealed invasive ductal carcinoma in 33 patients.
Among these 36 patients, 23 had the unique double BRCA2 mutation c.2254_2257del/c.5351dup, identified exclusively in breast cancer patients from the Jordanian population. This mutation was previously reported by our institution as a potential founder mutation and has not been identified in other populations. Conducted at the King Hussein Cancer Center, the median age at diagnosis was 42.1 years (range: 20-76). Family history of cancer was positive in 95.7% of the cases. Notably, cascade testing was performed on 10 family members of the 23 patients with the double mutation, and all were found to have the same mutation, suggesting it is most likely a cis mutation, indicating that both mutations are on the same allele.
The other 13 patients had various combinations of P/LP: CHEK2 and PMS2; EPCAM and MSH2; BRIP1 and MUTYH; BRCA2 and CHEK2; BRCA2 and RAD51D; ATM and BRCA2; BRCA2 and PALB2; BRCA2 and PTCH1; ATM and BRCA2; BRCA1 and PTEN; ATM and RAD50; ATM and MUTYH. One patient had three P/LP variants: CHEK2, PALB2, and RAD51D.
No significant differences were found between the unique double BRCA2 mutation c.2254_2257del/c.5351dup group and the group of patients with various combinations of P/LP in terms of age at diagnosis (p=0.981), sex (p=0.446), TNBC status (p=0.174), presence of other cancers (p=0.689), histopathology (p=0.174), and family history of cancer (p=0.674).
Conclusion: The BRCA2 double mutation c.2254_2257del/c.5351dup was identified exclusively in the Jordanian population. Future research should focus on the implications of these mutations on treatment outcomes and long-term prognosis.

P1-03-12: Reprogramming SREBP1-dependent metabolism and inflammation in high-risk breast to prevent cancer: the example of licochalcone A

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Breast cancer (BC) risk reducing drugs have minimal impact on cancer incidence due to their adverse effects and low acceptance by risk-eligible women. Further, they are ineffective against hormone receptor negative (HR-) BC. Novel non-endocrine agents with reduced toxicity and efficacy not limited to HR+ BC are needed. Sterol regulatory element binding protein 1 (SREBP1) has been shown to have a key role in tumorigenesis in the breast through reprogramming immune and microenvironmental factors, epithelial to mesenchymal transition, cell cycle, and programmed cell death. It has a prognostic value in breast cancer patients and is an independent factor of 5-year overall and disease specific survival. Targeting this metabolic vulnerability presents opportunities to reverse tumorigenesis in several tissues such as breast and endometrium. We recently presented data which revealed that in the microstructures from the contralateral unaffected breast (CUB) of postmenopausal women with unilateral BC, LicA significantly upregulated antioxidant pathways and downregulated NF-kB dependent inflammatory pathways as well as SREBP1-dependent lipogenesis. We also showed that metabolic flux in the CUBs treated with LicA supports these effects. In addition, LicA exhibited suppression of proliferation in pre-malignant and malignant HR+ and HR- breast cell lines and in their xenografts in mice. Now we present confirmatory evidence that the mechanism through which LicA prevents HR+ and HR- BC is by reducing SREBP1-dependent metabolism and inflammation. We also show the promising PK profiles of novel oral formulations we have developed.
We performed NanoString metabolism panel analysis in MDA-MB-231 (HR-) and MCF-7 (HR+) breast cancer cells treated with LicA (5 μM) for 24 h. We also performed western blots to confirm the significantly modulated metabolic pathways. In addition, we evaluated spatiotemporal changes in the concentrations of cholesterol in the inner leaflet of plasma membrane of these cells. Four proprietary novel formulations of LicA were developed and administered orally to female BALB/c mice and Sprague-Dawley rats followed by LC-MS/MS analysis of the plasma and mammary tissue. Using SAAM II we modeled their pharmacokinetic (PK) profiles.
We observed significant (adj $P < 0.05$) upregulation of antioxidant (up to 9-fold), and downregulation (up to 6-fold) of NF-kB-dependent inflammatory pathways such as prostaglandin E2 synthesis. These results were consistent with our previous observations in animal models and in the high-risk women's CUBs. We also observed significant downregulation of SREBP1 dependent lipogenesis genes such as ACAT2, FASN, and SCD in these cells, which is in line with our data in the CUBs. Our western blot analysis showed a significant suppression of SREBP1 (4-fold) particularly in HR- cells, along with the reduced phosphorylation of PI3K (5-fold) and AKT (6-fold, on Ser 473). These results were confirmed by the depletion of cholesterol in the inner leaflet of plasma membrane in HR- (8-fold) and HR+ (4-fold) cells. Consistent with our data from the CUBs and in vivo models, we

observed 2- to 3-fold reduction in proliferative markers such as MKI67, BCL2, and RRM2 in HR+ and HR- cells after 24 h exposure to LicA. These results provided additional evidence that LicA exerts its antiproliferative effects by suppressing SREBP1-dependent lipogenesis and inflammation. Two of the novel LicA oral formulations showed promising PK, sufficient for efficacy with less frequent dosing which make them suitable for BC prevention.
Our data suggests that suppression of SREBP1 dependent metabolism and inflammation can prevent BC and presents evidence that LicA is an excellent candidate for HR+ and HR- BC prevention through this mechanism. We will test LicA's oral formulation in intraductal models of precancer lesions in immunocompetent animals to further establish its preventive efficacy.

P1-03-14: Lympho-Vascularized Breast-Skin Platform for Modeling Lymphovascular Space Invasion in Advanced Breast Cancer

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Inflammatory Breast Cancer (IBC) is an aggressive form of breast cancer, accounting for 10% of breast cancer deaths despite its relatively rare occurrence. A significant characteristic of IBC is the invasion of cancer cells into the lymphatic and blood vessels near the skin, leading to the formation of tumor emboli and lymphovascular space invasion (LVSI). Eventually, LVSI progresses into the overlying skin through the breast stroma and causes dermal lymphatic invasion (DLI). The mechanisms underlying LVSI and DLI remain poorly understood. Current experimental platforms, including 2D cell cultures, animal models, and more recent 3D in vitro models, have limitations in recapitulating the complexity of the tissue microenvironment, biomechanical cues, and cell behavior intrinsic to IBC. These limitations make it challenging to elucidate the mechanism of LVSI and develop effective therapeutics. While most 3D tumor models typically incorporate the blood vasculature, they often overlook the crucial role of the lymphatics, despite compelling evidence that for certain breast cancer subtypes, lymph vessels serve as the predominant pathways for tumor metastasis. Existing experimental systems also do not incorporate surrounding metastatic sites. Therefore, multi-tissue on-a-chip platforms (MTCP) that possess integrated blood and lymphatics would have tremendous value for elucidating cell migratory patterns and underlying mechanisms of the immune response and cancer metastasis. In this study, we created the first breast-skin platform with functional blood and lymphatic vessels that are connected between tissue layers of a breast tumor and skin. The platform's fabrication involved polymerizing type I collagen for the matrix around parallel 22-gauge needles (0.718mm). To mimic breast tumor tissue, 7 mg/mL collagen solution seeded with 1 million MDA-IBC3 cells/mL were injected into the platform and underwent polymerization. The skin platform was created using a layer of 4 mg/mL collagen solution seeded with 0.1 million cells/mL of primary normal human dermal fibroblasts (NHDFs) at the top of the breast tumor layer and then polymerized. Next, needles were extracted to create hollow vessels. Vessels were perfused with 1×107 cells/mL mKate-labeled tumor microvasculature endothelial (TIME) cells for blood vessels and 1×107 cells/mL RFP

Expressing Human Dermal Lymphatic Microvascular Endothelial Cells (HDLMVECs) for lymphatics. All Breast-Skin platforms were then placed in 6 well plates with 1.5-2 mL of complete endothelial cell media in each well surrounding the platform and put back on the rocker for 3 days to allow formation of a confluent vessel. Utilizing this platform, we have shown that MDA-IBC3 breast cancer cells invaded the skin through the stroma. We observed angiogenic sprouting occurs when TIME cells begin to bud from the peripheries of the endothelial vessel which advances towards MDA-IBC3 cells. Also, we have shown MDA-IBC3 emboli surrounded by these vessels, attempting to intravasate. The observation of vascular endothelium sprouting, and adjacent tumor emboli is solely facilitated by interactions between tumor and endothelial cells, representing the in vivo behavior of IBC. This novel platform allows for the high-throughput and controllable study of LVSI, DLI, and skin metastasis. It serves as a valuable tool for dissecting the metastatic process, generating functional cell selection and signaling targets for further research. Multiple functional endpoints, such as collagen alignment, immunofluorescent staining, live cell imaging, and cytokine quantification, can be assessed simultaneously. Our goal is to use the Breast-Skin platform to further our understanding of the mechanisms that drive skin metastasis in breast cancer and ultimately identify effective therapeutic targets.

P1-03-15: Characterization of metabolic and energetic risk factors and preventative breast surgical decisions in a cross-sectional survey cohort of hereditary cancer pathogenic variant carriers

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Background: About 10% of all breast cancers (BC) result from an inherited pathogenic variant (PV). Energetics and Lifestyle in Inherited Syndromes (ELLIES) Project is a survey-based study examining diet, exercise and metabolic risk factors in individuals with hereditary cancer pathogenic variants (HCPVs). This study describes metabolic risk factors and lifestyle habits among the first 750 participants in this cohort. We are interested in exploring how metabolic and energetic risk factors such as BMI, diet, and physical activity (PA) levels influence breast surgical decisions in both preventative and therapeutic settings.

Methods: The online self-administered survey, developed in REDCap, includes questions on demographics, personal health, cancer risk factors (menopausal status, reproductive history, hormone use, breast density, family history of cancer), and metabolic risk factors (weight, height, hypertension, diabetes, and hyperlipidemia). Physical activity (PA) levels were assessed using a modified International Physical Activity Questionnaire (IPAQ), and dietary intake was evaluated with the 14-item Mediterranean Diet Adherence Screener (MEDAS). Participants aged ≥18 years with a HCPV provided electronic consent and received a $10 e-gift card upon survey completion. The survey, disseminated through social media and the advocacy group Facing Our Risk of Cancer Empowered (FORCE), also includes a Spanish version. The survey remains open with the goal of reaching 1,000

responses. Descriptive statistics were used to analyze participant demographics, cancer risk factors, and metabolic risk factors.

Results: From August 2019 to December 2023, 750 participants completed the survey; mean age: 45.1 years (SD=12.6) and 96.1% were female. Self-reported race/ethnicity was 91.5% White, 3.9% Black/African American, 2.3% Asian, 2.8% Hispanic, 17.1% Ashkenazi Jewish, and 2.3% Other. Among female participants (n=721), 66.6% are previvors and 33% are survivors. In previvors, 47% have a BRCA1 or BRCA2 PV and 33.5% of previvors have had risk reducing mastectomy (RRM); mean age at RRM was 42 years (SD=11.0). Rates of RRM in previvors by PV were 42.7% BRCA1, 38.3% BRCA2, 38.9 PALB2, 28.6% ATM, 14.3% CHEK2. Family history of BC was higher in previvors with RRM (87%) compared to those without (80%). Mean BMI 26.7 (SD=6.6) for previvors with RRM and 27.3 (SD=6.7) for those without. Previvors without RRM reported moderate intensity PA (43.9%) compared to those with RRM (38.5%). Over half of previvors (62.3%) reported they were currently trying to lose weight; 183 (38%) reported ≥5 meaningful weight loss attempts in their lifetime. Perceptions of weight, diet, and PA as important cancer risk factors were similarly distributed among previvors with and without RRM. In survivors, 137 (57%) have a BRCA1 or BRCA2 PV and 91 (37.8%) of survivors have had RRM; mean age at cancer diagnosis was 43 years (SD=10.9); mean age at RRM was 45.8 years (SD=10.2). Family history of BC was reported in 64.8% of survivors with RRM compared to 61.7% without. Mean BMI was 26.4 for survivors with RRM (SD=6.0) and 25.6 (SD=5.4) for those without. Nearly half of survivors without RRM (49%) engaged in moderate intensity PA compared to survivors with RRM (38.5%). Of survivors, 55.6% reported trying to lose weight and 32% reported ≥5 meaningful weight loss attempts in their lifetime.

Conclusion: Individuals with a HCPV may elect to undergo bilateral mastectomy in the preventative setting or at the time of a BC diagnosis to decrease their risk of a future BC. A better understanding of how metabolic risk factors, diet and PA impacts BC risk in PV carriers is needed. Based on this initial descriptive analysis, examination with longitudinal follow up of the cohort will further explore how these metabolic and energetic factors impact breast surgical decisions.

P1-03-16: Multimodal analyses of clinical, radiology, pathology and genomic information for enhanced prediction of response to neoadjuvant therapy in breast cancer

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Background: Neoadjuvant chemotherapy (NACT) is the standard of care for early-stage breast cancer patients (pts) with high-risk clinical features and pathologic complete

response (pCR) is considered the best predictor of favorable long-term outcomes. We developed machine learning models to predict pCR from a series data modalities available at the time of diagnosis. We compared the predictive value of unimodal radiology, pathology, genomic, and clinical features, and their multimodal integration to improve pCR prediction.

Methods: We analyzed a multimodal cohort of 1,192 early-stage breast cancer pts treated with NACT at Memorial Sloan Kettering (MSK) between 2014 and 2021. Clinicopathologic features including hormone receptor subtype, demographic information (self-reported race, age at diagnosis), grade and stage were collected and curated for all pts, and combined to build a logistic regression prediction model. Digital pathology whole-slide images (WSIs) from pre-NACT tumor biopsies were collected for 1,101 patients. We used a pre-trained transformer model and attention-based multiple instance learning (MIL) to predict pCR from H&E WSIs. Pre-NACT magnetic resonance imaging (MRI) exams, which included T2-weighted images, T1-weighted precontrast images and T1-weighted post-contrast images, were collected for 838 pts, and were used to build a deep learning (DL) model to predict pCR. Tumor biopsies and matched blood specimens from 315 pts underwent targeted DNA sequencing using the MSK-IMPACT platform, which identified mutations, copy number changes, and structural rearrangements in a selected panel of up to 505 cancer genes. These genomic features were combined using an elastic net regularized logistic regression model for pCR prediction. Multimodal integration was performed using existing methods, and results were compared for early and late fusion strategies. Predictive performance was measured by computing the area under the receiver-operator curve (AUC) metric.

Results: Individual data modalities exhibited varying levels of predictive performance for different receptor subtypes. For example, clinical and pathology models performed better for HR+ subtypes, while the genomic prediction model outperformed the rest in the triple negative breast cancer (TNBC) set. Automated DL models using MRI inputs achieved moderate predictive performance on the HR+/HER2-, HR+/HER2+, and HR-/HER2+ subtypes. Subtype-specific models tended to outperform subtype-agnostic models trained in the larger, pan-subtype cohort. End-to-end DL pathology and radiology models that can be deployed and run automatically without human curation to predict pCR directly from images had comparable performance to clinical models using variables curated by expert pathologists and radiologists. Multimodal predictors combining the genomic data with the other three data modalities readily available at initial presentation exhibited the best overall predictive performance, but the gain in predictive performance was small and may not justify the increased requirements in terms of data acquisition and model complexity for certain receptor subtypes.

Conclusions: Multimodal data can be incorporated into machine learning models for improved prediction of pCR to NACT in breast cancer. Additional work is needed to validate the clinical utility of these types of multimodal approaches, as well as to determine the most informative data modalities for each hormone receptor subtype and the subset of patients that are most likely to benefit from the use of these models. Automated workflows using DL pre-trained models may provide valuable clinical decision support, guiding escalation and

de-escalation therapeutic and monitoring strategies to improve outcomes for pts with high-risk early breast cancer.

P1-03-17: Disseminated tumor cell (DTC) quantities are associated with Ki67 and pCR rate in breast cancer

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Background: The prognostic relevance of disseminated tumor cells (DTCs) in the bone marrow (BM) of patients with primary breast cancer was reported manifold and therapeutic strategies are still controversially discussed. Despite successful treatment of the primary tumor, recurrence occurs in about 30% of breast cancer patients. One reason might be hematogenous spread as an early event in the disease, when single tumor cells split of the primary tumor and migrate into the lymphatic and blood system or into the bone marrow, where they are believed to become dormant and hence persistent against chemo-therapy. Little is known concerning the association of DTC quantities and the clinic-pathological data, including Ki67proliferation index in primary tumors and affected lymph nodes, though.
Methods: Between January 2019 and December 2023, we collected 430 BM aspirates from the anterior iliac crest of patients with primary (n=399) and recurrent (n=32) breast cancer during oncologic surgery. Patients either had neoadjuvant chemotherapy (NACT, n=153), neoadjuvant endocrine (n=90) or no neoadjuvant treatment (n=184).
After density gradient centrifugation, cell suspensions were transferred onto glass slides and subjected to immunocytochemical staining against pan-cytokeratin. DTCs were visualized using alkaline phosphatase and short counterstaining with hematoxylin. DTCs were semi-automatically detected and enumerated using a microscope based scanning system with a rare events algorithm to identify DTC candidates according to color, shape, intensity and size. We correlated DTC quantities to clinico-pathological parameters using Pearson correlation and Chi-Square tests.
Results: Per patient about 4 million BM cells were analyzed. The DTC-positivity rate was 40%. Patients were divided into subgroups based on the immunohistochemically determined subtype of the tumor: of the patients with luminal A tumors (n=210) 39.5% were DTC positive (mean=11), with luminal B/HER2- tumors (n=31) 41% (DTC mean=14), of Luminal B/HER2+ (n=28) 53.5% (DTC mean=12), of TNBC (n=70) 41.4% (DTC mean=15.2), and of HER2-enriched (n=33) 24.2% (DTC mean= 15) were positive.
The DTC mean was elevated in clinical nodal positive patients (n=92; DTC mean: 15.6) compared to clinical nodal negative patients (n=326; DTC mean:11.7; p=0.122) except for cases with luminal B/HER2+ tumors.
We found a positive correlation between the DTC count and the proliferation index Ki67 of the core needle biopsy (CNB; p=0.008; r=0.279). Additionally, we separated the patients into different groups with Ki67 from 1-9%, 10-29% and >30% as determined in the CNB (DTC mean: 11 vs. 13.3 vs.14), the affected lymph nodes (LN; DTC mean: 12.6 vs. 13.1 vs.

17.1) and the resected tumor tissue (DTC mean: 11.5 vs.11.3 vs.17.6). Among DTC-positive patients, the mean DTC number was increased gradually with increasing Ki67 in all 3 specimens (CNB, LN and tumor tissue).
Comparing the different neoadjuvant strategies, we found an increased DTC mean in all molecular subtypes, except Luminal B/HER2+, when the patients did not receive NACT (n=184; DTC mean: 14.5) compared to patients undergoing NACT (n=153; DTC mean: 11.9; p=0.134). Ki67 index was elevated in the lymph node metastases compared to the tumor biopsy and the resected tissue: LN_Ki67>CNB_Ki67>tissue_Ki67 among all molecular subtypes in patients receiving NACT.
Looking at the pCR rate of DTC-positive patients (46%; 25 of 54), it was lower compared to DTC-negative patients (57%; 54 of 94) receiving NACT underlining the higher risk profile for DTC-positive patients.
Conclusion: Our data suggest that DTC quantities are equally high among the different intrinsic subtypes. DTC-positive patients are less likely to achieve a pCR. Additionally, we found a positive correlation between the DTC quantity and the proliferation index Ki67.

P1-03-18: Correlates of Breast Adipose Inflammation after Primary Breast Cancer Diagnosis in the Pathways Study

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Background: Excess adiposity is associated with higher rates of breast cancer recurrence and increased mortality. Insights into the mechanisms by which adiposity influences these outcomes may lead to improved disease management. A histologic marker of white adipose inflammation, crown-like structures of the breast (CLS-B), is associated with excess adiposity, adipocyte hypertrophy, and increased in-breast aromatase levels. CLS-B are comprised of dead or dying adipocytes surrounded by macrophages, and may be measured in slides obtained from breast surgical specimens. We are conducting a prospective cohort study of these inflammation-related markers and breast cancer outcomes. We describe here select correlates of CLS-B and adipocyte diameter in 630 women diagnosed with breast cancer in the Pathways Study.
Methods: The Pathways Study is a prospective cohort study with 4,504 women who were diagnosed with invasive breast cancer from late 2005 through May 2013 in the Kaiser Permanente Northern California healthcare system. From women who underwent mastectomy, formalin-fixed paraffin-embedded tissue blocks from definitive surgery enriched in white adipose tissue were obtained, sectioned and stained by hematoxylin & eosin and CD68 immunohistochemistry. Slides were then examined for adipocyte diameter and CLS-B. Blood specimens collected around the time of enrollment into the cohort were also assayed for high-sensitivity C-reactive protein (hsCRP). Data related to cancer diagnosis, demographic characteristics, anthropometry, and other variables were obtained

from baseline in-person visits, surveys, and electronic health records.
Results: To date, 630 women have complete data on adipocyte diameter and presence of CLS-B. Most women were diagnosed with Stage I-III disease, 79% had estrogen-receptor positive-tumors, and 19% had HER2-positive tumors. Mean and median adipocyte diameters were 103.6 and 103.4 μ, respectively, with an inter-quartile range of 94.4 to 113.9 μ. CLS-B were observed in 267 (42.4%) of the 630 women, and among these women, median CLS-B density was 0.3 per cm2, with an interquartile range of 0.16 to 0.77 per cm2. In univariate associations, there was some suggestion that adipocyte diameter varies by self-reported race and ethnicity ($p=0.056$), with largest mean values for Blacks (n=50, adipocyte diameter 110.4 μ) and smaller for Whites, Asians, and Latinas (n=390 and 102.8 μ; n=97 and 103.8 μ; and n=76 and 103.7 μ, respectively). These differences aligned with differences in body mass index (BMI) by race and ethnicity, and were strongly associated with BMI ($p<0.0001$), with adipocyte diameter across increasing BMI categories (kg/m2) of 95.8 μ for $18.5 \leq BMI < 25$, 106.2 μ for $25 \leq BMI < 30$; 109.0 μ for $30 \leq BMI < 35$, and 111.7 μ for $35 \leq BMI$. Similar associations were observed for waist circumference and waist-to-hip ratio, as well as hsCRP (all $p<0.0001$). Presence of CLS-B differed somewhat across race and ethnicity ($p=0.097$), with 58.0% of Blacks, 39.2% of Whites, 49.5% of Asians, and 38.2% of Latinas having CLS-B present. Presence of CLS-B was also strongly associated with BMI, waist circumference, waist-to-hip ratio, and hsCRP. For example, across the increasing BMI categories above, the proportions of women with CLS-B were 27.2%, 44.8%, 51.3%, and 63.6%., and across increasing hsCRP quartiles were 22.5%, 34.5%, 53.6%, and 56.9%.
Summary: These observations indicate that breast adipose tissue inflammation and adipocyte size correlate with anthropometric measures of adiposity and systemic inflammation, and vary by race and ethnicity. As we continue to obtain measurements of these variables, we will determine how they relate to more precise body composition compartments and whether they are also associated with recurrence, mortality, and other outcomes after breast cancer.

P1-03-19: Comprehensive Analysis of ADC Target Expression in Invasive Lobular Carcinoma

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Introduction: Antibody-drug conjugates (ADCs) targeting specific surface antigens have represented a notable advance in therapeutic efficacy in solid tumors. Herein, we analyzed RNA sequencing (RNA-seq) data from invasive lobular carcinoma (ILC) samples to identify novel cell surface protein candidates for designing new and highly specific ADCs, aiming to minimize side effects and improve patient outcomes.
Methods: RNA-seq data from the BostonGene internal breast cancer (BC) cohort (non-ILC n=518, ILC n=138), TCGA-BRCA (non-ILC n=815, ILC n=233) cohort, and normal tissue samples in the Adult GTEx dataset (n=7862) for 82 cell surface protein genes were assessed

together. BC samples were classified as histo-molecular ILC if they showed at least one of the following: ILC histology, CDH1 truncation, gene loss, Z-scores of < -1.5 for gene expression. Among the ILC samples, some were classified as classic (low-grade and Lum A or B; n=224) and non-classic ILC (high-grade, HER2-enriched, or Basal-like; n=28). We also classified all ILC samples as high- (HG-ILC, n=142) or low-grade (LG-ILC, n=229) based on molecular grading. Information on ADC targets and relevant clinical trials were extracted from ClinicalTrials.gov.
Results: Among the 82 cell surface protein genes assessed, 18 demonstrated high expression levels defined as ≥4 log2 transcripts per million (TPM) in at least one subtype and include LYPD3*, FOSL2, CD276*, CRIM1*, PRLR*, PVRL4*, TPBG*, VTCN1*, ERBB3**, ALCAM, CD46, ERBB2**, SLC39A6**, ST14*, CD74, TACSTD2*, GPNMB, and MUC1*. Genes marked with * showed low expression in normal tissues (<2 log2 TPM), while genes marked with ** showed low expression in normal tissue and are being investigated in ≥5 clinical trials.
Both non-ILC and ILC groups showed high expression of ERBB3**, ERBB2**, ALCAM, CD46, SLC39A6**, ST14*, CD74, TACSTD2*, GPNMB, and MUC1*.
Among the ILC samples, CRIM1*, PRLR*, and PVRL4* showed high expression in LG-ILC and classic ILC. LYPD3* was highly expressed only in non-classic ILC. FOSL2, CD276*, TPBG* and VTCN1* showed low expression in HG-ILC. However, TPBG* and VTCN1* were highly expression in LG-ILC and classical ILC.
Interestingly, the low expression of several targets in normal tissues (marked * and **) indicate their suitability as ADC targets for treating ILC while minimizing adverse effects.
Conclusion: We defined distinct ADC target expression landscapes in classic and non-classic ILC, further distinguished from non-ILC. While both classic and non-classic ILC show high expression of several ADC targets, they differed notably in TPBG, CRIM1, PRLR, and VTCN1 expression. The low expression of several analyzed genes in normal tissues revealed their potential as targets for highly specific ADCs with anticipated reduced adverse effects. Our findings underscore the need for clinical trial designs encompassing ADC target expression data across ILC subtypes to optimize efficacy and safety of ADC-based treatments, subsequently improving patient outcomes.

P1-03-20: Role of MCM2 and the licensing complex in predicting endocrine and CDK4/6 inhibitor resistance in ER+ breast cancer

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Background: Metastatic ER+/HER2- breast cancer (BC) is inevitably associated with resistance to endocrine therapy (ET) and CDK4/6 inhibitors (CDK4/6i). However, there remains a need for comprehensive understanding of resistance mechanisms and better predictive biomarkers for patient selection or new treatment strategies. We previously reported that in the NeoPalAna trial (NCT01723774), high expression of the origin licensing

complex (LC) was associated with resistance to CDK4/6i and a similar trend, though lessened, was observed for ET. The LC factors ORC1-6, CDC6, and CDT1 load the MCM complex (MCM2-7) onto the origin in G2/M, priming it for initiation of DNA replication in S phase. Within the LC, we identified MCM2 to be the gene most significantly associated with resistance. We hypothesize that high expression of MCM2/LC presents a new, highly effective predictor of resistance to ET and CDK4/6i in ER+ BC.
Methods: Publicly available primary (TCGA, PanCancer Atlas; METABRIC, Nature 2012 & Nat Commun 2016) and metastatic (The Metastatic Breast Cancer Project, Provisional 2021) BC datasets in cBioPortal were used to define copy number (CN) aberrations and their correlation to mRNA expression data for LC components. Clinical subtype for METABRIC regression analyses was refined using genomic data and the mRNA expression distribution of clinically defined ER, PR, and HER2 tumors (PMID 30867590, 34725325). K-means clustering was performed on MCM2-7 complex and entire LC gene sets for signal discovery. Number of k was determined using the elbow and silhouette analyses. Logrank p-values and hazard ratios were derived from Cox-proportional hazard models examining the association between mRNA expression by RSEM in METABRIC of LC components, individually or as gene sets, with breast cancer-specific survival (BCSS) or distant recurrence-free survival (DRFS) using R (v4.3.1) and calculated via log rank. Nominal p-values underwent Bonferroni correction as needed. For all analyses, datasets were filtered for clinical ER+/HER2- status.
Results: cBioPortal genomics revealed that MCM2 is amplified in 20% and <1% in metastatic versus primary BC and increases to 58% and 9-17%, respectively, when also considering CN gains. This trend was largely consistent for most other LC components as well. Higher MCM2 mRNA was associated with CN amplification and gains of the gene. Furthermore, high MCM2 transcript levels correlated with PR negativity (p=2.51e-05), Luminal B versus A status (p<2e-16) and high tumor grade (p<2e-14). MCM2 expression was also associated with worsened DRFS and BCSS, both in univariate (HR=1.51, p=7.23e-12; HR=1.49, p=2.17e-11) and multivariate regression analyses (HR=1.35, p=6.88e-05; HR=1.23, p=5.35e-03), after adjusting for PR, grade, age, and PAM50 intrinsic subtype. BCSS also decreased with high MCM2 mRNA regardless of type of endocrine therapy received (log rank p=2.32e-08). Low versus high MCM2-7 and LC clusters were associated with better DRFS (HR=0.52, p=7.89e-09; HR=0.56, p=3.63e-07) and BCSS (HR=0.57, p=1.14e-07; HR=0.57, p=3.91e-07). DRFS for MCM2-7 and LC clusters remained significant in same multivariate analysis (HR=0.68, p=4.13e-03; HR=0.74, p=2.89e-02).
Conclusions: Our analysis demonstrates that MCM2 and the LC gene set are independently poorly-prognostic for both DRFS and BCSS in a large, well-annotated BC dataset. This impact is significant and does not wane after multivariate analysis. Most LC components, including MCM2, are not routinely included in targeted next-generation sequencing panels, and their incorporation can help validate the predictive nature of these genes in real-world cohorts. Our results also provide a strong rationale for clinical development of a set of predictive biomarkers including these genes, particularly MCM2, in the setting of ER+/HER2- BC.

P1-03-21: Improving gene signatures for predicting the progression of ductal carcinoma in situ

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Introduction: Ductal carcinoma in situ (DCIS) is a non-invasive form of breast cancer characterized by abnormal cells confined within the milk ducts. While DCIS is not life-threatening, its clinical significance lies in its potential to progress to invasive ductal carcinoma (IDC). This progression is driven by molecular imbalances from genetic and epigenetic alterations, including increased expression of matrix metalloproteinases (MMPs). Our recent study demonstrated that MMP1 expression modestly predicts DCIS progression to IDC, independent of tumor subtype (AUC = 0.76). In this study, we aimed to identify additional informative genes that could enhance the predictive potential of MMP1 based on molecular data from DCIS clinical studies.
Methods: We analyzed molecular and clinical data from 380 DCIS specimens across three cohorts: the Sweden Cancerome Analysis Network - Breast (SCAN-B, n = 85), the Translational Breast Cancer Research Consortium (TBCRC, n = 216), and the Resource of Archival Breast Tissue (RAHBT, n = 79). Potential gene candidates were identified in TBCRC and RAHBT cohorts. First, DESeq2 v 1.40.2 was employed to select differentially expressed genes between cases with ipsilateral breast events (n = 121, considering both DCIS and IDC) and without reported events (n = 95) within five years in the TBCRC study. Additionally, we considered the genes encoding the 37 proteins analyzed by ion beam imaging by time of flight (MIBI-TOF) technology in the RAHBT cohort. Multiple score values based on the percentile expression of selected genes were evaluated in the SCAN-B cohort (n = 85). AUCs were computed using pROC version 1.1.8.
Results: We identified 89 genes that improved the predictive power (AUC > 0.8) when combined with MMP1 on the TRCRC and RAHBT studies. These genes were combined into four-gene signatures using the formula: (MMP1+X)/(Y+Z), generating 92,070 unique combinations. 3,821 of these signatures improved the AUC of MMP1 alone. To avoid redundancy in the information, we restricted combinations to genes uncorrelated with MMP1 (28 dividend genes and 34 divisor genes) and tested the predictive performance in an independent cohort (SCAN-B). We identified that the ratio between the percentiles of (MMP1+MMP27)/(CD36+IGFBP6), showed a strong predictive performance in the SCAN-B cohort (AUC = 0.91). Other genes that enhanced the predictive signature included transcriptional regulators (SP1, SF3B4, and CTNNBIP), metabolic enzymes (GNMT, TKT, and PGD), and mitochondrial proteins (NDUFB7, MRPL9, and FH).
Conclusions: The identified four-gene ratio signature significantly enhanced the prediction of DCIS progression to IDC across different study cohorts. We have designed and are developing an assay based on immunohistochemistry (IHC) to assess these proteins in FFPE tissues, enabling retrospective exploration of DCIS cohorts. Our current studies are focused on validating this signature in larger independent cohorts and exploring its utility in prospective clinical settings. This approach has the potential to refine diagnostic algorithms for DCIS patients, ultimately improving clinical decision-making and patient outcomes.

P1-03-22: Comprehensive characterization of the androgen receptor in male breast cancer

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Background: Male breast cancer (BC) accounts for less than 1% of new BC cases annually. Androgen receptor (AR), a member of steroid and nuclear receptor superfamily is emerging as an important factor in pathobiology of BC. While the estrogen receptor (ER) is well-studied in BC, the role of the AR is less understood, particularly in male patients. Here, we aimed to characterize the molecular and immunological features of AR gene expression in male BC.
Methods: 191 samples from male breast cancer patients were tested by NGS (592, NextSeq; WES, NovaSeq) and WTS (NovaSeq; Caris Life Sciences, Phoenix, AZ). MSI was tested by IHC and NGS. Tumor mutational burden (TMB) totaled somatic mutations per tumor (high>10 mt/MB). Immune cell fractions were calculated by deconvolution of WTS: Quantiseq. Tumors with AR-high(H) and AR-low(L) RNA expression were classified as above or below the 50th percentile, respectively. Real world overall survival (OS) and treatment-associated survival was obtained from insurance claims and calculated from tissue collection to last contact using Kaplan-Meier estimates. Statistical significance was determined by chi-square and Mann-Whitney U test with p-values adjusted for multiple comparisons (q<.05).
Results: AR-H male BC had lower frequency of TP53 mutations (20% AR-L vs 7% AR-H, p=0.02) compared to AR-L male BC tumors. AR-H had numerically higher frequency of PIK3CA (34.8% vs 27.2%) and CHEK2 (3.7% vs 1.1%), but lower frequency of BRCA2 (7.1% vs 13.6%) and PTEN (2.3% vs 6.9%) compared to AR-L, all p = 0.1-0.2. AR-H male BC had lower frequency of TMB-high (3.45% vs 12.22%) and PD-L1 positivity (5.08% vs 18.57%), all p<0.05. Analysis of inferred immune cells revealed that AR-H had higher infiltration of NK cells (3.58% vs 2.56%), dendritic cells (2.43% vs 1.98%), and B cells (5.93% vs 5.26%), all p<0.05. AR-H had higher T-cell inflamed score (19 vs -72) and MAPK activation score (-0.24 vs -1.6) but lower IFNg score (-0.38 vs -0.33), all p<0.05. AR-H had higher expression of immune checkpoint genes (CD274, FOXP3, HAVCR2, LAG3; FC: 1.3-1.5) and stem cell-related genes (CD34, CD44, POU5F1, KLF4, ALDH2; FC: 1.2-1.4) compared to AR-L male BC, all p<0.05. AR-H male BC had higher AR protein (IHC) expression (100% vs 86.8%, q<0.05) and numerically higher frequency of AR-fusion variant (3.2% vs 0%, p=0.08) compared to AR-L male BC. AR-H male BC had worse OS (mOS: 35.9 vs 92.4 month; HR 1.6, 95% CI 1.0-2.5, p = 0.043) compared to AR-L BC. When analyzed by TP53 mutation status, AR-H with TP53-wt had numerically better survival (mOS: 35.6 vs 25.7 months, HR 0.52, 95% CI 0.20-1.35, p=0.17) compared to TP53-mt. Similarly, AR-L with TP53-wt had numerically better survival (mOS: 48.3 vs 23.2 months, HR 0.58, 95% CI 0.22-1.33, p=0.18) compared to TP53-mt.
Conclusions: Our analysis suggests a strong association between AR expression and TP53

mutations, TMB-H, and PD-L1 positivity, immune cell infiltration, immune checkpoint and stem cell-related gene. Also, T cell inflamed and IFNy score were inversely related. Further exploration of specific alterations and immune-oncology markers associated with AR expression may help in clinical trial design for male patients with BC.

P1-03-23: Identification of Stage 1 Breast Cancer Biomarkers Using Extracellular Vesicle Proteomics and Machine Learning

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Abstract: Breast cancer is one of the most common cancers among women worldwide, and early detection is critical for successful treatment and improved survival rates. However, early-stage breast cancer presents significant diagnostic challenges due to the small size of tumors and the limited presence of tumor-specific biomarkers in the bloodstream. Traditional diagnostic methods often struggle to detect these early signs, making it imperative to develop more sensitive and specific approaches.
Circulating extracellular vesicles (EVs) have emerged as a promising solution for early breast cancer detection. EVs are stable proteomic substrates that allow for the deep enrichment of biologically relevant and informative biomarkers from plasma. This enables comprehensive analysis of multiple tumor-associated and host response proteins. Cancer and tumor microenvironment cells produce approximately 20,000 EVs per day, providing a substantial pool of circulating biomarkers. EVs offer a means to detect and monitor small tumors through both direct detection of tumor-associated biomarkers and host response/tumor microenvironment biomarkers within the EV milieu.
Methods: To identify novel biomarkers, we performed proteomics analysis on EVs purified using size exclusion chromatography and a proprietary buffer system that enhances EV and corona protein recovery. TrueDiscoveryTM data-independent acquisition (DIA) mass spectrometry (MS) analysis was conducted on EVs from 30 Stage 1 Breast cancer patients and 75 normal patient plasma samples. This was followed by performing an in-house developed machine learning pipeline to identify candidate multiplexes with an Accuracy > 0.98.
Results: After quality control correction, an average of 2,562 proteins was identified per sample. Comparative analysis between Stage 1 Breast Cancer and Control cohorts using the in-house Machine Learning Pipeline identified 585 differentially expressed proteins (t test FDR corrected qvalue < 0.01, Logistic Regression cross validation AUC > 0.5, and absolute log2 fold change > 0.5). Of the 585 differentially expressed proteins, 71 have an AUC > 0.85. Support Vector Machine Learning revealed 43 significant 3-plexes with Accuracy > 0.98, consisting of 38 candidate proteins. Interestingly, 18 of the 38 candidate proteins appear in multiple 3-plexes. Finally, we performed ELISA assays of top candidate proteins showing differential expression between cancer EVs and normal patient EVs, indicating we can translate discovery proteomics to fast, easy, and scalable immunoassays.
Conclusions: Key biomarkers from breast cancer patient EVs indicate the potential to detect

and diagnose early-stage and low tumor burden cancers using our methods. Interestingly, no significant differences were noted when biomarkers were evaluated in pre-EV enriched patient plasma. This highlights the unique nature of the EV microenvironment and suggests that early detection of breast cancer may be effectively captured in the EV microenvironment of the patient. An unbiased approach was used for EV proteomics to examine all EVs in the patient. By taking this unbiased approach, we can expand our discovery of biomarkers that may be associated with the patient's response to disease presence.
These findings confirm that the EV microenvironment represents a distinctive oncological ecosystem with highly specific and sensitive disease biomarkers that do not appear dysregulated in whole plasma. Finally, the ability to translate discovery proteomics to ELISA based immune-assays for a clinical assay highlights the power of our approach and data. This novel approach to cancer proteomics holds great promise for the future of cancer diagnostics and monitoring, allowing for the identification of biomarkers indicative of early-stage breast cancer.

P1-03-24: Genomic and Clinicopathologic Profiling of Breast Invasive Lobular Carcinoma in Patients with Germline Predisposition

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Background: Invasive lobular carcinoma (ILC) is the second most common histologic subtype of breast cancer (BC), characterized by biallelic CDH1 inactivation. While germline pathogenic/likely pathogenic variants (P/LPVs) in certain cancer susceptibility genes (CSGs), such as CDH1, have been linked to ILC, the comprehensive germline landscape of P/LPVs in ILC remains to be unveiled. This study aims to elucidate the spectrum of germline P/LPVs affecting CSGs in ILCs, compared to non-lobular BCs, and to characterize clinicopathologic features and the repertoire of somatic genetic alterations in this group.
MATERIALS AND METHODS
The sequencing data from 5,160 BC patients who underwent genetic testing and/or tumor sequencing with the FDA-cleared MSK-IMPACT assay was queried, including 586 patients with ILC and 4,574 patients with non-lobular BCs. Variant pathogenicity was reviewed according to ACMG criteria. Loss of heterozygosity (LOH) was determined using FACETS. The frequency of germline P/LPVs was compared between ILCs and non-lobular BCs. ILCs were subclassified following the WHO criteria. The clinicopathologic features and repertoire of somatic alterations of ILC with germline P/LPVs (gILC) were compared to those of consecutive sporadic ILCs (sILCs) at a 3:1 ratio.
Results: We identified 80 P/LPVs affecting CSGs in 77 (13%) ILC patients. CHEK2 (16%), MUTYH (10%), BRCA2 (9%), and ATM (6%) were the most frequently altered CSGs, followed by CDH1, BRCA1, BRIP1, LZTR1 and PMS2 (5%, each). Compared to patients with non-lobular BCs who underwent genetic testing, patients with ILCs more frequently had

germline P/LPVs in CDH1 (0.68% vs 0%; OR=Inf; P<0.001) and PMS2 (0.68% vs 0.15%; OR=4.5; P=0.03) and fewer P/LPVs in BRCA1 (0.7% vs 2.4%; OR=0.28; P=0.006) and APC (0% vs 0.8%; OR=0; P=0.02). Median age at diagnosis in gILC patients was 54 (30-84) years. 23% (18/77) had a first degree relative with BC. gILCs were mostly classic ILCs (55%), followed by ILC variants, including pleomorphic/pleomorphic features (35%), solid/alveolar (4%), and mixed ductal-lobular BCs (6%). Most gILCs (87%) were ER+/HER2- and 10% were HER2+. Most patients with gILCs (67%) were node-positive on excision. Compared to sILCs, gILCs were enriched for HER2+ disease (10% vs 3%: P=0.017). No differences in other clinicopathologic features were observed. Tumor-specific bi-allelic inactivation of the CSGs affected by P/LPVs, by LOH (n=29) or somatic inactivating mutation (n=1), was seen in 42% of gILCs. Analysis of somatic genetic alterations revealed CDH1 (75% and 84%), PIK3CA (31% and 43%) and TP53 (22% and 29%) as the most frequently altered cancer genes in primary and metastatic gILCs. Compared to primary sILCs, genetic alterations in various chromatin remodelers, such as KMT2C (17% vs. 5%; P=0.01), ARID1A (17% vs 4%, P=0.001) and KMT2D (11% vs. 3%; P=0.03), were enriched in primary gILCs. In the metastatic setting, gILCs showed enrichment in genetic alterations in ERBB2 (29% vs 14%; P=0.03), MYC (12% vs 4%; P=0.04) and the chromatin remodelers KMT2B (13% vs 2%; P=0.01) and KDM6A (6% vs 0; P=0.01), compared to sILCs.
Conclusions: The spectrum of CSGs affected by germline P/LPVs in ILC is heterogenous and encompasses genes playing key roles in genomic instability. Despite the similarities between ILCs arising in the germline and sporadic settings, significant differences in their clinicopathologic and genomic features were identified, such as a higher rate of HER2-positivity and an enrichment in somatic genetic alterations in ERBB2 and in chromatin remodeling genes. These findings highlight opportunities for treatment personalization in patients with ILC who have germline predisposition.

P1-03-25: Characterization of the tumor immune microenvironment (TIME) and somatic landscape of metaplastic breast cancer (MpBC)

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Background: MpBC is a rare, aggressive subtype with a dismal prognosis. Standard of care treatments are limited and novel therapies are needed. Recent clinical studies have demonstrated a response to immune checkpoint inhibitors. However, characterization of the immune and genomic environments in MpBC is paramount to inform new biomarker-selected strategies. Here, we investigated the TIME and somatic landscape of MpBC.
Methods - We retrospectively analyzed de-identified next-generation sequencing data from unique patients with a breast cancer diagnosis (n=13,512) in the Tempus database. We selected patients with MpBC (n=171, 1.3%) and non-MpBC (n=13,341,98.7%) histologies based on clinical documentation reported within 180 days of sample collection. For patients with multiple samples sequenced, the sample closest to the date of reported diagnosis was utilized. Tumors were sequenced with the Tempus xT DNA (648-gene panel) and/or xR

RNA assays. Somatic alterations, immune cell infiltration predicted from gene expression patterns, PD-L1 from IHC, TMB (tumor mutational burden), and MSI (microsatellite instability) were evaluated. Wilcoxon rank-sum and Pearson's Chi-squared/Fisher's exact tests assessed statistical significance ($p<0.05$, $q<0.05$ for false discovery rate correction for multiple testing).
Results: The MpBC and non-MpBC cohorts comprised a diverse population (White, 72% vs 73%, Black/African American, 12% vs 15%, Asian, 7.9% vs 4.4%, Other, 7.9% vs 7.7%) ($p=0.5$). The median age at diagnosis in the MpBC and non-MpBC cohorts were 61 and 57 years, respectively ($p<0.001$). Breast cancer receptor subtype distribution for MpBC vs non-MpBC was HR+/HER2- (20% vs 58%), TNBC (72% vs 24%), and HER2+ (1.9% vs 9.2%) ($p<0.001$). PD-L1 expression (CPS ≥ 10) was higher in the MpBC (35%) vs non-MbBC group (14%) ($p<0.001$). In MpBC, the proportion of M1 macrophages and neutrophils was higher vs non-MpBC ($p<0.001$ for both), and the proportion of B and NK cells was lower ($p<0.001$ for both). There was no clinically significant difference in the percentage of patients with TMB-H or MSI-H status ($p>0.05$ for both). The top five somatic alterations that were more frequent ($q<0.001$) in MpBC vs non-MpBC were: TERT (23% vs 1.3%), CDKN2A (23% vs 5.1%), CDKN2B (21% vs 5.1%), MTAP (16% vs 3.3%), and PIK3R1 (13% vs 3%). Additional potentially therapeutic-relevant alterations associated with PI3k pathway were prevalent in the MpBC and non-MpBC groups: PTEN (25% vs 10%, $q<0.001$), PIK3CA (26% vs 32%, $q=0.2$) and AKT1 (1.8% vs 3.7%, $q=0.3$). Within the MpBC group, there was a similar incidence of PD-L1 expression (CPS ≥ 10) in both TNBC (38%) and HR+/HER2- subtypes (33%). No significant difference was observed between the immune cell subsets within MpBC subtypes ($p>0.05$). Somatic alterations between TNBC and HR+/HER2- subtypes in the MpBC group were analyzed and there was a trend towards differences in PIK3CA (23% vs 39%) and PTEN (22% vs 33%) ($q=0.6$ for both).
Conclusion: In this large, real-world analysis, patients with MpBC displayed a distinct molecular phenotype compared to non-MpBC patients. In patients with MpBC, TNBC was more common, whereas the HER2+ subtype was rare. Patients with MpBC had higher PD-L1 expression and therapeutically relevant alterations, including those within the PI3k pathway, were frequently encountered in MpBC. Although limited by sample size, this is one of the first studies to compare the molecular phenotypes between subtypes within MpBC. These findings are hypothesis-generating and provide further rationale for developing novel combinatorial therapeutic clinical trial strategies for MpBC.

P1-03-26: Genomic Landscape of prImary vs seconDary Endocrine Resistance in HR+/HER2- advanced breast cancer: the GLIDER study

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Background: Resistance to endocrine therapy (ET) in HR+/HER2- advanced breast cancer (BC) can be classified as primary (PETR) or secondary (SETR). It is unclear if the two clinical patterns are characterized by distinct molecular mechanisms. In the GLIDER study, we analyzed the genomic landscape of PETR and SETR BCs to identify the possible drivers of the different resistance patterns.
Methods: PETR and SETR were defined as per ABC 6-7 consensus definitions (PETR: relapse while on the first 24 months of adjuvant ET or progressive disease within the first 6 months of 1st line ET for advanced BC; SETR: PETR criteria not met). Clinical-genomic data were retrieved from a cohort of HR+/HER2- metastatic BC that received any lines of ET (single agent or combination) and that were subjected to Next Generation Sequencing (NGS) using tumor-only panels between March 2016 and July 2024 at the European Institute of Oncology. Genomic data were compared between BCs showing PETR vs SETR. Signaling pathways were identified as previously described in The Cancer Genome Atlas. The occurrence of oncogenic/likely oncogenic genomic alterations (OncoKB) was described in PETR and SETR BCs, and compared with Fisher's exact test and logistic regression. Progression-free survival (PFS) under ET was estimated with Kaplan-Meier and compared with Cox regression. Median follow-up (FUP) was calculated using the Reverse-Kaplan Meier method. No family wise correction was applied for multiple hypothesis testing.
Results: A total of 122 patients were included, of whom 17.21% (n=21) with de novo metastatic BC: 59.32% (n=45) patients received aromatase inhibitor and 38.14% (n=45) fulvestrant, as monotherapy (22.13%, n=27) or combined with CDK4/6 inhibitors (CDK4/6i) (72,95%, n=89). ET was received mostly in 1st line (94.17%, n=113). With a median FUP of 49.0 months (IQR 25.4-81.9) and 105 (88.98%) PFS events, median PFS was 20.7 months (95%CI 17.8 to 25.1) to the first-ET received in the metastatic setting. 17.21% (n=21) patients displayed PETR and 82.79% (n=101) SETR. NGS was performed on tissue (23%, n=28) and liquid biopsy (77%, n=94). A total of 437 genetic alterations were identified, with PIK3CA (50%, n=61) and ESR1 (46%, n=56) being the most commonly altered genes. Tumors showcasing PETR exhibited more PTEN deletions/indels (14.3% vs. 1.9%, Odd-ratio [OR] 16.0, P=0.03), MDM2 amplifications (9.5% vs. 0.0%, P=0.02), IGF1R amplifications (9.5% vs. 0.0%, P=0.02), MUTYH alterations (9.5% vs. 1%, OR 10.2, P=0.08) and MYC amplifications (14.3% vs. 2.9%, OR 5.3, P=0.06), while showing lower occurrence of ESR1 alterations (14.3% vs. 31.7%, P=0.18). At the pathway level PETR exhibited a higher prevalence of alterations involving the MYC-pathway (14.3% vs. 2.9%, OR 5.3, P=0.06) and tyrosine kinase receptors (TRK) (23.8% vs. 9.9%, OR 3.2, P=0.13), while presenting less cell-cycle alterations (4.8% vs. 11.9%, OR 0.4, P=0.4). In the multivariable model accounting for altered signaling pathways, MYC (OR 12.1, P=0.02) and TRK (OR 4.2, P=0.052) status appeared to predict PETR. BCs harboring altered TRK (18.9%, n=23) (mPFS 8.1 vs. 18.5 months, hazard ratio [HR] 1.29, P=0.3) and MYC (6.6%, n=8) (mPFS 11.5 vs. 18.0 months, HR 2.2, P=0.06) displayed inferior PFS with ET. Neither the type of endocrine agent nor progesterone receptor status at immunohistochemistry appeared to induce specific pathway alterations; tumors exposed to CDK4/6i exhibited less cell-cycle (OR 0.26, P=0.02) and ESR1 alterations (OR 0.41, P=0.04). Further subgroup analyses and PFS adjustments will be provided in the full poster.

Conclusion: In GLIDER study, we provide evidence that distinct molecular mechanisms may connote primary (MYC and TRK alterations) vs secondary (ESR1 mutations) endocrine resistance, warranting ad hoc therapeutic approaches.

P1-03-27: Circulating tumor DNA (ctDNA) dynamics as a predictor of treatment response: a review of Liquid Biopsy-RECIST (LB-RECIST) criteria in Metastatic Breast Cancer (MBC)

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Background: Radiological imaging currently represents the cornerstone for monitoring disease response in patients with MBC. However,issues exist with imaging assessments despite standardized response criteria such as RECIST and iRECIST. Recently, dynamics of ctDNA emerged as an alternative to evaluate molecular treatment response through the introduction of LB-RECIST criteria, with a proposed cut-off of 10% in the Variant Allele Frequency (VAF) variation to define patients with radiographic progression. However, specific assessment in setting of MBC is lacking.
Methods: This study retrospectively analyzed 107 patients (pts) with MBC enrolled in a prospective, longitudinal study, NU16B06 trial at Northwestern University (2016-2021), who were longitudinally characterized for ctDNA at baseline (BL), first radiological evaluation (EV1), and progression (PD). For validation, a cohort of 104 pts with MBC enrolled within a multicenter academic consortium, was analyzed. ctDNA characterization was performed through the Guardant360 NGS panel in both cohorts. VAF variation (VAFv) was defined as the percentage change in VAF between the analyzed timepoints. Differences in VAFv across subgroups were assessed using the Mann-Whitney test, and a receiver operating characteristic (ROC) curve was applied to determine the optimal cut-off for distinguishing progressors from non-progressors.
Results: In the clinical study, hormone receptor positive (HR-positive) MBC was the most represented subtype (56 patients, 52%), followed by triple-negative (28 patients, 26%) and HER2-positive (23 patients, 22%). The most common metastatic site was bone (49 patients, 45%), followed by lymph nodes (43 patients, 40%) and liver (26 patients, 24%). Considering BL and EV1 timepoints, with a median interval of 4.4 months, a significant difference in VAFv ($P<0.001$) emerged between progressors (30 pts, 28.3%) and non-progressors (76 pts, 71.7%) at first radiologic evaluation (VAFv = +48%, Inter Quartile Range - IQR 0% to 230% and VAFv = -72%, IQR -99% to -29%, respectively). The best cutoff to define PD was evaluated through a ROC curve as -1.32% (sensitivity 86.7%, specificity 80.3%); however, a cut-off of 0% showed the same predictive capability and was therefore considered optimal. Among the 61 patients with PD samples and a previous sample within 6 months, the median VAFv was 188% (IQR 0% to 1585%). A VAFv ≥ 10% was observed in

42 pts (68%), while utilizing the ≥0% cut-off 50 pts (82%) were included.
In the validation cohort of 104 pts with BL and PD samples, the median VAFv between baseline and progression was 27% (IQR -40% to +440%), with a median interval of 3.1 months. A VAFv ≥ 10% was observed in 53 pts (51.0%), while 74 pts (71.2%) were correctly categorized considering the cut-off of VAFv ≥ 0%.
Conclusions: This analysis confirms the potential of utilizing ctDNA dynamics as a surrogate biomarker for radiographic treatment response. While a 0% cut-off appears to better capture progression compared to a 10% cut-off in both the initial and validation cohorts, prospectives studies are ongoing to standardize and validate LB-RECIST and determine whether optimal VAF cutoffs may be assay and disease context dependent.

P1-03-28: Agreement between the DESTINY-Breast04/06 VENTANA 4B5 HER2 IHC clinical trial assay and other comparator assays for HER2-low breast cancer: Overall results of a large-scale, multicenter global ring study

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Background: Treatment with trastuzumab deruxtecan improved outcomes compared with standard of care for human epidermal growth factor receptor 2 (HER2)-low (immunohistochemistry [IHC] 1+ or IHC 2+ with in situ hybridization negative) metastatic breast cancer (BC) in DESTINY-Breast04 and DESTINY-Breast06. The VENTANA Pathway 4B5 IHC assay, approved in the US as a companion diagnostic, was used in both trials. This global ring study assessed concordance between VENTANA Pathway 4B5 and comparator assays (CAs) in identifying HER2-low BC, with the first phase of the study including laboratories in the US, Canada, and Europe. Concordance in the first phase varied, with positive percent agreement (PPA) tending to be high, especially with 4B5 laboratory-developed tests (LDT). Here, we report the overall results combining the first and second phases of the ring study.
Methods: 50 clinical BC samples were chosen from a cohort of 300 by a steering committee of expert pathologists. Samples were stained using VENTANA Pathway 4B5 and scored as HER2 IHC 0, 1+, 2+, and 3+ by a central laboratory and a panel of experts before being sent to laboratories for HER2 IHC testing per American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) 2018 guidelines. Laboratories were actively scoring HER2 IHC for BC in a clinical setting, had two independent pathologists, and did not routinely use VENTANA Pathway 4B5. The second phase analyzed data from laboratories in Australia, New Zealand, Brazil, Chile, China, Hong Kong, Taiwan, Malaysia, and the Philippines. Pathologists first scored samples with their routine protocols and assays (HercepTest [Omnis or Link48], Leica Oracle, non-4B5 LDT, or 4B5 LDT); then, following virtual alignment on interpretation of HER2 IHC scoring guidelines, they rescored the samples 2 weeks later. Postalignment scores were compared with the reference VENTANA

Pathway 4B5 scores. The primary endpoint was PPA and negative percent agreement (NPA) for HER2-low versus HER2 IHC 0 (includes both ≤10% faint, incomplete membrane staining and no membrane staining) based on postalignment scoring.
Results: A combined 6580 scores from 135 pathologists at 70 laboratories were recorded before virtual alignment. Of these, 129 pathologists from 68 laboratories received alignment guidance, and 6270 postalignment scores were available for analysis. Following alignment, PPA (agreement in identifying HER2-low), NPA (agreement in identifying HER2 IHC 0), and overall agreement were 84.8% (95% CI, 83.6-86.0), 69.2% (95% CI, 67.0-71.2), and 79.4% (95% CI, 78.3-80.5), respectively, with Cohen's κ of 0.54 (corresponding to moderate agreement). Virtual alignment did not substantially affect PPA, NPA, or overall agreement (prealignment scores were 85.1%, 69.5%, and 79.7%, respectively). Postalignment, PPA by assay type ranged from 61.6% (Leica Oracle, N = 196) to 95.5% (HercepTest Omnis, N = 467) and NPA ranged from 36.9% (HercepTest Omnis) to 81.7% (Leica Oracle). PPA by regional subgroup ranged from 62.0% (Latin America, N = 335) to 95.6% (France, N = 391), while NPA ranged from 52.8% (Europe [Other], N = 776) to 89.0% (Australia/New Zealand, N = 395).
Conclusions: Interassay and interlaboratory variability was observed in concordance between VENTANA Pathway 4B5 and CAs in identifying HER2 low versus HER2 IHC 0. PPA tended to be higher than NPA, suggesting more consistent detection of HER2-low compared to HER2 IHC 0. Awareness of available novel treatment options, deliberate pathologist training, and optimization of analytical assay methods and their choice are indicated for accurate identification of patients with clinically actionable HER2 expression levels.=

P1-03-29: Targeted Human Plasma Metabolomics for Lobular Breast Cancer Biomarker Discovery

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Background: Women with lobular breast cancer (LBC) face several unique challenges compared to those with invasive ductal carcinoma (IDC). LBC often presents with several histological and clinical features, such as a diffuse growth pattern and exhibit poorer long-term outcome and a unique pattern of metastasis. Overall, LBC presents diagnostic and therapeutic challenges due to its unique growth pattern. Despite these unique features, the exact metabolic pathways involved in LBC development remains unclear. Metabolomic profiling of plasma from women with LBCmay help to identify new biomarkers to understand the molecular pathways involved in the clinical characteristics of LBC. The purpose of this study is to identify a panel of metabolomic biomarkers that would improve clinical assessment of LBC using plasma samples, and to understand the intersection between LBC and IDC.
Methods: Our study included a total of 185 plasma samples from women with biopsy-confirmed BC and 56 samples of healthy controls. All biospecimens were obtained from the Cooperative Human Tissue Network (CHTN) biobank. A targeted, quantitative mass

spectrometry (MS)-based metabolomics approach was used to analyze 138 metabolites in plasma samples using a combination of direct injection (DI) MS and reverse-phase high performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS). A large-scale stochastic approach was implemented, generating 500,000 logistic regression models with balanced class weights. Each model utilized a randomly selected subset of features. The variables from all high-performing models as measured by subtype-specific performance thresholds (>98% accuracy for lobular carcinoma vs. healthy controls, >95% accuracy for ductal carcinoma vs. healthy controls, and >95% AUC for ductal vs. lobular carcinoma) were aggregated to create a comprehensive set of potentially significant features.
Results: Our multi-stage feature selection approach yielded distinct sets of discriminative features for each comparison, demonstrating high accuracy in differentiating between breast cancer subtypes and healthy controls. For the lobular carcinoma vs. healthy controls comparison, our stochastic modeling process identified 231 feature subsets with an accuracy exceeding 98%. Subsequent frequency-based feature selection resulted in a parsimonious set of four features (three metabolites and age). This refined feature set achieved accuracy and AUC values of 100%. In the ductal carcinoma vs. healthy controls comparison, we retained 33 models with accuracies above 95%. The frequency-based feature selection identified a set of five discriminative features containing four metabolites and age. This feature panel yielded an accuracy of 95% and a bootstrapped AUC of 97%.
Conclusions: This study presents a multi-stage approach for identifying discriminative biomarkers in breast cancer subtypes. Our methodology, which integrates stochastic modeling, ensemble techniques, and iterative feature selection, has demonstrated high efficacy in distinguishing metabolic profiling differences between ductal carcinoma, lobular carcinoma, and healthy controls. The identified panels offer promising avenues for non-invasive differentiation between ductal and lobular carcinomas. This may represent a step towards therapy improvement for target therapy in lobular breast cancer. Future research should focus on external validation of these findings and exploration of the biological significance of the identified metabolites using larger cohorts.

P1-03-30: Genomic risk score distribution and outcomes of patients with early-stage breast cancer diagnosed during pregnancy

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Background: Breast cancer (BC) diagnosed during pregnancy has been associated with worse prognosis, potentially due to pregnancy-associated changes in the local and systemic hormonal and immune environments, and cytokine profiles. Oncotype DX Breast Recurrence Score® test is a prognostic biomarker, predictive of chemotherapy benefit in patients (pts) with early-stage, estrogen receptor-positive (ER+), HER2-negative (HER2-)

BC, though there are limited data regarding its use in pregnant women. The aim of this study was to evaluate the distribution of Recurrence Score® (RS) results and the long-term outcomes of pts with ER+, HER2- BC diagnosed during pregnancy.
Methods: Women with stage I-III, ER+, HER2- BC diagnosed during pregnancy were identified from a prospective cohort study of pts newly diagnosed with BC at age ≤40 years, enrolled from 2006 to 2016. Pts were classified as pregnant or not pregnant at the time of BC diagnosis. The non-pregnant group was subdivided into nulligravid or in the postpartum period. RS were obtained from banked samples when not clinically performed. RS results were classified as low (<11), intermediate (11-25) or high (> 25) and were correlated with clinicopathological characteristics and outcomes. Descriptive analyses were summarized for pts characteristics. RS results were compared by Wilcoxon Rank Sum test. Distant recurrence-free interval (DRFI) was estimated with Kaplan-Meier methods.
Results: From 403 pts with stage I-III, ER+, HER2- BC, with clinical or research-obtained RS results and available gravidity history, 16 (4.0%) were pregnant and 387 (96.0%) were not pregnant at BC diagnosis (117 [29.0%] nulligravid and 270 [67.0%] postpartum). Median follow-up was 11.1 years. Median age at diagnosis was 36 and 37 years, node positivity (N+) rate was 8 (50.0%) and 146 (37.7%), and chemotherapy was administered to 15 (93.8%) and 276 (71.3%) pregnant and non-pregnant women, respectively. Among pts diagnosed during pregnancy, 15 (93.8%) had had prior pregnancies and 5 (31.3%) were diagnosed with stage I, 7 (43.7%) with stage II, and 4 (25%) stage III BC. From those with N+ BC, 6 pts (37.5%) had N1, and 2 pts (12.5%) had N2 disease. Within the pregnant group, median RS was 30.5 (range 21-68), with 6 (37.5%) pts having an intermediate RS and 10 (62.5%) a high RS. This contrasts with non-pregnant patients whose median RS was 20 (3-77, P=0.0002). The 11-year DRFI rates for pregnant pts with node-negative BC were 100% for RS 11-25 and 75% for RS > 25; while for N+ BC, 11-year DRFI was 100% for RS 11-25 and 50% for RS > 25.
Conclusion: Pts with early-stage, ER+ BC diagnosed during pregnancy had genomically intermediate- or high-risk tumors. RS results were higher among pregnant pts compared to non-pregnant women. Most pregnant pts had prior pregnancies, which may have influenced the biological characteristics of the BC diagnosed during a subsequent pregnancy. Although most pregnant patients received chemotherapy, distant relapse rates were elevated among pts with high RS.

P1-04-01: Impact of Obesity, Skeletal Muscle Index, and Comorbidities on Chemotherapy-Related Outcomes in Early Breast Cancer: A Retrospective Subanalysis

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Introduction: Women with early breast cancer (EBC) generally have a very good prognosis, with 5-year survival rates approaching 90%. Chemotherapy can cause significant side effects that decrease quality of life. Many patients with EBC also have comorbidities, but it is

unclear which of these is more strongly associated with chemotherapy intolerance. In our previous retrospective study, we found correlation between sarcopenia detected by bioelectrical impedance spectrometry and worse chemotherapy tolerance; in addition, older age and the presence of multiple comorbidities were also associated with greater chemotherapy toxicity in unadjusted analyses. Here we seek to better understand the relationship between specific comorbid conditions and chemotherapy tolerance.
Methods: This retrospective sub-analysis included 323 patients who received chemotherapy for EBC Patient characteristics, treatment details, and toxicity-related outcomes were obtained. Multivariate logistic regression models were used to associate sarcopenia status with toxicity endpoints, adjusting for other patient characteristics. Age (<65 years old), obesity (BMI>30), and sarcopenia (low SMI <6.75 kg/m2) were forced into the final model. Analysis included 16 comorbidities: hypertension (HTN) , diabetes mellitus (DM), congestive heart failure, cirrhosis, renal disease, chronic obstructive pulmonary disease, tobacco use, hypothyroidism, previous breast cancer, previous cancer, osteoarthritis (OA), rheumatic arthritis, coronary artery disease, peripheral vascular disease, osteoporosis/osteopenia, and stroke. Significant comorbidities were identified through a backward elimination procedure.
Results: Obesity was significantly associated with higher chemotherapy toxicity (OR = 3.58, 95% CI: 1.39-9.24, p=0.02). Patients with sarcopenia also had a notably increased risk of chemotherapy toxicity (OR = 6.94, 95% CI: 3.07-15.62, p<0.0001), as did those with HTN (OR = 1.98, 95% CI: 1.01-3.87, p=0.048).
Regarding chemotherapy dose delay or reduction, patients with an SMI below 6.75 kg/m^2 were more likely to experience dose delays (OR = 2.48, 95% CI: 1.10-5.56, p=0.03). Patients with renal disease had an elevated risk of dose delays as well (OR = 4.41, 95% CI: 1.17-16.66, p=0.03). Early termination of chemotherapy was more common in obese patients compared to those of normal weight (OR = 4.67, 95% CI: 1.36-16.07, p=0.006), and in patients with osteoporosis (OR = 3.46, 95% CI: 1.24-9.58, p=0.02).
Hospitalization rates were significantly higher among obese patients (OR = 8.00, 95% CI: 1.75-36.64, p=0.003), those with SMI below 6.75 kg/m^2 (OR = 8.70, 95% CI: 2.53-30.30, p=0.0006), patients with DM (OR = 3.22, 95% CI: 1.07-9.67, p=0.04), renal disease (OR = 7.09, 95% CI: 1.56-32.171, p=0.01), and those with OA (OR = 4.34, 95% CI: 1.35-13.98, p=0.01).
For neuropathy, likelihood was higher in obese patients (OR = 3.62, 95% CI: 1.20-10.87, p=0.006) and in those with an SMI below 6.75 kg/m^2 (OR = 5.92, 95% CI: 2.27-25.38, p=0.0003). No other comorbidity was significantly associated with neuropathy status.
Conclusion: Obesity and low SMI significantly increase the risk of adverse chemotherapy outcomes in EBC patients. Notably, specific comorbidities such as HTN, kidney disease, DM, and OA play a crucial role in predicting chemotherapy-related toxicity and complications. Our subanalysis provides a more detailed examination of how individual comorbidities uniquely contribute to chemotherapy tolerance. This underscores the importance of a comprehensive, personalized approach in managing EBC patients, considering not only body composition but also the presence of specific comorbidities. Tailoring interventions to address these factors could improve treatment outcomes and quality of life. Future research

should aim to develop and validate personalized interventions in clinical settings to manage these risks better.

P1-04-02: Longitudinal multi-omics study reveals potential targets against chemotherapy induced peripheral neuropathy during taxane treatment

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Background: Taxanes are effective but pose a significant clinical challenge due to the risk of chemotherapy-induced peripheral neuropathy (CIPN) that can result in treatment discontinuation or dose reduction. In a multi-omic, longitudinal study, patients with early-stage breast cancer undergoing taxane therapy as part of their (neo)adjuvant treatment regimen were evaluated over 12 months. Using selected panels of mRNAs, miRNAs, cytokines, and metabolites obtained from blood, we examined changes in molecular markers over time to elucidate biological pathways involved in CIPN and identify potential targets for therapeutic intervention. We hypothesized that a biomarker profile could be identified to track CIPN predisposition and its progression over time.

Methods: 400 breast cancer patients were evaluated at 7 time points—pre (visit 1), during (visits 2-4), and after taxane chemotherapy (visits 5-7). CIPN was measured using the Chemotherapy-induced Peripheral Neuropathy 20-item (CIPN20) questionnaire. Patients with linearized CIPN20 scores (range 0-100) of 8 points above baseline at a time point were classified as having neuropathy. Panels of 194 mRNAs, 798 miRNAs, 13 cytokines, and 85 metabolites were assessed at each of the 7 time points. Changes in molecular markers from pre-treatment baseline through treatment and post-treatment phases were analyzed using a semi-parametric approach, the OmicsLonDA package. Changes in mRNA expression over time were used to examine the activation (Z score > 0) and inhibition (Z score < 0) of selected molecular pathways associated with CIPN, mapped using Ingenuity Pathway Analyses (IPA).

Results: 99 mRNAs, 55 miRNAs, and 10 metabolites showed significant differences between patients with and without CIPN over six time intervals; no significant differences were found for the cytokine panel (P>.05). For example, among the mRNAs, expression of OPRM1 was significantly higher in the CIPN negative group from visit 3 to 7 (int3-7), while expression of CAMK1D mRNA was significantly higher in the CIPN positive group for int2-6 (FDR P<.05). Among the miRNA candidates, miR.31.5p.0 was significantly higher in the CIPN negative group for int3-6, whereas miR.184.0 was significantly higher in the CIPN positive group for int6-7 (FDR P<.05). Among the 10 statistically significant metabolites, tyrosine was significantly higher in the CIPN positive group compared to the CIPN negative group for int2-7 (FDR P<.05).

Total 99 significant mRNA candidates were identified in 120 pathways over time, including 18 genes in cAMP-response element binding protein (CREB) signaling, 14 in opioid signaling, 6 in neuropathic pain signaling, and 10 in endocannabinoid neuronal synapse pathways. Additionally, interval-specific (i.e. between visits) comparative analyses of these pathways longitudinally revealed general predicted activation (Z-score > 0) of CREB signaling in int1-2, inhibition (Z-score < 0) from int2-3 to int5-6, and further activation at int6-7 in the CIPN negative group. A similar activation-inhibition pattern was also observed for the opioid signaling pathway.

Conclusions: Using our multi-omic approach, we observed that CIPN is associated with changes in CREB signaling and opioid signaling pathways, which may provide novel opportunities for therapeutic intervention and biomarker development.

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P1-04-03: Model-predicted Neurokinin-1 (NK1) Receptor Occupancy of Netupitant versus Aprepitant Over an Extended Time Period: Implications for Controlling Nausea and Vomiting Associated with Antibody-Drug Conjugates (ADCs)

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Background: Nausea and vomiting (NV) are expected side effects of anticancer treatment and have traditionally been assessed up to 120 h (5 days) post-treatment. However, following a recent systematic review by Chow et al. (Supp Care Cancer 2023) which assessed the prevalence of “long-delayed” NV (beyond 120 h), attention has begun shift to this unmet need, particularly as data emerges with novel targeted therapies such as ADCs. Evolving data with ADCs in breast cancer indicates NV are not only among the most frequent treatment-related side effects but can occur for a longer time, potentially resulting in detrimental effects on quality of life and on maintaining dose-intensity. Using guideline-recommended antiemetic prophylaxis [i.e., an NK1 receptor antagonist (RA)-containing regimen] when initiating ADCs is critical to optimize patient outcomes. Netupitant (NETU), a highly selective NK1 RA used in combination with palonosetron, has shown favorable comparative efficacy to an aprepitant (APR) regimen up to 168 h, likely due in part to its longer half-life. As receptor occupancy (RO) is assumed to serve as proxy for clinical efficacy, we conducted an analysis aimed at evaluating the RO in the brain striatum with netupitant versus aprepitant for an extended time interval.
Methods: Mean plasma concentration-time curves of NETU and APR from previous pharmacokinetic (PK) studies in the Caucasian population were analyzed by compartmental PK modelling upon single administration of the therapeutic doses of 300 mg and 125 mg po, respectively. Emax pharmacodynamic (PD) models were fitted to the % NK1 RO data in the striatum region after NETU and APR administrations as a function of PK model predicted plasma concentrations. The % NK1 RO in the striatum region up to 480 h for 300 mg NETU

on day 1 versus 3-day APR (125 mg day 1 and 80 mg days 2-3) were then assessed by PK/PD model predictions.
Results: The RO patterns for NETU and APR were quite distinct. The NK1 RO reached 90% by 3 h for both agents. RO remained high up to 120 h (day 5) for both NETU (76%) and APR (81%) but then dropped substantially for APR and gradually declined for NETU. At 168 h (day 7), the RO was 70% for NETU and 39% for APR. The RO for NETU and APR, respectively, at 192 h (day 8), 240 h (day 10), and 480 h (day 20) was 67% vs 19%, 60% vs 3%, and 22% vs 0%.
Conclusions: There was a persistent and gradual decline of % NK1 RO over a period of 20 days following a single dose of NETU compared with a rapid decline in RO for APR after 6 days. These findings suggest that NETU has the potential to prevent NV over a prolonged duration, making it a promising antiemetic for prevention of NV with new anticancer targeted therapy like some ADCs. Preserving patients' quality of life and ensuring uninterrupted treatment is vital to optimizing outcomes and maximizing treatment effectiveness.

P1-04-04: evelopment and validation of a nomogram for axillary lymph node metastasis risk in breast cancer

Xiaoyun Mao, Shijing Wang

Purpose: Preoperative assessment of axillary lymph node (ALN) status is essential for breast cancer treatment planning. To prospectively analyze risk factors for ALN metastasis in breast cancer patients, comparing high-resolution computed tomography (HRCT) imaging with pathology, and to develop and validate a nomogram predicting the status of each node.
Methods: From April 2023 to May 2024, patients with pathologically proven breast cancer were enrolled in the study. All patients underwent a chest HRCT examination within one week prior to surgery. ALN specimens were subjected to pathological examination and anatomically matched to HRCT imaging. The least absolute shrinkage and selection operator (LASSO) regression was employed to refine the features for the metastasis lymph node (MLN). Subsequently, the final selected features were determined through multivariate logistic regression analysis and utilized to construct a nomogram. The discrimination, calibration, and clinical utility of the predicting model were evaluated using the concordance index (C-index), area under the curve (AUC), receiver operating characteristic (ROC) curve, calibration plot, and decision curve analysis. Internal validation was conducted through bootstrapping validation.
Results: A total of 302 ALN from 98 patients were included in this study. The patients were divided into two groups based on pathology: a MLN group (71 nodes) and a non-metastasis lymph node (NMLN) group (231 nodes). The predictors included in the prediction nomogram encompassed the mean CT value, short diameter, border, and shape of ALN, as well as the Ki-67 status and histological grade of the primary tumor. The model exhibited satisfactory discrimination, with a C-index of 0.869 [95% confidence interval (CI): 0.826-

0.912] and an AUC of 0.862 (95% CI, 0.815-0.909). Moreover, in the interval validation, the model demonstrated its high performance, achieving a C-index of 0.845 and an AUC of 0.857. The calibration curve demonstrated a high degree of concordance between the predicted and actual probabilities. The decision curve analysis demonstrated that the prediction nomogram was clinically useful when the threshold for intervention was set at the metastasis possibility range of 1% to 86%.
Conclusion: The prediction nomogram combined with preoperative pathology and HRCT imaging have the potential to improve the evaluation of ALN status. This integrated approach can serve as a valuable reference for the management of breast cancer patients.

P1-04-05: Stroke-Related Mortality Trends Among Breast Cancer Patients in the United States from 1999 to 2020: A Retrospective Analysis

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Background: Breast cancer is the second most common cancer worldwide, and the most diagnosed cancer in women. It is associated with an increased risk of stroke, which may prove fatal. However, contemporary data regarding stroke-related mortality in breast cancer patients in the United States (US) is limited. We aim to assess the temporal trends in stroke-related deaths in women with breast cancer in the US from 1999 to 2020, stratified by race/ethnicity, age groups, census regions, and urbanization.
Methods: We used the Centers for Disease Control and Prevention's Wide-ranging Online Data for Epidemiologic Research database for death certificate data of all decedents with stroke as the underlying cause of death and breast cancer as a contributing cause of death. Crude mortality rates (CMRs) and age-adjusted mortality rates (AAMRs) with 95% confidence intervals (CIs) were calculated per 100,000 people, and the temporal trends were analyzed by determining the annual percentage change (APC) and the average APC (AAPC) using Joinpoint regression.
Results: From 1999 to 2020, a total of 12,767 stroke-related deaths in patients with breast cancer were recorded in the US. We observed a declining trend in AAMRs from 1999 to 2014 (APC -5.68, 95% CI -6.70 to -4.97), which stabilized thereafter till 2020 (APC 2.46, 95% CI -0.64 to 11.17). The highest AAMR was exhibited by Non-Hispanic (NH) Blacks or African Americans (0.202) and the lowest by NH Asian or Pacific Islander (0.083). AAMRs declined till 2013 for NH Blacks or African Americans and till 2014 for NH Whites, stabilizing afterward. Older individuals showed much higher CMRs, with about five-fold greater rates in the 85+ years age group (5.291) than in the 75 to 84 years age group (1.325). While the CMRs initially declined for all age groups, they stabilized for the 55 to 64 years group after 2016 and the 65 to 74 years group after 2013, and increased for the 75 to 84 years group after 2018 and the 85+ years group after 2016. Regional variation was evident, with higher AAMRs in the Midwest (0.197) and the West (0.181) compared to the South (0.157) and the Northeast (0.154). Initially, declining AAMRs were observed in all regions, with stable trends in the Northeast after 2018, the Midwest after 2012, and the

South after 2014. However, rising AAMRs were observed in the West from 2014 to 2020. Greater AAMRs were exhibited by rural areas (0.19) than urban areas (0.166), with rural areas showing a decline in AAMR throughout the period but urban areas showing an initial decline till 2012 and stable AAMRs thereafter till 2020.
Conclusion: While stroke-related deaths in breast cancer patients initially declined in the US, the mortality rates have stabilized in recent years. The highest burden of deaths was observed in NH Blacks or African Americans, older individuals, residents of the Midwest, and those living in rural areas. Focused efforts are needed to reduce the disparities and effectively mitigate stroke-related deaths in breast cancer patients.

P1-04-06: Reasons for Cannabis Use and Perceptions in Breast Cancer Patients Compared to Other Cancer Patients

Cristina Truica

Background: Cannabis use has increased significantly in the US with 38 states having passed laws allowing access to cannabis (C) and among cancer patients and survivors the estimated prevalence rates range from 8% to 40%. While the evidence supporting the efficacy of medical C for cancer symptoms is generally limited, some studies suggest benefits in managing pain, sleep disturbances, anxiety, nausea and vomiting, which are common symptoms in breast cancer (BC) patients. This study explores C use and attitudes/perceptions among cancer patients in general and BC patients specifically, and how BC patients may differ. Previous reports relied exclusively on online questionnaires, potentially excluding disadvantaged patients. We used an in-person survey to broaden our reach given our predominately rural catchment area. Methods: Between June 2023-June 2024, we conducted in-person surveys using anonymous paper questionnaires of adult (≥18y) cancer patients diagnosed within the previous year who visited the Penn State Cancer Institute. Responses from BC patients were compared to patients with other cancer types using Fisher's exact test. Results: Out of 186 surveyed patients, 50 (26.9%) reported having BC, with other common sites being lung (14.5%), melanoma/skin cancer (11.8%), non-Hodgkin Lymphoma (9.7%) and leukemia (9.1%). Of the full sample, 51.4% lived in rural areas and 14.7% were economically disadvantaged, reporting finding it difficult or very difficult to get by on present income. BC patients were significantly younger (mean = 58.9 years) and more likely to be employed and have early-stage cancer. The C use rate in our general sample (26.1%) was lower than in other studies done in predominantly urban centers. Compared to other cancers, BC patients had higher rates of C use since diagnosis (36.0% vs. 23.4%), C use during cancer treatment (30.6% vs. 22.2%) and current use (26.0% vs. 18.6%), although these differences were not statistically significant. BC patients were more likely than other patients to have used C instead of opioids to manage pain (47.6% vs. 23.1% of C users, p = .05) and were less likely than other patients to be concerned about C interacting with other medicines (3.1% vs 18.4%, p = .04). The most common reason for C use in our sample was to help with sleep and BC patients were more likely to have used C to improve sleep than others (88.2% vs. 54.8%, p = .03). The most

common reason for not using C in our full sample (52.2%) and in BC patients (40.6%) was that their health care providers had not suggested or recommended it. Most BC patients (60.0%) thought that C is safe for cancer patients to use and a large majority (92.0%) thought there are benefits related to C use, citing pain management most often (93.5%). However, most BC patients (69.4%) also thought there are risks related to C use, with an inability to drive (50.0%) cited most, while 32.7% thought that C is addictive. 52.0% of BC patients reported being mostly open or very open to C use and 49.0% reported being at least moderately confident about their C knowledge, with 46.0% wanting their provider to talk to them about C use. Importantly, most BC patients (55.1%) reported being likely or very likely to consider participating in research studies involving C, with the preparation most likely to interest them being in food such as brownies (69.6%) followed by oral intake (60.9%) or topically (60.9%). Conclusions: The prevalence of C use among the BC patients in our sample was higher than in other cancer types. BC patients were more inclined to use C instead of opioids for pain management and to utilize C to improve sleep. The BC patients perceived C as safe and potentially beneficial, expressing interest in research with C all the while acknowledging associated risks.

P1-04-07: Incidence and Risk Factors of Immune-Related Adverse Events in Metastatic Breast Cancer Patients: Findings from a Multi-Institutional Study

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Background: For patients (pts) living with metastatic breast cancer (mBC), treatment with immune checkpoint inhibitors (ICI) offers the promise of improved outcomes. However, immune-related adverse events (irAE) are a potentially irreversible, dose-limiting toxicity of treatment and can significantly impact quality of life. There is a lack of real-world data on incidence of and risk factors for irAE among patients with mBC.
Methods: This multi-institutional, retrospective study identified pts with mBC treated with ICI between 2014-2024. Pts on ICI therapy at time of study were excluded. Demographic, clinical, treatment, and irAE data were collected. Events were considered irAE if the treating team indicated toxicity was related to or likely related to ICI. Descriptive statistics were used to describe incidence of irAE. Pearson's chi-squared, two-sample T tests, simple and multivariable logistic regression models were used to evaluate risk factors for irAE including demographics, body mass index (BMI), comorbidities, breast cancer subtype, menopausal status, Programmed Death-Ligand 1 (PD-L1) expression, baseline lab values, ICI type, number of ICI cycles, combination vs. monotherapy, and clinical trial participation.
Results: 277 mBC pts were included. Mean age at start of ICI was 54 (SD 13.7, range 23-89). The study population was racially diverse (63.8% White, 8.7% Black, 12% Asian, 2.5% Latino), mostly pre-menopausal (52.3%), and more likely to have triple negative breast

cancer (62.8%) than hormone receptor positive (31.0%) or human epidermal growth factor receptor 2 (HER2) positive (6.1%) disease. 18.1% had documented PD-L1 positivity. 15.9% had a history of autoimmune disease at time of ICI initiation. 128 (46.2%) experienced a total of 173 irAEs, most commonly thyroiditis (20.8%), rash (20.2%), and colitis (19.1%). 24.0% of irAEs were grade 3-4 at onset. IrAEs per patient ranged from 0-4; pts who experienced irAE had 1.35 (SD 0.63) on average. Pts were on ICI for an average of 138.4 (range 0-1380) days before their first irAE. Compared to those without irAE, mBC pts with irAE were more frequently post-menopausal (54.3% vs. 42.0%, p=0.04), more likely to have hyperlipidemia (HLD; 33.9% vs. 22.0%, p=0.03), had more comorbidities (1.0 vs. 0.7, p=0.01), and had higher baseline hemoglobin (Hb; 12.4 vs. 11.5, p<0.01). In unadjusted logistic models, being post-menopausal (OR 1.30, p=0.03) and having HLD (OR 1.78, p=0.03) were associated with greater likelihood of irAE. Similarly, for each additional baseline comorbidity (among type II diabetes, hypertension, lung disease, HLD, chronic kidney disease, and coronary artery disease) and for each increased point of baseline Hb, the odds of irAE increased by 30% (OR 1.30, p=0.02) and 40% (OR 1.40, p<0.01), respectively. Increasing cycles of ICI had a modest but statistically significant increased association with irAE (OR 1.04, p<0.01). Adjusted for demographics, clinical factors, comorbidities, baseline labs and treatment characteristics, higher baseline Hb (OR 1.30, p=0.02) and increasing number of ICI cycles (OR 1.03, p=0.03) were associated with increased likelihood of irAE.
Conclusions: For a diverse population of women with mBC treated with ICI, real-world data demonstrates that irAEs are more common than previously reported in trials, with 46.2% of pts affected. The most common irAE, thyroiditis, often leads to irreversible physical effects. Pts that developed irAE were more frequently post-menopausal and had a greater number of baseline comorbidities. Adjusting for covariates, higher baseline Hb levels and increasing number of ICI cycles were associated with increased risk of developing irAE. This study identifies the frequency of and risk factors for irAEs among mBC pts, for whom balancing quality of life with prolonged courses of ICI therapy is paramount. This study may help mBC patients and their providers make informed decisions about ICI treatment.

P1-04-08: Feasibility Evaluation and Implementation of a Digital Health Intervention (DHI) Mobile Health (mHealth) Electronic Patient-Reported Outcome Measure (ePROM) Based Platform for Telemonitoring Patients with Breast Cancer Undergoing Chemotherapy

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Background: Breast cancer (BC) is the most prevalent cancer globally and a leading cause of morbidity and mortality among women. Symptoms and treatment-related side effects often go undetected during routine follow-ups. Digital Health Interventions (DHIs) offer promising tools for real-time monitoring and personalized care, potentially improving

clinical outcomes through enhanced symptom detection and informed decision-making. We aimed to implement and evaluate the feasibility of a DHI mobile health (mHealth) electronic Patient-Reported Outcome Measure (ePROM) based platform (ThummiOnco) for telemonitoring patients with BC undergoing neoadjuvant or adjuvant chemotherapy.
Methods: A prospective observational study was conducted at the Women's Outpatient Clinic of Barretos Cancer Hospital in Brazil. The study enrolled patients with localized breast cancer (TNM stages I-III) who were initiating adjuvant or neoadjuvant chemotherapy with any therapeutic regimen. Participants were monitored using the ThummiOnco platform for 4 to 6 months, following a standardized protocol. The feasibility of ThummiOnco telemonitoring was assessed through platform usage, resolution of patient-reported symptoms, and healthcare outcomes. Platform usage was defined as the median number of platform accesses per patient and the frequency of daily symptom reports. The resolution of patient-reported symptoms was determined by symptoms reported by patients that were resolved (i.e., no further complaints regarding the reported symptom) according to the healthcare team within 48 to 72 hours. Healthcare outcomes were measured in terms of number of complementary consultations, dose reductions, treatment interruptions or discontinuations, hospitalizations, and treatment-related mortality. Statistical analysis was performed using descriptive statistics.
Results: Between October 11, 2022, and June 21, 2023, 67 patients (median age 51) were enrolled. The majority (52%) had stage III disease, and 62% received neoadjuvant chemotherapy. The median number of app accesses per patient was 38, with an average of 6.65 symptom reports daily. In total, 919 patient-reported symptoms were recorded. Of these symptoms, 67.7% (n=622) were fully resolved within 48 hours, 26.5% (n=243) were partially resolved, and 6% (n=54) remained unresolved and under monitoring. Among the partially resolved or unresolved symptoms at 48 hours (32.3%, n=297), 23.9% (n=71) were fully resolved and 62% (n=184) partially resolved within 72 hours. The overall resolution rates at 72 hours were 75.4% (n=693) for full resolution and 20.0% (n=184) for partial resolution. Regarding symptom resolution by grade, 83% of grade I, 69.5% of grade II, and 54.8% of grade III symptoms were fully resolved, exclusively with the use of the ThummiOnco platform. Complementary consultations were required for 34 patients, with 30 conducted in person and 4 via teleconsultation. Dose reductions occurred in 10 patients (14.9%), treatment interruptions or discontinuations in 35 patients (52.2%), and hospitalizations in 7 patients (10.4%). One patient died due to progressive disease.
Conclusions: ThummiOnco telemonitoring facilitated early symptom identification and management, reducing the need for in-person consultations. Most reports were fully resolved using the ThummiOnco platform, with minimal additional demands on the medical team. Continuous monitoring of patient symptoms through e-PROM-based platforms is helpful in the management of patients with cancer under active chemotherapy for early BC.

P1-04-09: Evaluating the Necessity and Impact of Cardiac Imaging on Breast Cancer Care in Northwestern Ontario

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Background: Over 25,000 women are annually diagnosed with breast cancer in Canada. Their survival rates have improved significantly due to advances in screening and treatment. However, many treatments are cardiotoxic, and cardiovascular disease is currently the leading competing cause of death in older breast cancer survivors. Baseline left ventricular ejection fraction (LVEF) is a reliable predictor of heart failure (HF) in patients receiving anthracyclines (AC) and/or trastuzumab. Identification of reduced LVEF can promote interventions to prevent HF and improve patient outcomes. Accordingly, pre-treatment cardiac imaging is supported by the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines.

Research Question:Despite the perceived necessity of cardiac imaging before and during breast cancer treatment, the recommendations underlying its use is mostly based on expert opinion rather than specific data. This research aims to analyze local data to determine the impact of cardiac imaging on treatment outcomes for breast cancer patients receiving AC and/or trastuzumab and offer evidence-based guidance for ordering physicians at the Thunder Bay Regional Health Sciences Centre (TBRHSC).

Methods: This is a retrospective cohort study including all female patients seen at the TBRHSC who were treated with AC and/or trastuzumab for newly diagnosed breast cancer between January 1, 2012, and December 31, 2017. Data, including baseline characteristics, treatment regimen, imaging tests ordered from diagnosis until one-year post-treatment, and clinical outcomes were collected from the patient's medical records and recorded in a secure REDCap database. Patients were grouped into three cohorts based on treatment regimen: trastuzumab only (A), AC only (B), and both trastuzumab and AC (C). Initially, 125 patients were identified, but those who did not receive either treatment or had no imaging tests recorded were excluded from this study.

Results: A total of 93 patients met the exclusion criteria for this analysis, with an average age at diagnosis of 59.5 years (SD = 10.4). Invasive ductal carcinoma was the most common cancer (97.8%, n=91). Most cancers were diagnosed at stage 2 (51.6%, n=48), followed by stage 1 (24.7%, n=23), and stage 3 (14.0%, n=13); 9.7% (n=9) had unknown stages. Regarding receptor status, 69.9% (n=65) were ER-positive, 62.4% (n=58) were PR-positive, and 34.4% (n=32) were HER2-positive. BRCA1/2 status was unknown for 79.6% (n=74) of the patients included in this study.
In cohort A (n=3), 14 scans (4.67 per patient) led to 1 change in care (7.1%). Cohort B (n=60) had 75 scans (1.25 per patient) resulting in 10 changes in care (13.3%), including changes in chemotherapy (4.0%, n=3), care provider (5.3%, n=4), and medication (4.0%, n=3). Cohort C (n=30) had 144 scans (4.80 per patient) leading to 6 changes in care (4.2%).

Conclusion: This study found that the most significant changes in patient care based on cardiac imaging occurred in patients receiving only AC treatment, with changes happening in 13.3% of cases and each patient receiving an average of 1.25 scans. However, patients receiving only trastuzumab or a combination of trastuzumab and AC had fewer changes in

care (7.1% and 4.2%, respectively) despite having more scans per patient (4.67 and 4.80, respectively). This indicates that more frequent scans do not always lead to more useful information. The study highlights the importance of focusing cardiac imaging on those most likely to benefit, especially in areas with limited resources like Northwestern Ontario. Future research will aim to identify predictive factors for the optimal use of cardiac imaging to enhance resource allocation and patient outcomes.

P1-04-10: Peripheral blood cells as predictors of immune-related toxicity in early triple negative breast cancer (eTNBC)

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Background: Immune Checkpoint Inhibitors (ICI) acquired an impactful role in high risk early triple negative breast cancer (eTNBC). In particular, KEYNOTE-522 study has demonstrated that the addition of pembrolizumab to neoadjuvant chemotherapy (NACT) improves pCR rate and survival outcomes. Therefore, this novel regimen is characterized by an enhanced and potentially irreversible toxicity profile. The identification of biomarkers able to predict immune-related adverse events (irAEs) is ongoing in different neoplasms. Recent evidence showed a potential emerging role of blood cell counts and ratios, such as lymphocytes and monocytes.
With these premises, the aim of our study is to explore if peripheral blood biomarkers should predict irAEs in eTNBC patients candidate to receiving immunotherapy in peri-operative setting.
Methods: The study enrolled 20 eTNBC patients undergoing NACT containing pembrolizumab. Patients were divided into two subgroups based on the development of irAEs; patients who experienced toxicity were selected depending on the immunotherapy's discontinuation. Patients performed blood samples at baseline before starting treatment and Flow Cytometry analysis was used to characterized monocytes, circulating T cells (CD3, CD4 and CD8) and Myeloid Derived Suppressor Cells (MDSCs).
The statistical analysis was performed using Mann Whitney U Kruskal-Wallis ($p<0.05$) for estimating the frequency of monocytes, and T cells and MDSCs subtypes according to toxicity development.
These analyses were performed with R packages v. 4.3.1.
Results: Overall, 20 patients with eTNBC undergoing NACT were enrolled from July 2022 to February 2024. 12 patients developed irAEs (60%); severe adverse events (grade 3 or higher) were observed in four patients (20%). Particularly, 7 patients experienced hypotiroidism (35%), 3 patients developed a cardiac event (15%), 1 patient had adrenal insufficiency (5%), 2 patients developed acute kidney injury (10%). irAEs led to discontinuation of pembrolizumab in 10 patients (50%).
Considering the various types of peripheral white blood cells, patients with irAE had significantly higher level of monocytes compared to other ones ($p = 0.004$). Although, the

analysis suggested a statistical trend in an augmented expression of non-classical monocytes (CD14-, CD16+), the small sample size precluded statistical significance (p = 0.08). Patients experiencing irAEs showed also an increased expression of T-senescent lymphocytes (p= 0.08). Then, considering patients with irAEs who required treatment discontinuation we observed higher levels of monocytes (p=0.03), especially for the non-classical type (p=0.02).
Conclusion: the addition of immunotherapy to NACT is known to significantly increase the pCR rate and improve the survival outcomes. Despite that, the KEYNOTE-522 regimen is marked by a high rate of adverse events and predictive biomarkers useful to their identification are needed. Our study suggest that peripheral white blood count can be an accessible and easy way to early detect patients with higher risk to develop irAEs, but further studies with larger samples are needed to confirm our findings.

P1-04-11: Examining the risk factors for trastuzumab related cardiotoxicity in a predominantly Hispanic South Texas population: a descriptive study

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Background: Trastuzumab, a monoclonal antibody targeting human epidermal growth factor receptor 2 (HER2), stands as first line treatment for HER2 positive breast cancer. Despite its efficacy, trastuzumab's association with cardiotoxicity requires monitoring via serial transthoracic echocardiograms (TTEs). We aimed to evaluate the risk factors for trastuzumab related cardiotoxicity in a South Texas population that is uniquely a majority-minority Hispanic population.

Methods: A retrospective chart review study was conducted of all female patients with HER2-positive breast cancer who received trastuzumab treatment from 2015-2021. A total of 180 patients were identified. Patients without baseline TTE or a baseline left ventricle ejection fraction (LVEF) less than 53% were excluded. The final sample size included 132 patients. Cardiotoxicity was defined as a decrease in LVEF of more than 10% during the 1-year study period.

Results: The mean age at the time of diagnosis in the study population was 54.2 years. The average BMI of the study participants was 30.89. The incidence of cardiotoxicity in this study population was 6%. Among those who developed cardiotoxicity, 50% had hypertension, 25% had hyperlipidemia, 12.5% had type 2 diabetes mellitus, and 12.5% had previous coronary artery bypass surgery (CABG). In terms of cancer treatment, 12.5% of patients had a history of radiation, 25% had a history of anthracycline therapy, and 12.5% had their trastuzumab treatment ended prematurely. Of the cardiotoxicity population, 37.5% of patients were former smokers and 25% were former alcohol users. Hispanic/Latino patients composed 58% of the study population and represented 50% of patients who experienced cardiotoxicity. Conclusions: The incidence of trastuzumab-

related cardiotoxicity in this Hispanic/Latino majority-minority population was 6%, lower than the 9% observed in a predominantly white population in our previous study. However, further studies are needed to determine the factors contributing to this reduced cardiotoxicity rate in this population.

P1-04-12: Optimizing Tolerability of Pyrotinib in HER2-Positive Early-Stage High-Risk Breast Cancer: A Multicenter, Randomized, Three-Cohort Study

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Background: HER2-positive breast cancer patients with a high risk of recurrence are recommended extended treatment involving sequential therapy with anti-HER2 monoclonal antibody followed by maintenance therapy with HER2-targeted tyrosine kinase inhibitor (TKI). Pyrotinib, an oral irreversible pan-HER TKI, commonly induces diarrhea. This study aims to develop strategies to reduce the incidence and severity of diarrhea and evaluate the efficacy and safety of pyrotinib as an extended adjuvant treatment for early-stage HER2-positive breast cancer.
Methods: In this multicenter, prospective, randomized cohort study (ChiCTR2200060339), stage II-III HER2-positive high-risk breast cancer patients who had completed adjuvant treatment post-surgery, stratified by disease stage (II vs. III) and hormone receptor (HR) status (positive vs. negative), were randomized 1:1:1 into three cohorts to receive extended adjuvant therapy with 12 months of pyrotinib. Cohort 1 received pyrotinib 240 mg daily from D1-14, 320 mg from D15-28, and 400 mg from D29-364. Cohort 2 received pyrotinib 320 mg daily from D1-28 and 400 mg from D29-364. Cohort 3 received pyrotinib 400 mg daily from D1-364, combined with loperamide 4 mg thrice daily from D1-7 and twice daily from D8-28. The primary endpoint was the incidence of grade ≥3 diarrhea. Secondary endpoints included serious adverse events (AEs), other AEs, incidence of diarrhea, invasive disease-free survival, and overall survival.
Results: Between June 2022 and May 2024, 102 patients were enrolled, with 34 patients in each cohort. Twelve patients (35.3%) in Cohort 1, 13 (38.2%) in Cohort 2, and 13 (38.2%) in Cohort 3 had ≥4 lymph nodes involved post-surgery. Additionally, 19 patients (55.9%) in Cohort 1, 21 (61.8%) in Cohort 2, and 19 (55.9%) in Cohort 3 were HR positive. Grade ≥3 diarrhea was reported in 9 (26.5%) patients in Cohort 1, 9 (26.5%) in Cohort 2, and 5 (14.7%) in Cohort 3. The median time to the first occurrence of grade ≥3 diarrhea was 15 days (range: 8-296) in Cohort 1, 4 days (range: 1-43) in Cohort 2, and 3 days (range: 1-14) in Cohort 3. The median number of grade ≥3 diarrhea episodes was 1 (range: 1-3) in Cohort 1, 3 (range: 1-4) in Cohort 2, and 1 (range: 1-2) in Cohort 3. The median duration of each grade ≥3 diarrhea episode was 1 day (range: 1-2) in Cohort 1, 1 day (range: 1-4) in Cohort 2, and 1 day (range: 1-3) in Cohort 3. Dose reduction due to diarrhea occurred in 2 (5.9%) patients in Cohort 1, 7 (20.6%) in Cohort 2, and 10 (29.4%) in Cohort 3. Dose interruption for diarrhea was observed in 14 (41.2%) patients in Cohort 1, 15 (44.1%) in Cohort 2, and

16 (47.1%) in Cohort 3. Discontinuation of pyrotinib due to diarrhea was reported in 2 (5.9%) patients in Cohort 1, 4 (11.8%) in Cohort 2, and 2 (5.9%) in Cohort 3. The most common AEs were diarrhea (79.4% vs. 91.2% vs. 88.2%), nausea (17.6% vs. 20.6% vs. 44.1%), vomiting (11.8% vs. 8.8% vs. 35.3%), fatigue (14.7% vs. 17.6% vs. 26.5%), abdominal pain (8.8% vs. 11.8% vs. 20.6%), and abdominal distension (5.9% vs. 5.9% vs. 17.6%). Grade 1 constipation was observed in 1 patient (2.9%) in Cohort 1, 1 patient (2.9%) in Cohort 2, and 3 patients (8.8%) in Cohort 3.
Conclusions: Loperamide prophylaxis showed a numerically lower incidence of grade ≥3 diarrhea. Diarrhea is a predictable and manageable side effect of pyrotinib treatment, occurring early after initiation and rarely in the later treatment course. It is advisable to combine loperamide with pyrotinib to reduce grade ≥3 pyrotinib-induced diarrhea. Additionally, timely adjustment of loperamide dose is necessary to prevent other gastrointestinal AEs, such as nausea and abdominal distension.

P1-04-13: Comparison of Time-to-Pregnancy in Young Breast Cancer Survivors versus the general population: a Prospective Case-Control Study (FEERIC) from a collaborative research network

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Introduction: Breast cancer (BC) is the most prevalent cancer among women, and increasing attention is being paid to the side effects of treatments, including the potential impact on fertility. Concerns about pregnancy-induced recurrence have existed for decades, but recent data suggest that pregnancy after BC is safe. Additionally, there is a growing interest in understanding the fertility potential and pregnancy outcomes in BC survivors. However, data on fertility rates post-BC compared to the general population remain sparse.

Objectives: The FEERIC study aims to compare the time-to-pregnancy between women with and without previous BC who initiate pregnancy attempts over a three-year follow-up period.

Materials and Methods: The FEERIC (FErtility, ContracEption After Breast Cancer) study is a prospective case-control study involving women aged 18-43 years, with localized, relapse-free BC, and completed treatment. Controls were women of the same age group without BC. Data were collected via online questionnaires over three years. Participants were recruited through Seintinelles, a French social network for cancer research. The primary endpoint was time-to-pregnancy, with secondary endpoints including factors associated with time-to-pregnancy, use of ART, and pregnancy outcomes. Statistical analysis involved Kaplan-

Meier survival analysis and multivariate Cox Proportional-Hazards models, adjusted for confounding factors using Inverse Probability of Treatment Weighting (IPTW).

Results: From December 2018 to June 2019, 4351 women were enrolled, and a total 642 women (76 cases, 566 controls) initiated pregnancy attempts and had follow-up data available. Median time-to-pregnancy was 5 months (IQR: 2.0-7.0) for cases and 3 months (IQR: 2.0-6.0) for controls, with no significant difference between the groups (p=0.34). Most women (61 cases (80.3%) and 541 controls (95.5%)) attempted to conceive through unprotected intercourses only. A total of 7 cases (9.2%) and 25 controls (4.5%) used ART methods. In cases, only 8 women (10.5%) reused cryopreserved materials during their attempts. Most pregnancies occurred spontaneously: among these 50 cases and 402 controls who achieved pregnancy, 42 cases (84.0%) and 382 controls (95.0%) conceived after unprotected intercourses only, while 8 cases (16.0%) and 20 controls (5.0%) conceived after ART methods.
After univariable and multivariable analyses, age at pregnancy attempt, previous children at study inclusion, menstrual cycle regularity, BMI, and ART use were independent predictors of time-to-pregnancy, while the case or control status was not.
Conclusions: The probability of pregnancy for women attempting conception post-BC treatment is comparable to that of the general population. Future research should explore long-term reproductive health and the psychosocial impacts of fertility post-BC treatment to guide supportive care practices.

P1-04-14: Vaginal oestradiol pessaries for the treatment of genitourinary symptoms in women with early breast cancer: a single arm phase 2 study (OVGUS)

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Introduction: Genitourinary syndrome of menopause is common in breast cancer survivors. Guidelines recommend first-line non-hormonal vaginal treatments, and if these fail, consideration of vaginal oestrogens. Yet, product labels warning against their use continues and fear of cancer recurrence (FCR) is a perceived barrier to use.

Purpose: We aimed to determine the feasibility of a 12-week course of vaginal oestrogen in women with early breast cancer (EBC) on aromatase inhibitors (AIs).

Methods: We conducted a single arm phase 2 feasibility study of oestradiol (10mcg Vagifem Low™) administered intravaginally daily for 2 weeks then twice weekly for 10 weeks. Primary endpoint was adherence to treatment for 12-weeks (>70% participants completing >80% of doses). Secondary endpoints, assessed baseline, 2, 12-weeks, were: improvement in individual vaginal symptoms (dryness, pain, itch, irritation, pain on penetration), most

bothersome symptom (MBS) (none=0, mild=1, moderate=2 or severe=3), and urinary symptoms (ICIQ FLUTS); improvement in sexual function and quality of life (DIVA, EORTC QLQ C30); fear of cancer recurrence (FCRI SF); patient acceptance & ease of use; adherence to endocrine therapy; changes to serum hormone levels (oestradiol, oestriol, FSH); safety.

Results: We recruited 64 participants across 5 sites from November 2020 to July 2024, 2 participants withdrew. Here we report data for the first 56 participants (6 remain on study), final results will be available October 2024. Participants' median age was 57, 70% (39/56) were currently sexually active, 52% (29/56) postmenopausal at EBC diagnosis, and 21% (12/56) were on AI+ovarian function suppression. The primary endpoint is likely to be met with an 88% (49/56) adherence rate to date. We saw significant moderate-to-large improvements in MBS (2 weeks mean Δ -1.37, p<.001, 12 weeks mean Δ -1.48, p<.001) and each individual symptom after 2 weeks (dryness mean Δ -1.38, p<.001; itch mean Δ -0.37, p=.001; pain mean Δ -0.46, p=.001; irritation mean Δ -0.65, p<.001; dyspareunia mean Δ -0.92, p<.001) and 12 weeks (dryness mean Δ -1.46, p<.001; itch mean Δ -0.39, p.001; pain mean Δ -0.45, p<.001; irritation mean Δ -0.68, p<.001; dyspareunia mean Δ -1.08, p<.001). Additionally, day-to-day impact of vaginal ageing (DIVA questionnaire) domains daily living (mean Δ -0.36, p=0.01); emotional well-being (mean Δ-0.85, p<.001); sexual functioning (mean Δ-1.27, p<.001); self-concept & body image (mean Δ-0.79, p<.001) improved after 12 weeks. FCRI-SF scores indicated moderate FCR levels at baseline (M=17) but improved at 12 weeks (mean Δ-1.4, p=0.01), suggesting no short-term increased FCR with use of vaginal oestrogens. Few adverse events reported were reported and all were grade 1 or 2.

Conclusion: Women adhered to vaginal oestrogens over 12 weeks in our study. Our results suggest a randomised controlled trial involving vaginal oestrogen administration would be feasible and acceptable to women with EBC.

P1-04-15: Real-World Analysis of Safety and Efficacy of Metastatic Breast Cancer Patients Receiving Capivasertib

Mara Hofherr, Katherine Clifton

Background: CAPItello-291 was a randomized, double-blind, placebo-controlled trial which resulted in the FDA approval of capivasertib, an AKT inhibitor. Improvement in progression free survival (PFS) was only seen in patients with PIK3CA/AKT1/PTEN-alterations. For patients with these alterations, capivasertib with fulvestrant is an option after progression from at least one endocrine based therapy. To date, real world outcome analyses are limited.
Methods: In this retrospective, single center study we examined patients with metastatic breast cancer who received at least one week of capivasertib and one dose of fulvestrant. IRB approval was obtained. Patient specific alterations, previous treatments, weeks on treatments, and adverse drug events (ADE) were collected from a central EMR.
Results: 29 patients with metastatic breast cancer treated from November 30, 2023 – July 1,

2024 who received capivasertib and fulvestrant were analyzed. Mean age was 63.9. All patients were female and had a PIK3CA/AKT1/PTEN-alteration. 12 patients had ECOG 0, 13 patients had ECOG 1, 4 patients had ECOG 2-3. Patient previously received an average of 3.6 lines of treatment (1 – 11). 19 patients continue on capivasertib with an average of 17 weeks. 9 patients stopped capivasertib due to progression or intolerance with an average of 5.8 weeks on treatment. 25 patients (86%) reported any ADE. 9 patients (36%) reported grade 3 or higher side effects. Expected side effects included diarrhea (50%), rash (24%), nausea (24%), hyperglycemia (24%). Unexpected side effects included AKI (6.8%), DRESS (3.4%), LFTs 5x ULN (6.8%), colitis (3.4%). Median onset of diarrhea was 9 days (1-45 days). Patients were prophylactically prescribed cetirizine to prevent rash. Two patients with severe rash did not receive prophylactic cetirizine.
Conclusions: The authors saw similar rates of ADE that were described in CAPItello-291 and continue to explore underreported ADE. All ADE seen in this trial were appropriately managed. Median event free survival and progression free survival was not reached as of this analysis. Capivasertib appears to be safe and effective in a real-world population.

P1-04-16: Racial Disparities in the Pro-Metastatic Tumor Microenvironment of Treatment-Naive Breast Cancer

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Background: Black women have higher rates of distant recurrence and poorer survival in estrogen receptor-positive (ER+) breast cancer compared to white women. Such disparity may be attributed to underlying biological differences related to the pro-metastatic tumor microenvironment (TME). Our group has recently shown that residual estrogen receptor-positive (ER+) breast cancer after neoadjuvant chemotherapy (NAC) exhibits a higher density of Tumor Microenvironment of Metastasis (TMEM) doorways in Black women compared to white women. TMEM doorways are portals for hematogenous dissemination of cancer cells to distant organs. However, the baseline levels of TMEM doorways in treatment-naïve breast cancer tissues across different racial backgrounds have not been previously investigated. We hypothesize that racial disparity in pro-metastatic markers is due to baseline biological differences in the treatment-naïve tumor microenvironment between racial backgrounds. To address this question, we conducted a multi-institutional study to evaluate TMEM doorway score, macrophage density, and microvascular density in treatment-naïve breast tissues from a large cohort of 330 patients (184 Black patients and 146 white patients).
Methods: We performed TMEM doorway triple immunohistochemistry which visualizes each of the three components of the doorway: macrophage (CD68), tumor cell (panMena), and endothelial cell (CD31). Following staining, digitally scanned slides were imported into

and analyzed using the Visiopharm image analysis software. Self-identified race was used per patients' medical records. Patient demographics were analyzed with Wilcoxon rank sum test (continuous variables), Chi-squared tests, and Fisher's exact tests (categorical variables). Distant recurrence-free survival was analyzed using log-rank test and multivariate Cox regression model.
Results: Black patients compared to white patients had higher TMEM doorway scores in the entire cohort ($p<0.001$). This disparity was evident in the ER+/HER2- breast cancer subtype ($n = 142$, $p = 0.015$) but not in the TN breast cancer ($n = 79$, $p = 0.86$). In addition to TMEM doorways, we evaluated the individual components of TMEM doorways: macrophage density and microvascular density. While macrophages followed a similar pattern as TMEM doorways, microvascular density did not show any racial disparity. In the entire cohort, Black patients had higher BMI compared to white patients (mean=24.3) ($p=<0.001$). Black and white patients did not differ with respect to age, tumor stage, tumor grade, or lymph node stage. In this cohort, white patients were more likely to develop distant recurrence compared to Black patients (56% vs. 41%, $p=0.007$). Unadjusted Kaplan-Meier curves showed that higher macrophage density was associated with better DRFS ($p=0.04$), while TMEM doorway score and microvascular density were not associated with DRFS in the entire cohort. A multivariable Cox model showed no association between TMEM doorway score and DRFS ($p=0.15$).
Conclusion: In summary, our study shows that pro-metastatic tumor microenvironment (higher TMEM doorway score and macrophage density in the TME) is more pronounced in treatment-naïve breast cancer from Black patients compared to white patients. In contrast to our previous study's finding of an association between higher TMEM doorway scores and poorer DRFS in patients who received NAC, TMEM doorways in treatment-naïve tissue was not associated with DRFS in this cohort. This difference could be explained by changes in TME that occur after NAC and needs further evaluation.

P1-04-17: Exosomes as drivers and biomarkers of TAM recruitment and immune evasion in inflammatory breast cancer

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Background. Inflammatory breast cancer (IBC) is a rare and aggressive type of BC with a very poor prognosis and that accounts for 10% of BC-related deaths. Diagnosis and treatment of IBC are particularly challenging, as its symptoms resemble mammary infection, and the tumor has already metastasized at the time of diagnosis. Compared to non-IBC (nIBC), IBC tumors are enriched in pro-tumorigenic, M2-polarized tumor-associated macrophages (TAM) with immunosuppressive functions. Despite growing body of work, the mechanisms controlling IBC development and immune landscape are largely unknown. In particular, we still do not understand (a) how tumor cells drive infiltration of TAMs in the tumor microenvironment; (b) whether IBC intra-tumor TAMs dampen T cell

responses as in other types of cancers, thus allowing tumor growth; (c) how to identify patients at high risk of developing IBC and, once diagnosed, the individuals at risk of disease progression. Exosomes are small circulating extracellular vesicles that mediate cell-to-cell communication that are released by cancer cells locally in the primary tumor and in the blood circulation, thus potentially serving as predictive biomarkers and therapeutic targets for the systemic effects of the disease. We have previously shown that exosomes are functional determinants of BC progression and prepare distant sites for metastatic seeding by establishing favorable pre-metastatic niches, but their role in IBC outcomes is not known.
Methods. We have analyzed the proteomic profile of exosomes from IBC (MDA-IBC3, SUM-149, SUM-190, KPL4, and FC-IBC-02) and nIBC cell lines (MCF-7 and MDA-MB-231) as well as plasma exosomes from IBC and nIBC patients and healthy controls by liquid chromatography mass spectrometry. The functional role of cell line- or patient-derived exosomes was tested via incubation with THP-1 cells.
Results. We have identified a signature of IBC-specific proteins that were enriched more than 2-fold in IBC cell exosomes compared to nIBC cell exosomes, regardless of estrogen receptor (ER) or human epidermal growth factor receptor 2 (HER2) status. Among them, polypeptide N-acetylgalactosaminyltransferase 7 (GALNT7) was found up-regulated 5-fold in IBC exosomes. GALNT7 is an O-glycosylating enzyme that is highly expressed in cancer cells of BC patient biopsies. O-glycosylation in TAMs is associated with polarization towards a pro-tumorigenic, M2-like phenotype and significantly increases tumor growth. Moreover, interaction of macrophages with membrane O-glycans expressed on GALNT7-expressing tumor cells or other macrophages receiving GALNT7-enriched exosomes triggers the expression of activation markers found in IBC-associated TAMs, including CD206, CD163, and PD-L1. We also detected a specific, TAM-related protein signature in exosomes from plasma of IBC patients compared to nIBC or healthy controls that may help with stratification and treatment of IBC patients.
Conclusions. Based on our preliminary data, we propose that IBC cell-derived exosomes promote reprogramming of TAMs to shape an immune-suppressive tumor microenvironment and support disease progression, and as such may serve as “liquid biopsy” biomarkers for IBC diagnosis and prognosis.

P1-04-18: Mediator Subunit 1 (MED1) and tumor-secreted small extracellular vesicles (sEV) in breast cancer immune evasion and progression

Mahendra Jadhao, Yongguang Yang, Gregory Bick, Xioating Zhang

Breast cancer is the most common malignancy in women and HER2+ breast cancer has one of the poorest prognosis. HER2-targeted therapies in combination with immune checkpoint inhibitors (ICI) have shown promising preliminary results; however, acquired HER2 resistance limited the efficacy of advanced therapeutics. MED1 is a transcription regulator co-amplified with HER2 and plays a critical role in the progression and metastasis of HER2+

breast cancer. We recently generated a MMTV-HER2/MED1 double overexpression mouse model and observed increased tumor burden, stemness, and metastasis. Single-cell sequencing revealed MMTV-HER2/MED1 tumors had lower tumor-infiltrating CD4 and CD8 T cells and increased accumulation M2 macrophage population; which was further confirmed by IHC analysis. We also found that MMTV-HER2/MED1 tumor cells secreted more and larger small extracellular vesicles (sEVs) than MMTV-HER2 tumor cells. Importantly, MMTV-HER2/MED1 but not MMTV-HER2 tumor cell-secreted sEVs promote M2 polarization of BMDMs while depletion of sEVs abolished that effect. Next, we performed sEV miRNA-seq and identified mmu-miR125b as a top upregulated miRNA involved in M2 macrophage polarization. We found that mmu-miR125b mimic treatment promotes M2 polarization of mice BMDMs in a dose-dependent manner but miR125b-depleted sEVs lose that capability. Moreover, we have carried out in vivo MED1 depletion using p-HER2apt-siMED1 RNA nanoparticles in MMTV-HER2/MED1 mice and found greatly reduced tumor burdens. Consistent with the above, IHC analysis revealed MED1 depletion reduced M2 macrophage (F4/80+ and CD206+) population with an increased accumulation of cytotoxic TIL (CD8α+ and CD4+) populations. Interestingly, we have also observed the downregulation of IRF1/PD-L1 signaling axis in MMTV-HER2/MED1 tumor tissues after the treatment. We further confirmed upregulation of IRF1/PD-L1 in MMTV-HER2/MED1 tumor cells compared to that of MMTV-HER2 tumor cells while MED1 knockdown inhibited IRF1/PD-L1 signaling axis in both MMTV-HER2/MED1 and human BT474 cells. ChIP-qPCR results further supported the direct recruitment of MED1 on IRF1 promotors to regulate PD-L1 expression. Finally, p-HER2apt-siMED1 RNA nanoparticles cotreated with anti-PD1 antibody significantly attenuated MMTV-HER2/MED1 tumor burden; highlighting potential adjuvant effect of p-HER2apt-siMED1 RNA nanoparticles. Collectively, our studies revealed the roles of MED1 in regulating breast cancer immune microenvironment and the potential use of MED1 targeted therapy alone or in combination with current regimens for better treatment of breast cancer

P1-04-19: Regulating Stress to Improve Antitumor Immunity in Breast Cancer Models.

Gilberto Gastelum Martinez, Marc E. Lippman, Barry Hudson, Philip Miller, Courtney Pine, Yalini Anbalagan

Chronic stress significantly impacts cancer progression by fostering an immunosuppressive microenvironment that promotes tumor growth and diminishes therapeutic efficacy. Dysregulated stress hormones, like norepinephrine, mediate β-adrenergic stimulation, orchestrating a cascade of immunomodulatory events within the tumor microenvironment (TME). In estrogen receptor-positive (ER+) breast cancer, immunosuppression presents a challenge due to resistance to immunotherapy driven by molecular characteristics dampening antitumor immune responses.
We explore the hypothesis that chronic stress, mediated by dysregulated stress hormones, drives immunosuppression in ER+ breast cancer, hindering treatment efficacy.

Manipulating thermal stress in the laboratory setting provides a unique approach to studying β-adrenergic stimulation in cancer, particularly its impact on the immune microenvironment. Standard housing conditions for mice result in an environment below their thermoneutral point, inducing increased β-adrenergic stimulation. Conversely, housing mice near their thermoneutral point maintains basal levels of β-adrenergic stimulation, which is associated with delayed tumor growth and improved antitumor phenotypes in different cancer models. Leveraging a syngeneic ER+ breast cancer model, we investigated housing mice at thermoneutral temperatures to mitigate stress-induced immunosuppression.
Tumor-bearing mice housed at thermoneutral temperatures exhibited decreased stress hormone levels compared to those housed at standard conditions, correlating with delayed tumor growth. Further analysis revealed notable shifts in immune cell composition within the TME. Mice subjected to thermal stress regulation displayed increased accumulation of CD8+ T cells, reduced PD1 expression on CD8+ T cells, and decreased accumulation of regulatory T cells (Tregs), indicating a shift towards a more immunostimulatory microenvironment.
In conclusion, disrupting stress-induced immunosuppression and promoting cytotoxic T cell activity holds profound clinical relevance for individuals with stress-mediated diseases, particularly cancer. By exploring how stress influences immune responses within tumors, our research aims to uncover underlying mechanisms driving tumor progression and immunosuppression in ER+ breast cancer. These insights could inform the development of targeted therapies aimed at modulating stress-related pathways or enhancing immune function to improve treatment outcomes in patients with ER+ breast cancer. Furthermore, understanding the intricate interplay between stress and the immune system may have broader implications for managing stress-related comorbidities in cancer patients, such as anxiety or depression, which can influence immunosuppression, impact treatment efficacy, and overall quality of life. Therefore, the knowledge gained from our study could provide valuable insights into the role of stress in cancer progression and guide the development of therapeutic strategies to mitigate its detrimental effects on the immune response and overall disease outcomes in ER+ breast cancer patients.

P1-04-20: Difference in transcriptional expression and immune infiltration analysis between recurrent and de novo stage IV HER2-negative breast cancer in the NEWBEAT trial; WJOG9917BTR.

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Background: We have conducted a phase II trial (NEWBEAT) to evaluate the efficacy of triple therapy with nivolumab, paclitaxel, and bevacizumab in patients (pts) with HER2-negative metastatic breast cancer (MBC). Transcriptional expression is expected to differ

between recurrent and de novo stage IV breast cancer (BC); however, the data on this topic are limited. In an ancillary study (WJOG9917BTR), RNA sequence analyses were performed to elucidate this question.
Methods: The NEWBEAT study enrolled 57 pts and showed that the median progression-free survival (PFS) and overall survival (OS) were 14.0 months and 32.5 months, respectively, with a median follow-up of 29.5 months. In this biomarker study, transcriptional expression of tissue samples from surgical specimens or biopsies before treatment in pts treated with triple therapy was assessed by RNA sequencing. Immune infiltration analysis was performed using the CIBERSORT method.
Results: The biomarker study included 50 pts (36 with recurrent BC and 14 with de novo stage IV BC). Although the PFS and OS were not significantly different between recurrent and de novo stage IV pts (PFS: 14.3 and 18.8 months [p = 0.230], OS: 29.5 and 32.9 months [p = 0.1287], respectively), a significantly higher rate of responders (defined as PFS ≥1 year) was observed in de novo stage IV pts compared to recurrent pts (79% and 47%, respectively, p = 0.0393). Among 50 pts, 24 tumor samples were available for RNA sequence (RNA-seq) analysis, including 19 recurrent disease and 5 de novo stage IV. In recurrent disease, RNA-seq showed activation of the Wnt signaling pathway, the p53 pathway, and the endothelin signaling pathway, which are involved in angiogenesis and cell proliferation, compared to de novo stage IV. CIBERSORT analysis revealed a lower expression of plasma cells, gamma-delta T cells, and eosinophils in recurrent disease, indicating a lower immune infiltration status. Activation of these pathways may contribute to immune evasion and the formation of an immunosuppressive environment, suggesting that anti-angiogenic agents could potentially play an important role when combined with immune-checkpoint inhibitors in recurrent disease.
Conclusions: Our analysis showed a different immune status between recurrent and de novo stage IV BC. These data suggest that recurrent BC may require a different immunotherapeutic strategy compared to de novo stage IV BC. (UMIN000029590)

P1-04-21: Suppressing suppression: histone deacetylase inhibition primes the metastatic tumor microenvironment to respond to checkpoint inhibition by dynamic rewiring of cell interactions

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Immune checkpoint inhibition (ICI) is a type of immunotherapy that has been shown to promote durable responses in a minority of patients with metastatic, triple negative breast cancer expressing programmed death receptor ligand 1 (PD-L1), and only in combination with chemotherapy. In the breast expansion cohort of our Phase Ib trial (NCI-9844) we found that the combination of histone deacetylase inhibitor, entinostat, with dual ICI (anti-PD1 and anti-CTLA4), is safe, and led to a 25% objective response rate in heavily pretreated patients with metastatic HER2 negative breast cancer with expected rates of irAEs. In

addition, our preclinical studies support response in triple negative and HER2 positive mouse models of breast cancer. Evaluation of changes in the breast tumor microenvironment (TME) from these models revealed that decreased infiltration or suppression of myeloid derived suppressor cells (MDSCs) by entinostat is a potential mechanism of action. Here, we examined breast-to lung metastases from both HER2 positive and triple negative metastatic mouse models, Neu-N and 4T1, to gain a comprehensive understanding of changes to the tumor immune microenvironment within the metastatic niche induced by treatment with entinostat, and perpetuated following treatment combination with dual ICI. Single cell RNA sequencing of treated lung metastasis confirmed decreased granulocytic-MDSCs (G-MDSC) as seen in previous studies, and revealed significant shifts in C1q macrophages, metabolically activated macrophages, classic dendritic cells, activated and primed Th2 T cells, terminally differentiated T regulatory cells and some sub-populations of B cells. Multiparametric flow analysis was not concordant suggestive that functional changes are more likely contributing to mechanisms of response and decreased immune suppression. Entinostat treatment led to an increase in stemness in tumor cells and decreased mesenchymal gene expression. We then adapted cell circuit and CellChat analysis platforms to investigate cell interactions which revealed significant immune cell interactions affected by entinostat are MDSC- CD8-T cell, macrophage- CD8-T cell and NK- G-MDSC/ CD8-T cell interactions. Ligand receptor pair analysis then revealed signaling pathways controlling chemokine secretion, and cell adhesion are significantly decreased following treatment with entinostat and are preserved upon treatment with dual ICI. From this analysis, we are investigating 6 candidate targets on MDSCs and macrophages using ex-vivo suppression assays as compared to treatment with entinostat, to determine the contribution of these signaling axes in decreasing immunosuppressive function within the TME. We are also using multiplex immunohistochemistry to examine cellular interactions within breast-to-lung metastatic tumors. We will then validate findings in metastatic tumor samples from patients designated as responders from NCI-9844, treated with entinostat + dual ICI. Preliminary evaluation suggests a significant decrease in MDSC-CD8-T cell and Macrophage-CD8-T cell interaction in responders, supportive of our preclinical data. Overall, this investigation of breast-to-lung metastatic tumors reveals how entinostat decreases immune suppression via interference of MDSC and macrophage interaction with CD8-T cells as corroborated in patients who responded to treatment. Future studies will elucidate the molecular mechanisms of action and investigate the potential for identified targets as biomarkers of response and for potential as novel therapeutic targets to improve response rates in patients with advanced breast cancer.

P1-04-22: Loss of SMAD4 Drives Breast Cancer Invasion and Metastasis through E2F1-Mediated Activation of the PEG10-ERK Axis

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Objective: Although SMAD4 mutations are rare in breast cancer, its loss is strongly associated with metastasis and poor prognosis. This highlights the need to elucidate the

mechanisms by which SMAD4 influences breast cancer progression. In this study, we examined the invasive and metastatic properties of SMAD4-deficient breast cancer cell lines, alongside transcriptomic changes in SMAD4 knockout (KO) models, with the aim of uncovering the molecular pathways through which SMAD4 regulates breast cancer recurrence and metastasis.
Methods: We analyzed SMAD4 expression across multiple bioinformatics platforms and in 102 clinical breast cancer samples. Using CRISPR/Cas9, we generated SMAD4-knockout cell lines and conducted both in vitro and in vivo assays to assess the impact of SMAD4 depletion on tumor growth and metastasis. RNA sequencing (RNA-seq) was employed to identify downstream targets and pathways influenced by SMAD4, while Cut&Tag-seq provided direct evidence of SMAD4's role as a transcriptional co-regulator controlling the expression of key target genes.
Results: SMAD4 was found to be significantly downregulated in breast cancer samples, with this downregulation correlating with adverse clinicopathological features and increased risk of recurrence and metastasis. Mechanistically, we discovered that SMAD4 suppresses tumor progression via the PEG10/ERK signaling axis. Disruption of PEG10/ERK signaling abrogated the pro-metastatic effects of SMAD4 loss. Further, we demonstrated that SMAD4 interacts with the transcription factor E2F1, enhancing its transcriptional activation of PEG10, which in turn activates ERK signaling and promotes breast cancer cell invasion and metastasis.
Conclusion: Our study position SMAD4 as a pivotal transcriptional co-regulator in breast cancer, with important implications for its role as a prognostic biomarker and tumor suppressor. We also underscore the critical contribution of the SMAD4-PEG10-ERK axis in driving breast cancer metastasis, providing potential therapeutic targets for intervention.

P1-04-23: Subpopulations of tumor-infiltrating lymphocytes features in ER-low breast cancer

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Introduction: ER-low breast cancer (BC) is a special subgroup of tumors with the level of estrogen receptors (ER) in the range from 1% to 10% (total score according to IHC data – 3). Most often, either strong ER staining or its complete absence is determined, which of course makes it difficult to study cases with low ER expression. Overall, the prevalence of ER-low BC has ranged from 3% to 9%, however, due to the extremely high incidence of BC, the population of this subgroup is still of great importance. We analyzed the subpopulations of tumor-infiltrating lymphocytes (TILs) and tried to identify new predictive and prognostic factors to determine the immunological profile of this rare subgroup.
Materials and methods: This prospective trial included 90 patients (pts): 84 (93.3%) had 0-2 IHC scores according to Allred scoring and considered as triple negative BC (TNBC), 6 (6.7%) had 3 IHC scores and considered as ER-low. Both stromal and intratumoral

subpopulations of TILs was assessed with flow cytometry on a tumor sample obtained by core biopsy immediately before the start of neoadjuvant chemotherapy (NACT). All the pts received NACT with 4 cycles of dose-dense AC every 2 weeks (doxorubicin 60 mg/m2 + cyclophosphamide 600 mg/m2) then 12 cycles paclitaxel 80 mg/m2 + carboplatin AUC2 weekly in a N.N. Blokhin National Medical Research from 2018 to 2023.
Results: The level of TILs did not differ between the groups of TNBC (0.6%) and ER-low BC (0.8%, p=0.081). The analysis revealed a significant difference in the level of CD4+CD25+ activated lymphocytes: 8.0% in ER-low and 10.4% in TNBC (p=0.035). It is also worth noting quantitative, but not statistically significant differences in the level of NK cells (11.9% and 4.4%, respectively), NKT (11.1% and 9.3%, respectively) and B lymphocytes (4.6% and 2.0% respectively). Level of tumor infiltration with CD3+CD4+, CD3+CD8+, CD3+, CD8+, CD4+CD25highCD127−/low, CD3-CD19+, CD3-CD16+CD56+, CD3+CD16+CD56+, CD4+CD279+, CD8+CD279+, CD3-CD8+, CD3+HLA-DR+, CD3-HLA-DR+, HLA-DR+, CD8+CD28+, CD8+CD28-, CD8+CD28+/CD8+CD28-, CD4+/CD8+, 11b+28-, 11b+28+, 11b-28-, 11b-28+, CD8+CD4+, CD4+CD152+ lymphocytes subtypes did not differ significantly in the ER-low and TNBC groups. In the ER-low subgroup there were 16.7% of pathological complete responses (pCRs) versus 52.9% in TNBC (p=0.122).
Discussion: CD4+CD25+ and B-lymphocytes are known to be immunosuppressive and are important in terms of pCR reaching after NACT. It can be assumed that its level in ER-low tumors will be higher compared to TNBC, however, in our work, the data obtained do not agree with these deviations. The same applies to regulators of NKT cells, whose task is to regulate the immune response, both reducing and increasing the antitumor potential, including cytotoxic NK cells.

P1-04-24: Prognostic Value of Stromal Markers in Triple-Negative Breast Cancer: A Digital Image Analysis Approach

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Background: Triple negative breast cancers (TNBC), a heterogenous group of tumors represent about 15% of all breast cancers with limited biomarkers and treatment options. Tumor-stroma ratio (TSR) is a proven prognostic and predictive factor in various tumor types, including breast carcinomas, representing the stromal percentage in the most stroma abundant area of the tumor. The overall stromal ratio (OSR), which defines the stroma ratio of the entire tumor area on whole slide images (WSI) has scarcely been examined in the literature. Several studies suggest that changes in the extracellular matrix (ECM) composition and organization could promote tumor growth, but the results have not been consistent. The aim of our study was to investigate the prognostic value of stromal ratio and specific ECM proteins, including major structural components as type-I and type-III collagens as well as fibrillin-1. Understanding whether the expression of these proteins is associated with clinicopathological parameters and outcomes in the TNBC subtype offers valuable insight into the molecular mechanisms driving tumor development and metastasis.

We sought to determine if automated digital image analysis (DIA) methods could provide an objective approach for analyzing stromal ratio and ECM components on H&E and immunohistochemically (IHC) stained slides.
Methods: Our cohort included 101 female TNBC patients, primarily treated with surgery between 2005 and 2016. Representative H&E-stained WSIs were used to evaluate the TSR and OSR by two observers visually and through DIA. IHC was performed on tissue microarrays (TMAs) and scoring was determined both visually and through DIA for type-I collagen expression, type-III collagen expression and intensity, and fibrillin-1 expression and intensity. We employed the QuantCenter module of the SlideViewer program (v2.6; 3DHISTECH Ltd., Hungary), a DIA tool, to calculate the percentage of clusters representing the tumor, stroma, and background or cell-free area, as well as the intensity of the IHC staining. Statistical analyses were conducted using IBM SPSS statistics (v29 for Windows).
Results: The intra- and interobserver variability of the determination of TSR and OSR scores were good or excellent and significant ($r=.866-.979$, $p<.01$). The correlation between the visual and DIA assessed scores were good or excellent for both on the H&E and on the IHC-stained TMAs ($r=.927-969$), although only poor to moderate determining intensity of the IHC-staining ($r=.398-.566$). We found that high OSR correlates with worse overall survival, advanced pN categories, lower sTIL, lower mitotic index, and patient age ($p<.05$). TSR showed significant connections to the pN categories and mitotic index ($p<.01$). High type-I collagen (>45%), type-III collagen (>30%), and fibrillin-1 (>20%) expression levels were linked to significantly worse OS ($p=.004$, $p=.013$, and $p=.005$, respectively) and PFS ($p=.028$, $p=.025$, and $p=.002$, respectively) suggesting their roles in promoting tumor progression and metastasis. Multivariate analysis confirmed the independent prognostic value of high DIA OSR (HR=1.037, $p=.028$), type-I collagen (HR=3.075, $p=.01$), type-III collagen (HR=3.467, $p=.007$), and fibrillin-1 (HR=3.439, $p=.017$) for OS and type-I collagen (HR=2.725, $p=0.017$), type-III collagen (HR=3.825, $p=.011$), and fibrillin-1 (HR=3.815, $p=.018$) for PFS.
Conclusion: We identified promising extracellular matrix protein biomarkers that had not been previously examined in detail in TNBCs. Additionally, DIA proved to be a valuable tool in reliably separating the tumor tissue from its adjacent microenvironment in H&E-stained WSI for the objective measurement of TSR and OSR and to measure the expression of ECM proteins in IHC-stained samples.

P1-04-25: Fast and accurate detection of tumor-immune cell interactions with deep learning

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Background: Our previous studies demonstrated liquid biopsy-derived circulating tumor cells (CTCs) not only serve as predictive biomarkers of therapy response and overall survival of breast cancer patients, but also drive new metastasis, especially by multicellular

CTC clusters with enriched stemness and up to 50 times higher metastatic potential than single CTCs (Cancer Discovery, 2019 and 2023). However, CTC detection remains challenging, and its characterization is limited due to the rare frequencies in blood cells and labor-intensive manual validations of CTC images analyzed by CellSearch and a few other methods.
Methods: Utilizing FDA-approved CTC CellSearch platform and advanced single-cell sequencing technologies for CTC and immune cell profiling, we have analyzed over 2,000 blood biospecimens longitudinally collected from patients with advanced breast cancer (N=445), including CTC images and CTC interactions with immune cells. Harnessing the power of machine learning and deep learning algorithms for CTC and immune cell image analyses, this study investigates the significance of CTCs, immune cells, and CTC clusters in predicting patient prognosis.
Results: By analyzing CellSearch raw images obtained from blood of breast cancer patients, we developed a python-based CTCpose analysis platform for cell feature analyses in connection with clinical relevance of various cell populations and intercellular communications. Our analysis automatically identified CTCs, immune cells, and CTC clusters differentiated by homogeneity or heterogeneity in cellular composition. By utilizing artificial intelligence (AI) algorithms, we extracted cellular features and classifications to identify different cell types and cell clusters at an accuray and sensitivity over 97%. Specifically, we found expression patterns of biomarkers such as cytokeratin, CD45, and DAPI to discern the spatial distribution and intensity within samples.
Conclusions: The study has explored the potential prognostic values of CTCs, immune cells, and CTC immune cell interactions by correlating their presence, abundance, and spatial information, with clinical outcomes. These outcomes may include patient survival, disease progression, and treatment response. By combining multidimensional data derived from cell morphology, biomarker expression, and spatial relationships, we aim to develop predictive models capable of stratifying patients into unique risk groups.

P1-04-26: THE USE OF IMMUNE CELL HEATMAPS IN UNRAVELLING THE TUMOR MICROENVIRONMENT IN INFLAMMATORY BREAST CANCER.

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Background: Inflammatory breast cancer (IBC) is a rare but highly aggressive subtype of breast cancer. The tumor microenvironment (TME) plays an important role in its clinical phenotype, evolution and outcome. Cytotoxic CD8+ T cells (Tcyt) are linked to a better prognosis, while CD163+ tumor-associated macrophages (TAMs) seem to influence the rapid tumor progression of IBC and correlate with an unfavorable prognosis. Usually, immune cells in the TME are evaluated on the whole slide, but the methods used may differ significantly. Moreover, not only the quantity, but also the spatial localization of immune cells seems to be important. Therefore, we analyzed the density of CD8+ and CD163+ cells after heatmaps were generated based on their localization. Focusing only on the densities of

hot spots and cold spots could increase standardization and replace a time-consuming evaluation of the whole slide. Our aim is to demonstrate the feasibility of this approach and evaluate the correlation of this heatmap with clinicopathological characteristics and outcome parameters.
Method: In this retrospective cohort study, data were collected as part of an international collaboration since 1996. We included 142 patients with IBC who received their initial diagnosis and started treatment between June 1996 and December 2016. First, consecutive FFPE slides were stained with validated antibodies and digitized for further evaluation and quality control. Next, an image analysis algorithm in Visiopharm marked DAB+ immune cells and localized them spatially with XY coordinates. We then generated heatmaps showing hot and cold spots as areas of highest and lowest density (using the 90th and 10th percentile) and finally calculated the overlap of the CD8 and CD163 hot spots.
Results: Most tumors were hormone receptor (HR) positive (60%, 85/142). The median sTIL score of this cohort was 12.5% (95% CI [1-80]) and 39% of the samples were PDL1 positive. Evaluation of the density in only the hot spots of both CD8 and CD163 showed a very strong correlation compared to the density of the whole slide (ρ= 0.91 and ρ= 0.92 respectively, P< .001).
The presence of CD163+ TAMs correlated with more aggressive disease characteristics, both when evaluated with hot spots (HS) and on the whole slide (WS): nodal involvement (HS: P= .005 & WS: P= .04), higher tumor grade (HS: P< .001 & WS: P= .005), HR negativity (HS: P= .02 & WS: P= .04) and PDL1 positivity (HS: P< .001 & WS: P< .001). In contrast, CD8+ Tcyt correlated only with PDL1 positivity and more sTIL, and there was also no difference in evaluation of the WS or HS.
Both pathological complete response (pCR) and disease-free survival (DFS) were evaluated in 108 patients that presented without metastatic disease at diagnosis and received neoadjuvant chemotherapy (NACT). Patients who achieved pCR after NACT had more CD8+ and CD163+ cells, regardless of whether evaluation was done using the WS (CD8: P= .02 / CD163: P= .006) or only the HS (CD8: P= .03 / CD163: P= .01).
Finally, DFS was evaluated after dichotomizing the immune parameters. CD163+ TAMs were associated with shorter DFS (HR: 0.58, 95% CI [0.34-0.99], P= .04). This was borderline not significant in the HS analysis (HR: 0.60, 95% CI [0.34-1.05], P= .07). Although the presence of CD8+ Tcyt was not associated with longer DFS, an increasing overlap of the HS of CD8 with the HS of CD163 was associated with shorter DFS (HR: 0.59, 95% CI [0.33-1.03], P= .06), indicating the importance of immune cell interactions.
Conclusion: Using heatmaps, we evaluated the density of CD8 and CD163 in both hot spots and the whole slide. There was a strong correlation between the two evaluations, proving the feasibility and reproducibility of this approach. Moreover, the presence of CD163+ TAMs was associated with more aggressive disease characteristics and worse prognosis in IBC, both when using hot spot or whole slide evaluation.

P1-04-27: Characterizing Tumor-Infiltrating Lymphocytes and Their Clinicopathological and Prognostic Significance in Triple-Negative Breast Cancer: an Analysis Focusing on Natural Killer Cells and Spatial Distribution

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Background: Triple-negative breast cancer (TNBC) is a heterogeneous disease with a poor prognosis and limited treatment options. The tumor microenvironment, particularly tumor-infiltrating lymphocytes (TILs), plays a critical role in prognosis and response to therapy. High TIL levels generally correlate with better outcomes in TNBC, yet specific immune cell subtypes and their spatial distribution remain poorly understood. Our study aimed to characterize TIL composition, correlate these findings with clinicopathological features and patient outcomes, and investigate impact of heterogeneity of TILs on prognosis of TNBC patients.
Methods: We analyzed 36 TNBC samples through PanCancer Immune Profiling using NanoString nCounter assays to identify differentially expressed immune-related genes in relation to TIL levels. Next, we evaluated protein expression of selected markers through immunohistochemical staining on tissue microarray using 155 TNBC samples. Clinicopathological features and survival data were correlated with TIL infiltration and its subsets, including CD4+, CD8+, CD56+, CD57+, Granulysin (GNLY)+, and Granzyme B (GZMB)+ TILs. To comprehensively assess the expression of cytotoxic T lymphocyte (CTL) and natural killer (NK) cell markers, CTL-NK score was devised based on CD8+, CD56+, CD57+, GNLY+, and GZMB+ TIL levels, and its association with survival was analyzed. Additionally, the heterogeneity of TIL infiltration within tumors was evaluated and correlated with outcome of the patients.
Results: Differential expression of gene analysis revealed significant upregulation of NK cell-associated genes including GNLY, KLRC2, and GZMB in TIL-high TNBCs. Immunohistochemical validation confirmed that TNBCs with higher TIL had more CD56+, CD57+, GNLY+, and GZMB+ TILs, not only in absolute number but also in proportion relative to CD4+ or CD8+ TILs. High TIL infiltration correlated with some favorable clinicopathological features of tumor, including lower T stage and absence of lymphovascular invasion. In survival analysis, high CTL-NK score was found as an independent prognostic factor for better disease-free survival (DFS). Furthermore, uniformly high TIL infiltration was linked to better DFS, whereas cases with heterogeneous TIL infiltration showed no difference in survival compared with those with uniformly low TIL infiltration.
Conclusion: Our study showed that NK cell-associated gene expression and protein levels differ significantly according to TIL levels, and CTL-NK score and distribution of TILs within tumor have a prognostic value. These findings underscore the importance of both NK cells and the spatial uniformity of TIL infiltration in influencing outcome of TNBC patients,

providing valuable insights for refining prognostic assessments and guiding immunotherapeutic approaches.

P1-04-28: Comparison of tumor immune microenvironment of TNBC with different treatment outcome with neoadjuvant chemotherapy: Spatial analysis using multiplex fluorescent immunohistochemistry

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Background: Triple-negative breast cancer (TNBC) is known for its highly aggressive nature and poor prognosis. However, it has been noted to exhibit high immunogenicity compared to other breast cancer types, making it more responsive to immunotherapy. Recently, neoadjuvant chemotherapy (NAC) has become the standard treatment for early high-risk TNBC. Higher stromal tumor-infiltrating lymphocytes (TIL) predict increased pathologic complete response (pCR) and superior clinical outcomes in TNBC treated with NAC. Despite this general trend, high TIL does not always predict a favorable response, nor does low TIL always predict a poor response. This suggests that individuals with varying treatment outcomes might have different immune dynamics, even under similar TIL levels.
Objective: This study aimed to examine the tumor immune microenvironment (TIME) of pretreated TNBC samples that subsequently underwent NAC using multiplex immunofluorescence (mIHC). Our investigation explored the role of TIME in NAC response and elucidated differences in TIME among patients with similar TIL levels but varying treatment outcomes, as well as those with differing TIL levels but similar treatment responses.

Methods: We retrospectively collected medical records of 16 patients with stage II-III early TNBC treated with NAC at Gangnam Severance Hospital, Yonsei University, between January 2019 and August 2021. Two pathologists (SSJ and YJC) evaluated stromal TIL levels, tumor-stroma ratio, and PD-L1 (22C3) status (CPS). mIHC, including CK, CD4, CD8, CD20, and FOXP3, was performed for tumor cells (TC) and immune cells (IC). Spatial metrics, including the cellular densities of TC and IC, and cell-cell distances between IC, were analyzed.

Results: A total of 1,183,644 cells (761,976 TC and 421,668 IC) from sixteen patients (six pCR [4 high- and 2 low TIL] and ten non-pCR [4 high- and 6 low TIL]) were analyzed. There were no significant differences in baseline characteristics or IC densities between the pCR and non-pCR groups. In the pCR group, CD20+IC and CD8+IC were predominant IC subtypes, particularly in high TIL cases, whereas CD4+IC was the dominant fraction in the non-pCR group. Density plots of different IC types showed dense clustering of CD8+IC and CD20+IC near the TC in the pCR group. Regarding IC-IC interaction, the CD4 IC pair was the largest fraction overall. Within the pCR group, high TIL cases had a high proportion of CD20 IC pairs, whereas low TIL cases had IC pairs, including CD8+IC.

Conclusion: This study observed differences in IC composition and their distribution in pretreated TNBC samples based on pCR status and TIL levels. The findings suggest that tumoral CD8+IC and CD20+IC play crucial roles in determining the response to NAC in eTNBC. Particularly, significant infiltration of CD20+IC in high TIL conditions suggests the presence of B-T cell interaction.

P1-04-29: The expression patterns of the immune checkpoint molecules PD-1 and PD-L1 in the context of seroma development in breast cancer

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Background: Seroma formation is one of the most frequent complications after breast cancer (BC) surgery. One of the aims of the uni-center SerMa pilot study was to investigate the risk of patients for seroma development after mastectomy with or without implant reconstruction. The expression patterns of the programmed cell death protein 1 (PD-1) and its ligand (PD-L1) found in the tumor microenvironment (TME) of formalin-fixed paraffin-embedded (FFPE) tissue samples of BC patients were therefore of particular interest, since inhibitors against those molecules are currently used in BC treatment.
Methods: The checkpoint molecules PD-1 and PD-L1 were immunohistochemically stained in 80 FFPE tissue samples of patients of the SerMa pilot study cohort and positive cells were evaluated. PD-1 positive cells in the TME were examined and the presence (yes/no) was determined. To assess whether the tumor was positive or negative regarding PD-L1 expression, the immune cell (IC) score, combined positive score (CPS) and tumor proportion score (TPS) were assessed by pathologists. Cells isolated from aspirated seroma fluids (SFl) of a sub-cohort of 18 patients were analyzed using flow cytometry to identify CD3+ T cells and CD68+ macrophages with their PD-1 and PD-L1 expression.
Results: In 37 patients PD-1+ cells were detected in the TME and 15 out of the 80 were considered positive for PD-L1 (IC ≥1%; of those 4 had a CPS ≥10 and 3 a TPS ≥1%). Since we detected checkpoint molecules on our tissue samples, we were interested in their presence on cells of the SFl. We succeeded in measuring both PD-1 and PD-L1 on the surface of T cells as well as macrophages. Almost all analyzed SFl contained macrophages positive for PD-L1 (17 out of 18). In six out of the 18 SFl T cells were positive (>1%) for PD-1. In a next step, we compared the positivity of PD-1 and PD-L1 in cells of the SFl with the corresponding tissue samples (n=13). In three cases we found positive results for PD-L1 in both fluid and tissue, for PD-1 it was only 1 case. When comparing all patients with regard to negative or positive results for PD-1 and PD-L1 in tissue and SFl, there was no evidence of a significant positive or negative correlation.
Additionally, there was no significant difference in PD-1 and PD-L1 expression in the TME

between patients with and without seroma formation. However, dividing the cohort at the median age of 64 years we found significant differences between seroma developers and non-developers: the presence of PD-1+ cells in the TME of older patients (64 years and older) (p=0,032) as well as the CPS of younger patients (<64 years) (p=0,039) showed significance.
Discussion and Outlook: To the best of our knowledge, this is the first study showing PD-1 and PD-L1 expression on T cells and macrophages of SFl. However it is still unclear, whether there is a relation between the PD-1/PD-L1 positive tissue cells and the later emerging immune cells of the SFl, since the sub-cohort presented here is too small to draw any conclusions. Further research is needed to investigate the role of PD1/PD-L1 expressing immune cells in the context of seroma development in the bigger study cohort of the multi-center international SerMa study (EUBREAST 5). Our first results suggest that in older patients the presence of PD1+ cells in the TME decreased the risk of developing a seroma significantly while older age is associated with a higher risk for seroma formation.
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P1-04-30: Tumor-infiltrating lymphocytes around ductal carcinoma in situ on core needle biopsy can aid in the prediction of the presence of invasive carcinoma

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Background: Tumor-infiltrating lymphocytes (TILs) are immune cells that infiltrate tumors and their microenvironment and are associated with antitumor immune responses. TILs are reportedly useful in predicting the efficacy of chemotherapy and the prognosis of invasive breast cancer. However, there are few reports to date on TILs in noninvasive breast cancer; thus, the role of TILs is still unclear. Therefore, we decided to evaluate TILs around noninvasive carcinoma to determine whether it is possible to predict the presence of invasive carcinoma.
Methods: Our study included 212 patients diagnosed with ductal carcinoma in situ via core needle biopsy who underwent surgery from April 2014 to March 2022 at three hospitals, including our hospital. TILs were measured using needle biopsy specimens, and the percentage of lymphocytes in the stroma around noninvasive carcinomas was evaluated from 0% to 100% according to the measurement method of the International Immuno-Oncology Biomarker Working Group. We divided the percentage of TILs into two groups, high and low, with a cutoff value of 10% and examined their association with the presence of invasive areas in surgical specimens.
Results: The median patient age was 57 (30-88) years, and 66 (31%) surgical specimens showed invasive carcinoma. Of these, 152 (80%) were ER-positive and 40 (22%), HER2-positive (3+ by immunohistochemistry). There were three postoperative recurrences: two

in the axillary lymph nodes and one in the local skin. The average TIL values for the groups with and without tumor invasion were 11.1% and 7.5%, respectively, and tended to be higher in the group with tumor invasion (P =0.1517). When TILs were divided into low and high groups, 41 of 154 cases (27%) and 25 of 58 cases (43%), respectively, were found to have invasion. The high TILs group showed significantly greater invasion (p=0.0230). Univariate analysis in the presence of invasion showed significant differences in the high TIL group (HR: 2.09, 95%CI: 1.11-3.92, p=0.0230) and tumor diameter ≧14 mm on imaging (HR: 1.96, 95%CI: 1.01-3.83, p=0.0429). There was no significant difference in Ki-67≧30%, but this group tended to have greater invasion (HR: 2.16, 95%CI: 0.97-4.82, p=0.0626). Multivariate analysis using these three factors showed that a high TIL count was the most independent predictor of invasion (HR: 2.37, 95%CI: 1.12-5.04, p=0.0244).
Conclusion: These results suggest that the evaluation of TILs around noninvasive carcinoma on core needle biopsy may be predictive markers of invasion. TILs, along with other factors such as tumor size, may be used as predictive markers of invasion.

P1-05-01: Direct-to-Consumer “23andMe” Testing for Germline BRCA1 and BRCA2 Gene Variants among Arab Jordanians

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Background: Direct-to-consumer genetic testing allows individuals to access their genetic information independently, without the involvement of healthcare providers or insurance companies. Recently, the FDA approved the updated “23andMe” for selected variants in BRCA1 genes for marketing. This updated test, which analyzes human saliva samples from individuals aged 18 and older, now includes 44 specific BRCA1/BRCA2 variants, adding 41 new variants to the previously authorized three common among Ashkenazi Jews. However, thousands of pathogenic variants have been reported in BRCA1 and BRCA2 genes.
The included 44 variants cover over 90% of cancer-related BRCA1 and BRCA2 variants in individuals of Ashkenazi Jewish descent, 30-40% in African Americans, people of Europeans descent, and Hispanics or Latinos and 5-35% in Asians. Given this diversity, it is crucial to assess the prevalence of these variants in various populations. This study aims to evaluate the prevalence of BRCA1/2 variants included in the “23and Me” panel in the Arab Jordanian population.
Methods: Between January 2016 and June 2024, breast cancer patients underwent genetic testing at King Hussein Cancer Center. We analyzed the genetic data of breast cancer patients who were identified with pathogenic or likely (P/LP) variants. Specifically, we assessed the frequency of this cohort's 44 FDA-approved BRCA1/2 variants.
Results: Among the 5,896 breast cancer patients tested, 635 (10.7%) were identified as having P/LP variants. Of those, 421 (66.3%) had P/LP variants in the BRCA1 and BRCA2 genes. The median age at diagnosis with breast cancer was 40 years (range: 19-84 years). 150 (23.6%) breast cancer patients carried P/LP variants in the BRCA1 gene, encompassing 64 different variants and 271 (42.7%) were found to have P/LP variants in the BRCA2 gene,

which included 88 different P/LP variants. Only 44 (10.45%) patients were found to have P/LP variants listed in the “23andMe” panel, including 16 patients with BRCA1 variants and 28 patients with BRCA2 variants.
Almost half of the cases in our patients involved 8 highly frequent BRCA1 variants and 7 highly frequent BRCA2 variants. Among those, only BRCA2 c.2808_2811del, reported in 12 cases, is included in the 23andMe genetic testing panel. Eight BRCA1 variants matched those reported by “23andMe” in 16 cases: c.68_69del (2 cases), c.1961del (1 case), c.3756_3759del (1 case), c.3770_3771del (1 case), c.4065_4068del (5 cases), c.4327C>T (1 case), c.5123C>A (1 case), and c.5266dup (4 cases). For BRCA2, 6 variants matched those reported by 23andMe in 28 cases: c.658_659del (3 cases), c.771_775del (2 cases), c.2808_2811del (12 cases), c.3170_3174del (1 case), c.3847_3848del (5 cases), and c.7480C>T (2 cases).
Conclusions: The 23andMe test potentially missing 89.55% of P/LP BRCA1/2 variants in Arab Jordanians. Including frequently reported variants from different world regions should be considered in direct-to-consumer genetic testing to improve test sensitivity.

P1-05-02: Medium chain fatty acids shift metabolism towards the de novo serine pathway fostering epigenetic plasticity and oxidative DNA damage

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Introduction: We have investigated the breast microenvironment to identify factors that promote Estrogen Receptor Negative Breast Cancer (ERneg BC) and that may be disrupted for prevention. To that end, we have identified a lipid metabolism gene signature associated with the risk of ERneg BC. To better understand lipid metabolism in the breast, we studied the effect of fatty acids (FA) on non-transformed breast epithelial cells and tissues. FA exposure alters histone methylation, affecting gene expression and increase flux through serine, one-carbon, glycine (SOG) and methionine pathways. The association of the serine pathway and ERneg BC was first observed over a decade ago. A SOG pathway gene signature is significantly correlated with ERneg status. We hypothesized that the metabolism of FA results in a metabolic shift toward the de novo serine synthesis pathway (SSP), which ultimately increases S-adenosylmethionine (SAM), altering histone methylation, profoundly changing gene expression and fostering ERneg oncogenesis.
Methods: Non-transformed MCF-10A cells were used for in vitro metabolic and epigenomic analyses. Cells exposed to the medium-chain FA octanoic acid (OA) were utilized for proteomics and U13C-glucose tracing. SAM, glutathione (GSH) and 2-hydroxyglutarate (2-HG) concentrations were measured following treatment with OA ± blockade of the serine pathway. Reactive Oxygen Species (ROS)-induced redox changes were monitored live cells. Comet assay was performed to detect DNA damage. CUT&RUN was performed for H3K4me3. Human breast tissue derived microstructures were utilized for genomic

analysis. Single-cell RNA-seq (scRNAseq) was performed in microstructures exposed to ± OA. Metabolic flux analyses was performed using Compass.
Results: 13C flux analysis revealed that OA led to increased flux to methylation. OA significantly increased the main methyl donor SAM, the antioxidant GSH via the transsulfuration pathway and the oncometabolite 2-HG after 15 min exposure. Blocking the first and rate limiting enzyme in the SSP, PHGDH, prevented these increases. Proteomics revealed the overexpression of PHGDH following OA exposure. Upon exposure to OA, scRNAseq analysis revealed increased expression of the SSP transcription factor (TF) ATF3 and the SSP genes PHGDH and PSAT1 in epithelial and non-epithelial clusters. Upon OA the proportion of three subtypes within the epithelial compartment increased: basal BSL1, Hormone sensing HS1 and luminal progenitor LP3. Compass, an algorithm to characterize cellular metabolic states, revealed flux greatly increased through the three enzymes of the SSP: PHGDH, PSAT1 and PSPH secondary to OA exposure in BSL1, LP3 and HS1 cells. H3K4me3 CUT&RUN revealed 661 differential peaks (FDR < 0.05) comparing OA to control. Motif analysis revealed an overrepresentation of binding sites for SSP TFs ATF3/4 ($p < 0.05$). After 5 min OA exposure, mitochondrial and nuclear ROS increased significantly ($p < 0.01$), peaking at 15 min. OA exposure triggered DNA damage likely due to ROS increase in the nucleus. Compass predicted an increase in GSH metabolism and ROS detoxification in BSL1.
Conclusions: Protein levels of PHGDH are elevated in 70% of ERneg BCs. This cannot be explained by gene amplification alone as PHGDH gene amplification is observed in only approximately 6% of all breast cancers. This suggests that there are mechanisms other than gene amplification that contribute to PHGDH dysregulation. One of those mechanisms may be the lipid induced metabolic shift toward the SOG and methionine pathways that we have identified. The increased SAM and 2-HG foster epigenetic phenotypic plasticity via altered histone methylation. ROS increase shortly after OA exposure and are controlled by antioxidant defenses (e.g. GSH), which favors the survival of specific cell subtypes with acquired DNA damage which likely facilitates malignant transformation.

P1-05-03: Serum and breast tissue biomarker changes induced by Atorvastatin in women at increased risk for breast cancer

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Background: Breast cancer risk can be stratified using clinical models, genetic testing, and mammographic findings. These methods can identify those at high risk of breast cancer who might benefit from risk-reducing interventions. While Tamoxifen is highly effective at reducing the incidence of ER-positive breast cancers, there are no approved agents for reducing the risk of ER-negative cancers. Observational and preclinical evidence supports the study of statin drugs for breast cancer risk reduction.
Objective: This study aimed to investigate the effects of Atorvastatin treatment on serum and tissue biomarkers in patients at increased risk for breast cancer.

Methods: Women at increased risk for breast cancer were accrued prospectively into a biomarker modulation study at UTMDACC, Breast Medical Oncology High Risk Clinic. The study was approved by the IRB and all study participants signed informed consent. High risk was defined as having a previous history of DCIS, LCIS, or atypical hyperplasia, 5-year projected Gail risk >1.67 %, or lifetime risk >20 % calculated by clinical models. Patients were randomized to no treatment (n=15) or daily 10mg (n=16), 20mg (n=14), or 40mg (n=15) Atorvastatin for 3 months. Fasting blood and FNA biopsies were collected at baseline and study completion. Serum biomarkers were analyzed with ELISA (R&D Systems). The IGF1/IGFBP3 molar ratio was calculated using [IGF1 (ng/mL) x 0.13] / [IGFBP-3 (ng/mL) x 0.035]. The Wilcoxon matched-pairs signed rank test was used to determine significance. Lipidomic analyses were conducted on a Waters Acquity™ UPLC system with 2D column regeneration (I-class and H-class) coupled to a Xevo G2-XS quadrupole time-of-flight (qTOF) mass spectrometer under standardized operating procedures. Pre- and post-treatment serum lipids were compared in each treatment group. Paired t-tests with the Benjamini-Hochberg correction were used to determine significance. Results were also compared depending on menopausal status, BMI, and HMG-CoA Reductase genotypes. Tissue gene expression analyses will be conducted using the NanoString Breast Cancer 360 Panel.
Results: At baseline, IGF1 and IGFBP1 were significantly lower in participants with BMI>25 (p=0.017, 0.047), and the IGF1/IGFBP3 molar ratio was significantly lower in post-menopausal participants (p=0.025). Serum IGF1, IGFBP1, and IGF1/IGFBP3 molar ratio did not change significantly post-treatment. Serum IGFBP3 was decreased following treatment but was significant only in the 10mg treatment group (p=0.0034). Global changes in circulating lipids were seen following Atorvastatin treatment compared to no treatment. In the 40 mg treatment group, there was a significant reduction in several lipid subclasses, including glycosphingolipids, ceramides, and sphingomyelins, as well as reductions in selected phospholipids and triglycerides.
Conclusions: These results indicate that short-term treatment with Atorvastatin induced changes in circulating protein and lipid biomarkers. Further analysis on breast tissue will give greater insight into the effects of Atorvastatin treatment.

P1-05-04: Two years of experience of the first broad breast cancer program in Nuevo León, México

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Breast cancer is a complex disease that represents a significant health issue worldwide. Government involvement in oncology is mandatory, ensuring cancer prevention, early detection programs, and treatment for its population. Nowadays, to provide an optimal oncology treatment, a broad and multidisciplinary approach is needed. Unfortunately, this comes with a significant higher cost, leaving most patients in a difficult position. Public health program enhancements are required to provide a better outcome for the patients.

Mexico's public health system is complex, with different resources and coverage depending on federal and regional distribution and programs. Three federal health institutions hold up to 87% of the population, and private institutions only account for 2%. The remaining 11% of the population is often overlooked, depending on the regional government's efforts to ensure health coverage. Since 2022, the Nuevo León local government has raised the standard of care in public health in Mexico by implementing the first breast cancer program that includes whole coverage for diagnosis (including genetic testing for hereditary cancer), treatment, and risk reduction interventions.
In this abstract, we report the results of the first two years of experience in genetic testing and risk reduction interventions of a pioneer breast cancer program in Mexico. Patients identified in weekly multidisciplinary tumor board are referred to the clinical genetic service. After a clinical evaluation, 288/317 (90.85%) patients met the criteria for genetic testing according to current National Comprehensive Cancer Network guidelines. NGS multipanel allowed identification of at least one germline pathogenic variants (gPV) in 58 patients (20.3%). All patients received genetic counseling and followup.
The patients with gPV were analyzed by age and cancer type. Women younger than 50 had higher PV detection 49/240 (20.41%) than women older than 50 years 11/62 (17.7%). Regarding tumor type, we found that gPV were identified in around one-third of triple-negative breast cancer (TNBC) patients: 30/86 (34.88%). Finally, we evaluated the terminal efficacy of the program by the performance of risk reduction surgeries in the carriers. Risk reduction surgeries were recommended according to the type of gPV, age, parity, and clinical status of carriers. To date, 32 (83.7%) contralateral risk reduction mastectomies and 16 (40%) risk reduction salpingo-oopherectomies have been performed. The main reasons for incomplete coverage were patient decisions, age younger than 35 for oophorectomy, and advanced cancer diagnosis or palliative treatment. The experience gained in this model can be used as an example to replicate in other centers for the benefit of our oncologic patients.

P1-05-05: Genetic profile of pathogenic variants associated with breast cancer identified in a chilean inherited family oncological program

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Background: Hereditary breast cancer constitutes between 5-10% of total cases, whichare referred to oncological genetic counseling programs based on clinical criteria such as early onset, triple-negative, bilateral, male breast cancer, or family history of cancer(breast, ovarian, prostate or pancreatic cancer). Approximately 20-25% are attributable to pathogenic variants in the high-penetrance genes BRCA1 and BRCA2. However, with the application of multigene panels, testing for genes beyond BRCA1/2 has become more prevalent. The objective of this study is to identify the prevalence and spectrum of germline mutations in a Familial Hereditary Oncology Program in Chile.Methods: A total of 326 index cases were enrolled from Familial Hereditary OncologyProgram of Clinica Universidad de

los Andes between September 2020 to June 2024. Of these, 276 met clinical criteria for hereditary breast cancer, which were studied using a multigene NGS panel. The panel includes common hereditary breast cancer genes, such as homologous recombination repair genes and other DNA damage repair genes.Results: The detection rate of pathogenic or likely pathogenic variant was 17.4% (48/276).Among them, 16 patients (5.8%, 16/276) were identified as carrying BRCA1 mutation, 14 patients (5.1%, 14/276) as carrying BRCA2 mutation and 16 patients (5.8%, 16/276) as carrying non-BRCA germline variants detected in 8 genes: RAD51D (1.4%), MSH6(1.1%), BRIP1 (0.7%), CHEK2 (0.7%), PALB2 (0.7%), CDKN2A (0.4%), MUTYHheterozygous (0.4%) and NBN (0.4%). In addition, two patients (0.7%, 2/276) carrieddouble mutations in CHEK2-CDKN2A and BRCA2-CDH1. The most common variant was c.68_69del; p.(Glu23ValfsTer17) in BRCA1 present in three Ashkenazi Jewish patients. A novel mutation c.4614del; p.(Gln1538HisfsTer10) was identified in BRCA1. The majority of cases (26/48, 83%) were referred due to a personal history of breast cancer on average at 43.9 y.o., followed of ovary cancer at 52.7 y.o., prostate cancer at 63.3 y. Interestingly, the average age of diagnosis in non-BRCA carriers was later compared to BRCA2 and 1 (56 years, 48.9 years and 37.7 years, respectively). Significant differences were observed between the non-BRCA and BRCA1 groups (p=0.0022).Conclusions: Non-BRCA genes represent a third of the pathogenic/likely pathogenicvariants identified in patients with hereditary breast cancer, it is important to performmultigene panel to determine the hereditary susceptibility to cancer in our population and to identify who requires more specialized surveillance, demonstrating the relevance of a Familial Hereditary Oncology Program.

P1-05-06: The frequency of somatic mutations in the PIK3CA gene in Kazakhstani patients with metastatic HR+ HER2-negative breast cancer.

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Relevance: Up to 40% of HR+ HER2-negative breast cancer (BC) cases contain mutations in the PIK3CA gene and are a major cause of the development of hormone resistance. Activation of the PI3K protein (phosphatidylinositol-3-kinase) regulates proliferation and apoptosis, and somatic mutations in the PIK3CA gene can activate these processes. Information on the frequency and spectrum of PIK3CA mutations is important for determining the strategy of drug treatment. A combination of the selective PI3K inhibitor alpelisib and the estrogen receptor antagonist fulvestrant may be used as therapy for such tumors.
Objective of the study: To determine the frequency and spectrum of mutations in the PIK3CA gene in patients with “luminal HER2 (-) metastatic breast cancer in the Kazakh population.”
Materials and Methods: The presence of mutations in exons 7, 9, and 20 of the PIK3CA gene was determined in a sample of 320 tumor specimens from patients diagnosed with “luminal HER2 (-) metastatic breast cancer” who received antitumor therapy during the period of

2017-2023 at the Kazakh Institute of Oncology and Radiology. The age of patients ranged from 25 to 86 years, with a mean age of 65.6 years; all patients were women. Of the 320 patients, 183 (57.2%) were of menopausal age, 81 (25.3%) were perimenopausal, and 56 (17.5%) were premenopausal.
Patients with the luminal subtype “A” accounted for 134 (41.9±2.75)%, and those with the luminal subtype “B” accounted for 186 (58.1±2.75)%. Her2neu - 1+ was observed in 128 (40%) patients, and Her2neu – 0 in 192 (60%) patients. For patients with the luminal subtype “A”, 55 had Her2neu - 1+ and 79 had Her2neu – 0; for the luminal subtype “B”, 73 had Her2neu - 1+ and 113 had Her2neu – 0.
DNA extraction was performed using the GeneJET FFPE DNA Purification Kit from paraffin blocks. Amplification and detection of results were conducted using the PIK3CA Mutation Analysis Kit for Real-Time PCR (Entrogen), which includes the mutation spectra E542K, E545K, E545Q, H1047R, and H1047L.
Results: Among the 320 patients, 75 (23.4%) had various mutations in the PIK3CA gene, which is somewhat lower than global data. For example, according to cBioPortal, the mutation frequency of PIK3CA in breast cancer is 37% (1888/5129). The three most frequent missense mutations in the PIK3CA gene were p.H1047R - 36 (48%), mutation p.E542K - 22 (29.3%), and mutation p.E545K - 15 (20%). Another mutation, p.H1047L, was found in 2 (2.7%) cases, while the p.E545Q mutation was not detected in our sample.
PIK3CA mutations in the luminal subtype “A” were found in 33 (44%) cases, with Her2neu - 1+ status identified in 14 (42.4%) cases and Her2neu - 0 in 19 (57.6%) cases. In the luminal subtype “B” (n=42), Her2neu - 1+ was found in 18 (42.9%) cases, and Her2neu - 0 in 24 (57.1%) cases.
PIK3CA mutations were more frequently found in women of perimenopausal age - 43 (57.3%) cases, and in menopausal age - 32 (42.7%) cases. Among Kazakh nationality patients (179 in total), 35 (19.6±2.96%) were found to have the PIK3CA mutation.
Conclusions: The prevalence of PIK3CA mutations in the Kazakh population sample (n=320) was 23.4%, and the spectrum of PIK3CA mutations generally aligns with data from large international studies. PIK3CA mutations were somewhat more frequently found in patients of menopausal age (57.2%±2.76%), as well as in patients with the luminal subtype “B” Her2neu – 0. Among Kazakh women, the frequency of PIK3CA mutations was lower at 35 (19.6±2.96%) compared to women of other nationalities (28.4%).
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P1-05-07: Expression of ATP6AP2 in clinical breast cancer and the prognostic and therapeutic value in ERBB2 subtype of breast cancer

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Background: ATP6AP2 (ATPase H+ Transporting Accessory Protein 2), also known as the Renin/Prorenin receptor (RR), is a crucial protein associated with ATPases, playing a vital role in cellular intracellular pH homeostasis, energy conservation, protein degradation, and intracellular signalling. While its functions in non-cancer cells are becoming clearer, its involvement in cancer remains poorly understood. This study aims to assess the expression of ATP6AP2 in human breast cancer, examining its clinical implications and potential therapeutic value, with a focus on hormone receptor status and molecular subtypes.
Materials and Methods: We evaluated ATP6AP2 transcript expression using quantitative transcript analysis and assessed protein expression via immunohistochemistry. Expression levels were correlated with clinical and pathological parameters, hormone receptor status, molecular subtypes, and patient outcomes. Additionally, we investigated the association between ATP6AP2 expression and patient response to targeted therapies.
Results: Breast cancer tissues exhibited significantly elevated ATP6AP2 levels compared to normal mammary tissues (p=0.0024). High ATP6AP2 expression was observed in ER-positive and ERBB2-positive tumours. Patients with high ATP6AP2 levels had significantly shorter overall survival (OS) (p=0.039, hazard ratio: 3.75, 95% CI: 1.09-12.9) and marginally shorter disease-free survival (DFS) (p=0.087). Notably, among ERBB2-negative patients, high ATP6AP2 expression was associated with significantly shorter OS (p=0.022), which was not observed in ERBB2-positive patients. No significant difference was detected between triple-negative breast cancer (TNBC) and non-TNBC patients. Furthermore, high ATP6AP2 expression in breast cancer correlated with resistance to anti-ERBB2 treatment (p=0.0086).
Conclusion: ATP6AP2 is aberrantly expressed in breast cancer tissues and is associated with poor survival outcomes, particularly in ERBB2-negative tumours. Its expression pattern also correlates with response to anti-ERBB2 treatment, suggesting its potential as a predictive biomarker in breast cancer therapy.

P1-05-08: Germline Mutations in Early Triple-Negative Breast Cancer Brazilian Patients

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Background: Triple-negative breast cancer (TNBC) is pathologically defined by the absence of hormonal receptors and HER2 expression. This subtype accounts for approximately 20% of all breast cancers and is known for its worse prognosis. The literature demonstrates a high association of TNBC with germline mutations in BRCA1 and other genes. With the

development of targeted therapies, this information impacts the therapeutic decision for these patients.
Methods: Retrospective, single-institutional, descriptive study, which recruited consecutive patients submitted to neoadjuvant chemotherapy for early TNBC between 2010 and 2023. Data was retrieved from medical records. The primary objective was to describe germline hereditary breast and ovarian cancer (HBOC) test results in this population.
Results: 394 TNBC patients were included. The median age was 44, and most were premenopausal women (64%). Two hundred sixty-two patients (66.5%) had a positive family history of cancer, and 242 (61.4%) underwent a germline HBOC test. A germline mutation was present in 128 patients (52.9% of the tests): 24.4% of tested patients (15% of all patients) had variants of uncertain significance (VUS) and 31% of pathogenic mutations (19% of all patients). The most prevalent VUS were in BRCA2 (N=9; 3.7%), ATM (N=8; 3.3%), BRCA1 (N=6 2.5%), and mismatch-repair genes (N=9; 3.7%). The pathogenic mutations prevailed in BRCA1 (N=45; 18.6%), BRCA2 (N=9; 3.7%), and RAD15C (N=6; 2.5%). Together, germline pathogenic mutations in BRCANESS genes were present in 68 patients (28.1% of all tests). Two patients had co-pathogenic mutations in CHEK2 and TP53. Considering the tested cohort, 21.1% of > 60-year-old patients and 27.8% of patients without a family history of cancer presented pathogenic germline mutations.
Conclusion: The high prevalence of pathogenic mutations in this cohort highlights the importance of germline tests and genetic counseling for TNBC patients, despite age or family history criteria.

P1-05-09: High prevalence of germline BRCA1/2 pathogenic variants in TNBC patients who achieve pCR: results from the TRINITY study.

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Background: Several reports indicate that over 15% of triple negative breast cancer (TNBC) patients harbor germline BRCA1/2 pathogenic variants (PV). BRCA-associated cancers have distinct characteristics that may result in higher pathologic complete response (pCR) rates after neoadjuvant chemotherapy (NACT). We hypothesized that the population of TNBC patients who achieve pCR may be enriched with germline BRCA1/2 PV.
Methods: TRINITY is a retrospective, multicenter study, that enrolled patients with stage II–III TNBC who underwent surgery in Greece between 2016 and 2022. In this report we focused in the patient who received NACT and underwent BRCA1/2 germline genetic testing. We evaluate the prevalence of germline BRCA1/2 PV in the patients who achieved pCR and those with residual disease after NACT.
Results: Of the 230 enrolled patients in the study (10 centers), 113 (49.1%) received NACT. Median age was 52 years (range: 30.2-82.1). Of the patients with available data on family history, 45 (39.8%) reported breast cancer and 19 (16.8%) ovarian cancer family history.

Out of 113 patients, 61 patients had pCR and 52 incomplete response to NACT. BRCA1/2 data were available for 72 patients (63,7%): 45 and 27 patients from the pCR and residual disease group respectively.
More than half of the patients of the pCR group, 25 out of 45 (55.5%), had germline BRCA1/2 PV: 21 in the BRCA1 and 4 in the BRCA2. The prevalence of the PV in the patients with residual disease was 18,5% (5/27): 4 in the BRCA1 and 1 in the BRCA2. The age at diagnosis and the percentage of patients with family history didn't differ significantly in the two groups.
Conclusion: according to our study, the patients with TNBC that achieved pCR after NACT seems to have a high prevalence of germline BRCA1/2 PV. Genetic counseling and testing should be strongly recommended for these patients, despite the fact that they are not eligible for targeted adjuvant therapy.

P1-05-10: Investigating the Potential Role of Rare Germline Non-Coding Variants in Cancer Predisposition Genes in Patients with Triple-Negative Breast

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Current genetic screening for breast cancer predisposition is limited to the analysis of coding regions (exons) and intron/exon boundaries of BRCA1/2 genes. There is limited data on the prevalence and clinical significance of variants in the non-coding regions of these genes. Consequently, the majority of variants identified in these regions remain unclassified, and approximately 80% of germline BRCA1/2 tests are not considered in the daily management of patients with triple-negative breast cancer (TNBC).
Emerging evidence suggests that non-coding variants can impact cancer risk and response to treatment. This study aimed to investigate the prevalence of variants in the non-coding regulatory regions of BRCA1/2 and other breast cancer predisposition genes in TNBC patients selected based on age at cancer diagnosis and/or family history of cancer. Additionally, we sought to explore the functional role of identified variants of uncertain significance (VUS) through ongoing analyses.
We enrolled 144 TNBC patients who had previously tested negative for germline variants in the coding regions of BRCA1/2 and other cancer predisposition genes. Next-generation sequencing (NGS) analysis identified 635 rare variants in the non-coding regions of 28 selected genes involved in breast/ovarian cancer predisposition. In our TNBC cohort, we observed a higher prevalence of rare variants in the genes CDH1 (1.3%), STK11 (11.2%), ATM (10.7%), PTEN (7.40%), and PMS2 (5.04%).
Germline variants in BRCA2 were statistically significantly associated with worse overall survival (p-value=0.017). CDH1 rare variants were associated with the highest percentage

of non-pathologic complete response after neoadjuvant chemotherapy (p=0.0273). MLH1 and PALB2 rare variants were both associated with bilateral breast cancer (p=0.015 and p=0.0005, respectively). Rare variants of the ATM gene were associated with a positive family history (p=0.041).
Preliminary single nucleotide variant (SNV) data analysis showed that the most significant functional score for alterations were detected in the promoter of MSH6, potentially associated with chromatin effects. Further analyses are ongoing to elucidate the functional impact of these variants.
Due to the small sample size, these analyses should be considered exploratory, and larger studies are needed to confirm these findings and establish the clinical utility of screening for non-coding variants in TNBC patients.

P1-05-11: The timing of BRCA1/2 germline testing and patient's decision toward the risk-reducing mastectomy in breast cancer

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Background: BRCA1 and BRCA2 pathogenic variants significantly increase the risk of breast cancer. Risk-reducing mastectomy (RRM) is an option for these patients, offering substantial risk reduction. However, the decision to undergo RRM is complex and influenced by various factors. This study aims to analyze factors influencing RRM decisions in BRCA mutation carriers, with a particular focus on how the timing of BRCA testing affects the choice to undergo RRM.
Methods: We conducted a retrospective analysis of 499 breast cancer patients diagnosed with germline BRCA1/2 pathogenic variants at Seoul National University Hospital between 2005 and 2023. Exclusion criteria included patients with distant metastasis at the time of BRCA testing, those who underwent palliative surgery at initial diagnosis, patients who had already undergone contralateral total mastectomy at the time of BRCA testing, and those initially diagnosed with bilateral breast cancer.
Result: Of the 499 patients, 172 (34.4%) underwent RRM. Among the 262 patients who underwent BRCA testing before their initial breast cancer surgeries, 197 patients were aware of their BRCA mutation status prior to surgery, while for 65 patients, their BRCA test results became available after surgery. RRM rates were significantly higher in patients who knew their BRCA status before surgery compared to those whose results became available after surgery. (65.0% vs. 18.5%, p <0.001). Moreover, only 13.5% of patients who underwent BRCA testing after primary surgery subsequently underwent RRM.
In the subset of 68 patients diagnosed with contralateral breast cancer (CBC) during follow-up, RRM rates were higher among those aware of their BRCA status before surgery for CBC compared to those who learned results post-operatively (32.4%vs. 11.7%, p = 0.079). However, this difference was not statistically significant.
The results of the multivariate analysis showed that the timing of BRCA genetic testing emerged as a significant factor influencing the decision for RRM. Patients who underwent

testing before their initial surgery were 10.1 times more likely to undergo RRM compared to those tested after surgery (95% CI: 6.09-17.20). Additionally, BRCA1 mutation carriers were found to be 1.8 times more likely to undergo RRM than BRCA2 carriers (95% CI: 1.12-2.88). Additionally, patients who received neoadjuvant chemotherapy were 1.8 times more likely to undergo RRM compared to those who did not (95% CI: 1.07-2.84). In contrast, other factors including age at diagnosis, sex, family history of breast or ovarian cancer, personal history of ovarian cancer, and presence of bilateral breast cancer were not significantly associated with the decision to undergo RRM.
Conclusion: The timing of BRCA testing significantly influences decisions on risk-reducing mastectomy (RRM). This finding underscores the importance of considering genetic testing timing in breast cancer management. Clinicians should take this into account when planning BRCA testing to optimize patient decision-making regarding risk-reduction strategies.

P1-05-12: The 70-Gene Signature Assay vs. Traditional Clinical Risk Assessment: Impact on Adjuvant Chemotherapy Choices in Mexican Breast Cancer Patients

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Introduction: Breast cancer is the most frequent malignant neoplasm in mexican women; it is a complex disease with diverse clinical outcomes and multiple responses to treatment (1). Adjuvant systemic treatment decreases the risk of developing distant metastasis and death by approximately 30% in patients with node-negative breast cancer, but 70% of all patients on adjuvant therapy will experience toxicity without benefit from such treatment (2,3). Tools including St Gallen, NIH guidelines, risk calculators (PREDICT) or indexes such as the Nottingham Prediction Index are currently used as tools for administer adjuvant chemotherapy, although in certain populations the need for this therapy tends to be overestimated (5,6). The 70-Gene Signature Assay has shown a significant correlation with patient outcome. This tool was subsequently validated in several groups of patients in European countries and was approved by the Food and Drug Administration of the United States (7,8). Currently there is no study in mexican breast cancer patients, therefore, our study is the first on the signature of 70 genes in Mexico.
The 70-Gene Signature Assay was evaluated in Mexican breast cancer patients. An association was assessed between the 70-Gene Signature Assay with clinicopathological characteristics in these patients.
Methods: Forty eligible patients with breast cancer who underwent a 70-Gene Signature Assay test and had the result in their medical record were included. Data collection was performed from the clinical archive of the Hospital Angeles Leon, in the period from November 2012 to March 2023. The following criteria were considered: patients diagnosed with clinical stage I-II breast cancer, node-negative and had not received neoadjuvant therapy. Patients whose results were pending at the time of the study or those with a previous malignant neoplasm were not included. The concordance between the risk

predicted by the 70-Gene Signature Assay and commonly used clinicopathological guidelines (St. Gallen guidelines, National Institutes of Health [NIH] guidelines, the Nottingham Prognostic Index and the PREDICT tool) were assessed.

According to the St. Gallen guidelines, low clinical risk is defined as the sum of all the following criteria: estrogen receptor positive and/or progesterone receptor positive, a tumor size of 2 cm or less, a histologic grade 1, and an age of 35 years or older (9). The National Institutes of Health (NIH) guideline recommends that the low-risk group in the node-negative group have a tumor size of less than 1 cm and a favorable histologic subtype such as tubular and mucinous cancer (10,11).

The Nottingham Prognostic Index (NPI) is a prognostic measure that predicts operable survival of primary breast cancer. The NPI value is calculated based on tumor size, number of lymph nodes and tumor grade (11), The PREDICT program algorithm was developed from data of the East Anglia Cancer Registry and Information Centre (ECRIC) on patients with primary breast cancer treated in 1999-2003 and provides 5- and 10-year survival estimates with and without adjuvant systemic therapy (hormonal therapy, chemotherapy and trastuzumab) (13). The clinical low-risk group for this calculation was defined as patients with 10-year overall survival probabilities of at least 88% in ER-positive tumors and at least 92% if ER expression was observed in ER-negative tumors (13).

Statistical analysis was performed with SPSS version 15.0 (SPSS Inc., Chicago, USA). The p value was bilateral. Differences between the groups of interest were tested with Pearson's $\chi 2$ test or Fisher's exact test. A p value < 0.05 was considered statistically significant.

Results: Of the 44 eligible patients, 4 were excluded because of sampling failure (n = 4). A prognostic signature report was obtained for the remaining 40 patients. Twenty-five (62.5%) patients had a low-risk prognostic signature, and 15 (37.5%) patients had a high-risk prognostic signature. The mean age of eligible patients was 54.28 years ± 11.33 and the mean tumor diameter was 1.7 cm ± 0.9 cm. No significant differences in age, tumor size, histological grade, estrogen, or progesterone status were observed between the low-risk and high-risk groups according to the 70-Gene Signature Assay. Only histologic type was significantly different between groups p = 0.000. The mean tumor diameter was 1.80 cm (range, 0.9-4 cm) in the high-risk group and 1.64 cm (range, 0.2-3 cm) in the low-risk group (Table 1).

According to the St. Gallen criteria, 38 of 40 (95%) patients were at high risk, according to the NIH guideline, 34 of 40 (85%) patients were at high risk, according to PREDICT, 28 of 40 (70%) patients were at high risk and for the NPI, 16 of 40 (37.5%) patients were at high risk. (Table 2).

Discussion: The 70-Gene Signature Assay has been considered a powerful tool to predict metastasis or the outcome for breast cancer patients. Our analysis shows a notably proportion in the low-risk group (62.5% of the low-risk group, 37.5% of the high-risk group), these results differ from those reported in Korea, Japan and European populations for the low- risk group (13.9%, 20% and 19.6% respectively) and high-risk groups (86.1%, 80%, 80.4% respectively)(14). There were not statistically differences between age, tumor size, histological grade, estrogen, or progesterone receptors. Only the histological type was different between low and high-risk groups.

The clinical utility of the 70-Gene Signature Assay depends on its potential value in addition to using traditional prognostic factors. Therefore, we compared the signature's risk assessment to the clinicopathological risk assessment using the St. Gallen criteria, the NIH guidelines, PREDICT and Nottingham Prognostic Index. Overall, as compared with that of the clinicopathological risk guidelines, about two thirds of the patients were discordant with the 70-Gene Signature Assay. (Discordance of 67.5% compared to the St. Gallen criteria, 57.5% compared to the NIH guideline, 45% compared to PREDICT and 57.5% compared to the NPI). In a previous study, the discordant rate between the 70-Gene Signature Assay and other clinicopathological guidelines was 28.3-39% (15, 16). A discordant rate is not frequently mentioned in many of the earlier validation studies. However, a comparison between the 70-Gene Signature Assay and the clinicopathological guidelines suggests that this signature is a stronger predictor of disease outcome than the currently used clinicopathological guidelines (14).

As a result, these discordant rates imply that using this signature could lead to a significant reduction in the number of patients recommended for chemotherapy. Our findings show similar incidence in the low-risk group compared with European studies, this could implicate a cost and unnecessary cytotoxic treatment indicate in patient where classical clinicopathological risk assessment tools tend to overestimate the high-risk patients in Mexican population.

The limitations of this study were first the sample size, second there is not a follow-up evaluation to determine those patients who remains disease-free at 5 to 10 years. Further large-scale studies with a follow-up evaluation are required to assess whether the use of the 70-Gene Signature Assay can predict a prognosis for Mexican patients with breast cancer.

Conclusion: Our study is the first to evaluate the 70-Gene Signature Assay in Mexican breast cancer patients. The results show a higher proportion of patients classified as low-risk compared to traditional clinicopathological guidelines, which often overestimate the need for chemotherapy. The discordance rates suggest that the 70-Gene Signature Assay may offer more accurate risk assessment, potentially reducing unnecessary treatments. Although limited by sample size and lack of follow-up, these findings emphasize the value of integrating genomic tools with conventional methods to enhance personalized treatment. Future large-scale studies with follow-up are needed to confirm these results and fully establish the clinical utility of the 70-Gene Signature Assay in Mexican patients.

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P1-05-13: Rates of Adherence to NCCN Breast Cancer Screening Recommendations among Unaffected Women with a Hereditary Breast Cancer Predispositionosition

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Background: Hereditary predisposition accounts for approximately 5-10% of all breast cancer cases. It is clinically important to identify individuals with a hereditary breast cancer predisposition early in order for appropriate cancer risk management to be implemented. Although cancer screening guidelines exist for the high- and moderate-penetrance breast cancer predisposition genes, limited data are available on the breast cancer screening adherence rates among these women. Here we describe our experience among unaffected women with a hereditary breast cancer predisposition who were seen in a breast cancer screening and prevention program.
Methods: Our cohort included patients with a pathogenic variant (PV) in a breast cancer predisposition gene who were seen in the Magee-Womens Breast Cancer Risk Assessment and Prevention Program for consultation regarding cancer risk management at least once between January 1, 2017 and December 31, 2022. We conducted a retrospective medical chart review to collect data on risk reducing mastectomy, screening adherence, and breast cancer diagnosis. Patients who had a breast cancer diagnosis prior to genetic testing being performed were excluded. Patients not pursuing risk reducing mastectomy were recommended to follow screening guidelines as suggested by the National Comprehensive Cancer Network (NCCN). Breast cancer screening was not recommended after risk reducing mastectomy. We evaluated adherence to the recommended breast cancer screening recommendation over the past 18 months (between January 1, 2023 to June 30, 2024). Numbers of screen-detected and interval breast cancer diagnoses were described.
Results: A total of 336 patients were identified, of whom 36 were excluded due to insufficient EHR data to determine screening adherence. Of the remaining 300 patients, age at initial encounter ranged from 19 to 65 years. The majority of patients had a PV in BRCA2 (109, 36.3%) and BRCA1 (85, 28.3%), the remaining genes include ATM (32, 10.7%), CHEK2 (31, 10.3%), PALB2 (23, 7.7%), TP53 (5, 1.7%), CDH1 (4, 1.3%), BARD1 (3, 1.0%), RAD51C (2, 0.7%), and RAD51D (1, 0.3%). Sixty-one patients underwent risk reducing mastectomy (age range 21-63 years) with 5 breast cancers diagnosed at the time of surgery (3 with a BRCA2 PV, 1 with a BRCA1 PV, 1 with a CDH1 PV). Sixteen patients had a screen-detected breast cancer (5 with BRCA1, 5 with BRCA2, 3 with ATM, 1 each with CDH1, CHEK2 and RAD51C). There were two interval cancers (both BRCA1). Screening adherence was evaluated for the remaining patients over an 18-month period from January 2023 to June 2024. MRI was not recommended for individuals who were pregnant/breast feeding, were at a weight incompatible with MRI, or have metal implants. Mammogram was not recommended for individuals who were pregnant, or were younger than the age at which mammogram should be started. Annual MRI was recommended for 208 and annual mammogram was recommended for 190, 183 were recommended to have both MRI and mammogram. 149 of the 208 (72%) patients for whom MRI was recommended had it done within the past 18 months, while 162 of the 190 (85%) patients had mammogram as recommended. Of the 59 patients who did not have MRI, 35 (59.3%) were up-to-date with mammogram.
Conclusion: In this population of patients with a hereditary predisposition to breast cancer, the majority of patients have adhered to breast cancer screening recommendations in the past 18 months. Rates of adherence to screening were high, and was higher for

mammogram than MRI. Coordinated high risk management provided in an established cancer screening clinic is effective in promoting adherence to cancer surveillance.

P1-05-14: OnTarget crofelemer diarrhea prophylaxis trial: responder analysis of patients with breast cancer receiving targeted therapy with or without chemotherapy

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Background: Cancer therapy-related diarrhea (CTD) is a common adverse effect of targeted therapies (e.g., TKIs, CDK4/6 inhibitors), resulting in treatment modifications and poor clinical outcomes. Crofelemer is a chloride ion channel modulator of cystic fibrosis transmembrane conductance regulator. It is FDA-approved for HIV-associated diarrhea based on a responder analysis of the proportion of patients with improved diarrhea. Here, we present the prespecified responder analysis of the OnTarget study (NCT04538625) in the breast cancer subgroup receiving targeted therapies.
Methods: OnTarget was a randomized, multicenter, double-blind, placebo-controlled prophylactic trial evaluating crofelemer versus placebo in adults with solid tumors receiving targeted therapies. Patients were randomized to 125 mg of crofelemer twice daily or placebo for 12 weeks to prevent CTD. Diarrhea outcomes were captured using patient-reported outcomes (PROs). Key exclusions included immunotherapy, neratinib, irinotecan, colitis/ostomy/abdominal surgery ≤3 months, and any antidiarrheal or antibiotic use ≤7 days. The primary outcome was the mean weekly number of loose/watery stools (LWS) over 12 weeks. Secondary endpoints included the Patient Global Impression of Severity (PGIS) for gastrointestinal symptoms, and time to onset of durable response. Subgroup analyses for tumor types and targeted therapies were prespecified; anchor-based methods using PGIS determined optimal thresholds for the average number of weekly LWS for responders. The optimal cut-off values of the mean LWS per week to define responders were derived via receiver operating characteristics (ROC) curve analyses of PGIS anchors. Further, monthly responders met the cutoff for mean weekly LWS for at least 2 of 4 weeks per month. Since the time to onset of responder status does not address sustainable effects, continuous efficacy over the entire 3-month period was assessed per ordered categories (none, 1/3, 2/3, or 3/3 months) via ordered logistic regression. We analyzed the onset and sustained effect of treatment using response patterns that prioritized an early and durable response for each month. The patterns of the number of responding months were ranked from 0-8, with a rank of 8 designating a full responder for all 3-months. All p values are 1-sided.
Results: The OnTarget study enrolled 287 adults with 10 solid tumor types and 24 unique targeted therapies. The prespecified primary endpoint of the mean weekly LWS over 12 weeks was not met. Adverse events were similar in both arms. A total of 184 (64.1%) patients with breast cancer participated; median age 57 yrs [IQR: 48-65 yrs], 16.5% non-White); 96 (54.6%) received abemaciclib, 67 (38.1%) pertuzumab/trastuzumab, and 13

(7.4%) kinase inhibitors. PRO data was available for 176 patients with breast cancer. ROC analysis showed that weekly responders have ≤9 LWS/week in each week. Continuous responder efficacy analysis in patients with breast cancer having ≤9 LWS/week showed significant improvement with crofelemer over placebo (OR 1.77 [CI: 1.01-3.12]; p=0.0245). Patients with an ECOG performance status of 0-1 (n=170) showed improved response with crofelemer compared to placebo (OR 1.99 [95% CI: 1.11-3.56]; p=0.0104). Additionally, more patients with breast cancer receiving crofelemer (41/87; 47.1%) were responders for all 3 months compared to placebo (30/89; 33.7%) (OR 2.14 [95% CI: 1.10-4.14]; p=0.0123). Crofelemer had significantly faster onset and sustained efficacy compared to placebo (OR 1.80 [95% CI: 1.02-3.16]; p=0.021), including patients with ECOG status of 0-1 (n=170, OR 2.00 [95% CI: 1.12-3.56]; p=0.0095).
Conclusion: In this responder analysis of patients with breast cancer on targeted therapies, crofelemer CTD prophylaxis resulted in a greater proportion of monthly responders of diarrhea improvement compared to placebo, with better-sustained response in those with preserved functional status (ECOG 0-1).

P1-05-15: A real-world analysis of cancer prevention measures among individuals with hereditary breast and ovarian cancer (HBOC) syndromes and no personal history of malignancy.

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Background: Strategies exist to decrease cancer incidence among individuals with HBOC syndromes. These include: 1) surgeries such as risk-reducing mastectomy (RRM) and risk reducing bilateral salpingo-oophorectomy (RRBSO) and 2) risk-reducing medications with selective estrogen receptor modulators (SERM) or aromatase inhibitors (AI). RRM decreases the incidence of breast cancer by >90%. RRBSO decreases ovarian cancer risk by >80%, may decrease breast cancer risk, and decreases mortality in individuals with BRCA1 and BRCA2 mutations. Use of 5 years of SERM or AI reduces risk of future breast cancer by 40-50% in individuals at high risk, but data regarding efficacy in individuals with HBOC syndromes are limited. To date, few studies have explored cancer risk reducing treatment decisions among individuals with hereditary cancer susceptibility without personal history of malignancy.
Methods: Individuals evaluated in the Breast and Ovarian Cancer Risk Evaluation Clinic (BOCREC) at the University of Michigan Rogel Cancer Center with pathogenic germline variants (PGVs) conferring increased cancer risk without personal history of breast, ovarian, pancreatic, or prostate cancer from 1/1/2012 to 1/1/2022 were eligible for analysis. Basic demographic information (age, sex, and race/ethnicity), pursuit of cancer risk reduction measures, and subsequent cancer diagnoses were recorded. A chi-squared test was used to compare rates of RRM among carriers of high penetrance versus moderate penetrance breast cancer susceptibility genes.

Results: 402 individuals were included in this analysis. The majority were female (80%) and white (91%; Not listed 5%; Asian 3%; Black 1%). The most common PGVs identified were in BRCA1 (32%, n=134) and BRCA2 (32%, n=133), followed by CHEK2 (16%, n=66), ATM (7%, n=30), PALB2 (4%, n=18), BRIP1 (3%, n=14), RAD51C/RAD51D (2%, n=8), Lynch Syndrome associated PGVs (1.2%, n=5), CDH1 (0.5%, n=2), TP53 (0.5%, n=2), CDKN2A (0.2%, n=1), and PTEN (0.2%, n=1). Of the 295 individuals eligible for RRM (female, PGV in breast cancer susceptibility gene), 108 (37%) underwent RRM. Of those who had a RRM, the average time to surgery following PGV diagnosis was 21 months (range 0.5-97 months). RRM was most common in the 40-49 age group (31%, n=35), closely followed by the 30-39 age group (30%, n=34). Only 13% of individuals had a RRM below age 30 (n=15) and 9% of RRMs occurred in individuals aged 60 or older (n=10). Likelihood of undergoing RRM was significantly greater for individuals with a highly penetrant breast cancer PGV (42% for BRCA1, BRCA2, PALB2, PTEN, TP53, CDH1) versus a moderately penetrant breast cancer PGV (21% for CHEK2, ATM, RAD51C/D) ($p = 0.002$). Among 116 individuals eligible for RRBSO (female, PGV in ovarian cancer susceptibility gene) 86% underwent RRBSO within the timeframe recommended. Of the 178 patients eligible for breast cancer risk-reducing medication (breast cancer PGV, female, age ≥ 35, no RRM), 80% (n=142) had a documented discussion regarding risks and benefits of SERM or AI at time of PGV diagnosis and 7 patients (3.9%, BRCA1 n=2, BRCA2 n=2, CHEK2 n=3) initiated therapy. Of the 402 patients analyzed, 41 (11%) were subsequently diagnosed with cancer during the follow up period, 51% (n=21) of which were breast cancer. Of those diagnosed with breast cancer, 57% were carcinoma in situ and 24% were stage I.
Conclusions: In this cohort, nearly 40% of unaffected females with hereditary breast cancer risk opted to undergo RRM. Approximately 1 in 5 individuals with moderate penetrance breast cancer PGVs opted for RRM despite insufficient evidence of benefit. Compliance with RRBSO among those with hereditary ovarian cancer risk was high. Uptake of risk-reducing medication (SERM or AI) was exceptionally low despite its potential to significantly decrease breast cancer risk. Additional research regarding efficacy of risk-reducing medication in those with hereditary breast cancer predisposition is needed.

P1-05-16: Differential ctDNA-based genomic features of Triple-Negative Metastatic Lobular Breast carcinoma: insights into a rare and poorly understood disease

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Background: Invasive lobular carcinoma (ILC) comprises 10-15% of all breast cancers (BC), predominantly presenting as low grade and hormone receptor positive (HRpos). Triple-negative ILC (TN-ILC) is rare, accounting for approximately 2% of all triple-negative BC

(TNBC) and 0.1% of all BC. Due to its rarity, TN-ILC is poorly understood and lacks well-established treatment standards. This study aims to investigate the genomic characteristics of TN-ILC through circulating tumor DNA (ctDNA) profiling within a large multi-center consortium.
Methods: The study analyzed a retrospective cohort of 750 patients (pts) with HER2 negative metastatic BC with ctDNA testing using the Guardant360 NGS panel within a large multicenter academic consortium. HR and HER2 status were defined based on the most recent biopsy. Associations across single nucleotide and copy number variations (SNVs and CNVs), histology, and HR status were tested by multinomial logistic regression in terms of Relative Risk Ratio (RRR), adjusting for significant clinical characteristics (i.e., lines of treatment, metastatic sites). Oncogenic pathway analysis was defined based on Sanchez-Vega F et al, Cell. 2018. Prognosis was analyzed for overall survival (OS) defined from the time of ctDNA collection.
Results: Among the 750 pts analyzed, the cohort consisted of TN-ILC (N:16, 2%), HRpos-ILC (N:91, 12%), TN-invasive ductal carcinoma (IDC) (N:186, 25%), and HRpos-IDC (N:457, 61%). In TN-ILC, the most frequently altered genes were PIK3CA (50%), TP53 (44%), ERBB2 (25%), and CDH1 (25%). Less frequent alterations included BRAF, ALK, ARID1A, MET, RB1, and SMAD4, each occurring in 12-19% of cases. PIK3CA (48%) and TP53 (39%) had a similar alteration frequency in HRpos-ILC, and ESR1alterations were also common (36%). Multivariable multinomial logistic regression, designed with TN-IDC as reference, investigated differences in oncogenic pathway alterations across histologies and subtypes. In TN-ILC, a significant association was observed for RAS SNVs (RRR = 7.9, p = 0.038) and PI3K SNVs (RRR = 4.32, P = 0.015). In HRpos-ILC, cell cycle CNVs (RRR = 0.3, P = 0.013) and P53 SNVs (RRR = 0.26, P < 0.001) had a significantly lower prevalence than in TN-IDC, while RAS SNVs (RRR = 4.94, P = 0.022) and PI3K SNVs (RRR = 2.6, P = 0.003) were more common. Single gene alterations were investigated across significantly altered pathways. PIK3CA SNVs were more common in TN-ILC (50%) compared to TN-IDC (16%), but similar in frequency to HRpos-ILC (42%) (P<0.001). PTEN SNVs were higher in HRpos-ILC (11%) than in TN-IDC and TN-ILC (respectively 0% and 6%). KRAS SNVs were more represented in HRpos- and TN-ILC (respectively 6% and 8%), with respect to TN-IDC (1%) (P= 0.017). TP53 SNVs were significantly more detected in TN-IDC (68%) than in HRpos and TN-ILC (respectively 44% and 35%, P<0.001). A similarly unfavorable outcome was observed for TN-ILC and TN-IDC (median OS 12 and 13 months, respectively), compared to their HRpos counterparts (median OS 31 and 33 months, respectively) (P<0.001).
Conclusions: Our study highlights distinct genomic features of triple-negative ILC detectable through ctDNA. These findings reinforce the need for improved understanding of TN-ILC to define personalized treatment options for this aggressive and rare subtype.

P1-05-17: Structural Variants Affecting CDH1 in Breast Invasive Lobular Carcinoma

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Background: Invasive lobular carcinoma (ILC) is the most frequent special histologic subtype of breast cancer (BC). ILC displays a characteristic discohesive phenotype, caused by inactivation of CDH1 (E-cadherin) that plays key roles in cell-to-cell adhesion. CDH1 bi-allelic inactivation is due to loss-of-function CDH1 mutations associated with loss of heterozygosity of the wild-type allele (LOH) in most (~80%) cases. A subset of ILCs, however, lack CDH1 mutations despite displaying a lobular phenotype, and their molecular underpinning is yet to be determined. Here we sought to elucidate whether structural variants (SV) would be a mechanism of CDH1 genomic inactivation underpinning ILC.
Methods: We interrogated 4,376 primary and 5,105 metastatic BCs, previously subjected to clinical paired tumor/normal targeted sequencing using the FDA-approved MSK-IMPACT assay to identify BCs harboring SVs affecting CDH1. LOH was assessed using FACETS. The driver probability of fusion genes was examined using Oncofuse. A histopathologic examination of cases was conducted following the WHO criteria. Estrogen receptor (ER) and HER2 status were retrieved from medical records. E-cadherin expression was assessed by immunohistochemistry. Somatic genetic alterations of BCs harboring CDH1 SV were compared to those of ILCs with CDH1 loss-of-function mutations. Mutational signatures were inferred using SigMA in cases with ≥5 single nucleotide variants.
Results: We identified 21 BCs (6 primary and 15 metastatic BCs) harboring SVs in CDH1, encompassing out-of-frame fusion genes (n=6), out-of-frame intragenic deletions (n=5), in-frame intragenic deletions (n=5), out-of-frame duplications (n=4) and an out-of-frame intragenic inversion (n=1). The second CDH1 allele was affected in 71% (15/21) of cases in the form of LOH (n=12) or as somatic inactivating mutations (n=3). Three fusion genes (CDH1-RAD21, CDH1-TANGO6, CDH1-ZNF815P) were predicted to result in the loss of the cytoplasmic and/or transmembrane domains of E-cadherin, while the other three (SMPD3-CDH1 and two fusion genes affecting CDH1 and intergenic regions in chromosome 16 or 19) were predicted to lead to loss of CDH1 signal peptide, precursor and extracellular domains and the 5' regulatory region harboring the transcription start site. All 6 fusion genes were categorized as drivers by Oncofuse (median driver probability score=0.97; range, 0.84-0.99). Most BCs harboring CDH1 SVs exhibited a lobular phenotype (16/21; 76%), including pleomorphic ILC/ILC with pleomorphic features (n=8), classic ILC (n=6) and mixed ductal-lobular BCs (n=2), while 5/21 (24%) cases displayed a non-lobular histology. We observed loss of E-cadherin expression in 73% (11/15) of cases interrogated. Most BCs were of histologic grade 3/poorly differentiated (n=10) or grade 2/moderately differentiated (n=10). The majority were ER+/HER2- (12/20; 60%), followed by ER-/HER2- (6/20 (30%). Besides CDH1, the most frequently altered cancer genes in primary BCs harboring CDH1 SVs were PIK3CA and NSD3 (33%, each), while TP53 (40%), ESR1 (27%) and PIK3CA (20%) were the most frequently affected cancer genes in metastatic cases. Akin to ILCs with CDH1 mutations, most primary BCs with CDH1 SVs (66%) showed a dominant aging mutational signature, and most metastatic cases had a dominant aging (54%) or APOBEC (31%) mutational signature. We observed no differences in the repertoire of genetic alterations between primary and metastatic BCs harboring CDH1 SVs compared to primary

and metastatic ILCs harboring biallelic CDH1 mutations.
Conclusions: We demonstrate that CDH1 affecting SVs act as drivers of a subset of ILCs. Most of these SVs result in loss of E-cadherin protein expression and a lobular phenotype, akin to CDH1 mutations. ILCs harboring CDH1 SVs were enriched for aggressive histologic features and display a repertoire of genetic alterations resembling that of ILCs with CDH1 mutations. These findings support the notion that ILC is a convergent phenotype, driven by various molecular mechanisms that disrupt the cell-cell adhesion complex, including SVs affecting CDH1.

P1-05-18: Genomic Landscape in Advanced Breast Cancer Across Different Genomic Ancestries

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Background: The use of genomic profiling in advanced breast cancer (aBC) has recently increased, however, access disparities persist due to racial/ethnic inequities. This study evaluated aBC samples with comprehensive genomic profiling (CGP), stratified by HER2 and hormone receptor (HR) status, and examined genomic alterations across different genomic ancestries.
Methods: Hybrid-capture CGP targeting 324 genes was performed on advanced BC samples (FoundationOne®CDx). HER2 immunohistochemistry status was abstracted from pathology reports, and HER2 amplification status was obtained from CGP. Patients were classified according to genomic ancestry: African (AFR), South and East Asian (ASI), European (EUR), and Others (including Admixed American). Short variants (SV), copy number alterations (CNA), and rearrangements (RE) were extracted from CGP data.
Results:We analyzed 2083 aBC samples: HER2-positive (n=416), HR+HER2-negative (n=1140), and TNBC (n=527). Ancestry distribution was AFR (n=312), ASI (n=110), EUR (n=1447), and Others (n=214). In the HER2-positive subtype, the most frequent genomic alterations in the AFR group were SV in TP53 (75%), PIK3CA (39%), and ARID1A (8%); CNA in ERBB2 (96%), MYC (32%), and RAD21 (32%); and RE in CDK12 (5.6%). In the ASI group, the most frequent SV were in TP53 (79%), PIK3CA (62%), and ERBB2 (10%); CNA in ERBB2 (100%), RAD21 (34%), and MYC (31%); and RE in ATR (10%). In the EUR group, the most frequent SV were in TP53 (72%), PIK3CA (38%), and ARID1A (8%); CNA in ERBB2 (97%), RAD21 (29%), and MYC (22%); and RE in CDK12 (8%). In the Others group, the most frequent SV were in TP53 (64%), PIK3CA (45%), and ARID1A (11%); CNA in ERBB2 (98%), RAD21 (39%), and MYC (30%); and RE in BRCA1 (9%). In the HR+HER2-negative subtype, the most frequent genomic alterations in the AFR group were SV in PIK3CA (45%), TP53 (40%), and GATA3 (23%); CNA in RAD21 (26%), MYC (20%), and CCND1 (20%); and RE in NOTCH2 (5%). In the ASI group, the most frequent SV were in PIK3CA (56%), TP53 (40%), and ESR1 (20%); CNA in NSD3 (26%), FGFR1 (24%), and ZBF703 (24%); and RE in MAP2K4 (2%) and MYC (2%). In the EUR group, the most frequent SV were in PIK3CA (53%), TP53 (33%), and CDH1 (19%); CNA in CCND1 (23%), FGF19 (21%), and FGF3

(21%); and RE in NOTCH2 (1%). In the Others group, the most frequent SV were in PIK3CA (57%), TP53 (45%), and ESR1 (12%); CNA in RAD21 (23%), MYC (19%), and CCND1 (18%); and RE in NOTCH2 (3%). In the TNBC subtype, the most frequent genomic alterations in the AFR group were SV in TP53 (95%), PIK3CA (15%), and RB1 (11%); CNA in RAD21 (35%), MYC (35%), and MCL (15%); and RE in RB1 (5%) and SPEN (4%). In the ASI group, the most frequent SV were in TP53 (88%), PIK3CA (15%), and RB1 (15%); CNA in MYC (15%), NSD3 (12%), and FGFR1 (12%); and RE in RB1 (4%) and SPEN (4%). In the EUR group, the most frequent SV were in TP53 (90%), PIK3CA (25%), and PTEN (12%); CNA in RAD21 (23%), MYC (23%), and PTEN (9%); and RE in EZH2 (2%). In the Others group, the most frequent SV were in TP53 (91%), NF1 (12%), and RB1 (7%); CNA in MYC (37%), RAD21 (21%), and PTEN (14%); and RE in NF1 (3.5%) and EZH2 (3.5%). Conclusions: The distribution of genomic alterations is consistent across breast cancer subtypes, regardless of genomic ancestry. These findings highlight the critical need for equitable genomic testing access for all patient populations.

P1-05-19: Circulating Tumor DNA as a Prognostic Biomarker for CDK 4/6 Inhibitor Therapy in Metastatic Breast Cancer

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Background: Cyclin dependent kinase (CDK) 4/6 inhibition in addition with endocrine therapy (ET) is first line therapy for patients with hormone receptor-positive (HR+), HER2 non-amplified, metastatic breast cancer (mBC). Recognizing which patients are going to benefit from treatment and identifying which patients acquire resistance are still poorly understood. Molecular alterations in CCNE1, MYC, and RB1 from breast tissue samples have been associated with resistance to CDK4/6 inhibitor therapy. Circulating tumor DNA (ctDNA) offers a unique noninvasive method to capture mBC heterogeneity and treatment response. Studies have used ctDNA to monitor for emergence of mutations and monitor dynamic response to treatment in HR+ mBC. Here, we test the hypothesis that CCNE1, MYC, and RB1 can be detected in ctDNA and utilized as a biomarker to predict response to CDK4/6 inhibitor therapy. Methods: In this study, we analyzed a subset of patients from the Dallas Metastatic Breast Cancer Study comprised of patients with HR+, HER2 non-amplified, mBC who underwent treatment with a CDK4/6 inhibitor (palbociclib, ribociclib, abemaciclib) and ET, became resistant to therapy, and had ctDNA testing (n=102). Commerical testing results through Tempus xF and FoundationOne Liquid CDx, which detects cancer-relevant genetic alternations, were obtained and analyzed. The data was collected from multiple hospitals within a single academic medical center starting with the initial ctDNA collection from 2019 to 2024. 13 patients had ctDNA collected before the start of therapy, 84 patients had ctDNA collected after progression, and 5 patients had ctDNA collected before therapy and after progression. Results: 88 of 102 patients did not have CCNE1, MYC, or RB mutations present had a median progression-free survival (mPFS) of 13.5 months. Among the remaining 14 patients, two had CCNE1 alterations with a mPFS of 5.5 months: one had ctDNA collected before treatment and one had ctDNA collected after

progression. Three patients had MYC alterations with a mPFS of 19 months: one had ctDNA collected before treatment and after progression, and two had ctDNA collected after progression. Nine patients had RB1 alterations with a mPFS of 7 months: four had ctDNA collected before treatment and after progression, and five had ctDNA after progression. Of the patients that had both timepoints collected, one patient had acquired a MYC alteration at time of progression. Four patients had acquired RB1 alterations at time of progression. Among patients with RB1 mutations (n=9), 3 had loss of function mutations, 1 had a missense mutation, 1 had low coverage region, 5 had multiple single nucleotide variants (SNVs) and among these, 2 had splice site variants. Conclusion: There is an urgent need to develop predictive biomarkers that capture real-time changes in tumor biology. Tissue analyses from patients resistant to CDK4/6 inhibitors have demonstrated a 14%-28% expression of CCNE1, 16% of MYC mutations, and 9%-10% expression of RB mutations. In our study, we observe a lower rate of detection in ctDNA of each gene. This result suggests that correlation between tissue mutations and detectable ctDNA mutations may not occur in a linear fashion. This analysis further suggests that new research is needed to identify other mutations detected in ctDNA which could predict CDK4/6 inhibitor resistance beyond CCNE1, MYC, and RB. Broadening our understanding of other correlative mutations in ctDNA with therapy resistance could allow for improved clinical implementation. Our study was limited by the small sample size and lack of consecutive ctDNA collection. Further studies are needed to determine the temporal and dynamic changes of acquired tumor mutations detected through ctDNA and the predictive value of specific mutations on outcomes.

P1-05-20: Validation of the HER2 Activation Response Predictive Signature (HARPS) as a Predictive Biomarker for HER2+ Therapy Response in the Neoadjuvant Setting

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Background: Not all patients with breast cancer identified HER2+ by standard IHC and FISH assays benefit from HER2-targeted therapy, suggesting these assays are not optimized to predict response to HER2-targeted agents. We previously reported results from the I-SPY2 TRIAL wherein we identified a HER2 Activation Response Predictive Signature (HARPS) as a novel functional phosphoprotein-based measurement of HER2 activity in the baseline pre-treatment biopsy sample that is based on HER2 Y1248 and EGFR Y1173 co-phosphorylation (p). HARPS predicted neoadjuvant response to a variety of anti-HER2 agents in tumors classified as HER2+ and TNBC (PMID: 34741023; 32914002). Moreover, we found that activation of the PLK1 pathway through pFADD S194 predicted non-response to HER2 therapy (PMID: 38086377) in HER2+ patients. Based on this observation, we investigated the HARPS signature and PLK1 pathway activation as predictive biomarkers of response in a blinded and independent study set of pre-treatment tumor biopsy samples obtained from

women with HER2+ BC disease who received HER2-targeted neoadjuvant therapy.
Methods: Reverse phase protein microarray (RPPA) analysis of Laser Capture Microdissected (LCM) -enriched tumor epithelium was performed on FFPE tumor biopsies from patients with HER2+ BC (N=38; 31 HR+/7 HR-) under CAP compliance (#7223012). Anti-HER2 therapy consisted of Trastuzumab (T) + Pertuzumab (N=35), T alone (N=2), TDM-1 (N=1), pCR outcomes were kept blinded. Quantitative measurement of total HER2, pHER2 Y1248, pEGFR Y1173 and pFADD S194 was made. Previously described HARPS +/- cutpoints were imputed onto the pHER2 and pEGFR data and a pCR prediction (HARPS+ = pCR Yes (Y) vs HARPS- = pCR No (N)) was made on a subset (N=26) of patients whose tumors had the lowest and highest HARPS scores. After unblinding, ROC-based optimization of HARPS was performed on the full study set (N=38). Association between quantitative individual protein values, HARPS +/- determination and pCR was assessed using uncorrected, non-parametric t-tests and Fisher's exact tests.
Results: 14 tumors from the blinded set were determined to be HARPS-. After unblinding, 12 did not achieve pCR after therapy and 2 patients had pCR (12/14, 86% NPV). 12 tumors from the blinded set were determined to be HARPS+. After unblinding, 8 were pCR Y and 4 were pCR N (8/12, 67% PPV). There was a significant association (p=0.009) between the blinded outcome call vs the actual outcome. Independent analysis of pHER2 Y1248, pEGFR Y1173 and total HER2 were all found to positively predict pCR in the full study set (p=0.005, p=0.006 and p=0.009, respectively). pFADD S194 levels trended with non-response (p=0.09). A ROC-optimized HARPS signature applied to the full study set of 38 tumors correctly identified 11/14 pCR Y (80% PPV) and 20/24 pCR N (83% NPV). There was no significant association of misclassification based on HR status or HER2 treatment type.
Conclusions: The robustness and generalizability of the previously described HARPS signature to predict response to HER2 therapies was validated in a blinded independent study set of FFPE HER2+ tumors (HR+ and HR-). Response prediction of HARPS was independent of type of HER2 therapy and HR status. Moreover, we verified that PLK1 activation was a negative predictor of response and that PLKi inhibitors should be explored as a bona fide candidate for combination with HER2 inhibitors for HARPS- patients who will not respond to current anti-HER2 therapy regimens. Our results justify rigorous evaluation of HARPS as a reflex test for women with HER2+ disease in prospective clinical trials for HER2 therapy escalation/de-escalation.

P1-05-21: Prognostic implications of oncogenetic pathway alterations in advanced male breast cancer

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Background: Male breast cancer (MaBC) represents a rare entity, accounting for only 1% of all BC cases. In a previous work, we reported that MaBC recapitulates a unique mutational repertoire, harboring a distinct immune and genomic landscape across BC clinical subtypes.

However, prognostic implications of pathway alterations in MaBC have not been reported before, leaving uncertainty regarding their significance and clinical actionability in MaBC, and similarity with female BC (FeBC) counterparts. In this work, we analyzed the prognostic implication of single gene alterations on MaBC-related survival.
Methods: 17,759 breast tumors were tested by next-generation sequencing (NextSeq; WES, NovaSeq) and whole-transcriptome sequencing (NovaSeq; Caris Life Sciences, Phoenix, AZ). Real world overall survival (OS) was obtained from insurance claims and calculated from treatment start to last contact using Kaplan-Meier estimates. Statistical significance was determined by chi-square and Mann-Whitney U test with p-values adjusted for multiple comparisons ($q<0.05$).
Results: Of 17,759 BC patients, there were 226 (1.3%) MaBC and 17,533 (98.7%) FeBC. In FeBC, GATA3 mutations (MT) were associated with better OS post-endocrine therapy (mOS: 49.6 vs. 43.1 months, HR 0.83, 95% CI 0.71-0.9, $p<0.01$) compared to GATA3 wild type (WT); whereas in MaBC, GATA3-MT was not associated with survival post-endocrine therapy (mOS: 54.8 vs. 60.8 months, HR 1.4, 95% CI 0.63-3.2, $p=0.39$) compared to GATA3-WT. The presence of TP53 mutations was adversely associated in FeBC, with a trend in MaBC. Poorer outcomes in TP53-MT vs WT were observed in FeBC patients post-endocrine therapy (FeBC: ΔmOS 25.2 months, HR 1.7, 95% CI 1.6-1.7, $p<0.01$ vs MaBC: ΔmOS 16.3 months, HR 1.08 95% CI 0.43-2.7, $p=0.85$), with CDK4/6 inhibitor (FeBC: ΔmOS 18.2 months, HR 1.7, 95% CI 1.6-1.9, $p<0.01$ vs MaBC: ΔmOS 19.5 months, HR 1.35, 95% CI 0.46-2.7, $p=0.57$) and in the post-chemotherapy setting (FeBC: ΔmOS 18 months HR 1.3, 95% CI 1.3-1.4, $p<0.01$ vs MaBC: ΔmOS 34 months HR 1.7, 95% CI 0.93-3.4, $p=0.07$). Additionally, CDH1, BRCA2 and PIK3CA mutations appeared to have no prognostic role in both MaBC and FeBC. The proportion of HR+/HER2- subtype was 72.12% in MaBC and 54% in FeBC. The results in the HR+/HER2-MaBC subgroup were consistent with those reported from the overall cohort. The number of HR+/HER2- MaBC with mutations in ESR1 (n=7), AKT1 (n=2), PTEN (n=8), mTOR (n=0), CDK4 (n=0), Rb1 (n=2), as well as other cancer susceptibility genes were very infrequent.
Conclusions: MaBC may recapitulate a unique tumorigenic trajectory that differs from FeBC, as suggested by the dissimilar prognostic significance of selected genomic alterations. These data warrant further confirmation toward the goal of understanding sex-defined differences in BC and potential for tailored therapeutic strategies.

P1-05-22: Targeting human Endoplasmic reticulum oxidoreductin 1-Lα in novel therapy for triple negative breast cancer.

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Background: Triple negative breast cancer (TN breast cancer) has a poor prognosis and few therapeutic targets, and the development of novel therapies is desirable.Human endoplasmic reticulum oxidoreductin 1-Lα (hERO1-Lα) is an oxidase that exists in the endoplasmic reticulum and its expression is augmented under hypoxia. hERO1-Lα has a role

in the formation of disulfide bonds of secreted proteins and cell-surface proteins. We reported that hERO1-Lα is present in high levels in various types of tumors, and is a poor prognostic factor of TN breast cancer. We have focused on hERO1-Lα as a novel target molecule in TN breast cancer. In this study, we demonstrated that hERO1-Lα expression is involved in epithelial to mesenchymal transition (EMT), which is a process related to cancer metastasis and invasion, and analyzed its molecular mechanism. In addition, we investigated the effect of ERO1-Lα inhibitor (ERO1-Lα-I) on distant metastasis inhibition and anti-tumor effect for clinical application.

Materials and Methods: We generated MDA-MB-231 cells with hERO1-Lα knockdown (KD) using shRNA against hERO1-Lα, and with ERO1-Lα overexpression（OE） transfected with human hERO1-Lα cDNA. EMT changes in cells were analyzed by observation of cell morphology and western blotting (WB), and the same analysis was performed with the addition of ERO1-Lα-I (EN460). Cell motility was evaluated by scratch assay and invasive ability was evaluated by cell invasion assay. Since hERO1-Lα regulates the production of secreted proteins, we searched for EMT-related factors by comparing the results of cytokine arrays and ELISA using culture supernatants from each cell line. MCF7 with culture supernatant from OE cells was evaluated for induction of EMT using WB. The hERO1-Lα KD cells were transplanted into NOD/SCID mice and evaluated for distant metastasis. We also evaluated the anti-tumor and distant metastasis inhibitory effects of ERO1-Lα-I treatment and tumor pathology in NOD/SCID mice transplanted with TN breast cancer cell lines.

Results: EMT was suppressed in the hERO1-Lα KD cells and the ERO1-Lα inhibitor-added cells in WB, while it was enhanced in the OE cells. Cell motility and invasive ability were significantly suppressed in the hERO1-Lα KD cells ($p < 0.05$).Leukemia inhibitory factor (LIF), which has been reported to be involved in EMT, was detected relatively strongly in the culture supernatant of OE cells using cytokine arrays and was considered as a candidate molecule. ELISA showed a correlation between hERO1-Lα expression and LIF secretion. MCF cells with OE cells culture supernatant containing LIF showed EMT.Lung metastases were significantly suppressed in mice transplanted with the hERO1-Lα KD cells ($p < 0.001$) . Tumor growth was significantly suppressed in mice in the ERO1-Lα inhibitor group without toxicity ($p < 0.05$) .

Conclusions: hERO1-Lα suppression and hERO1-Lα inhibitors suppressed EMT of tumor cells, and LIF was identified as an EMT-related factor involving hERO1-Lα. Inhibition of hERO1-Lα suppressed distant metastasis, and addition of inhibitors showed antitumor effects. These results indicate that hERO1-Lα is involved in tumor growth and metastasis and may be a candidate for a new molecular targeted therapy for TN breast cancer.

P1-05-23: An observational cohort study of Multiplex8+: a spatially informed assay that uses multiplexed RNA-FISH guided laser capture microdissection followed by total RNA-sequencing

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We previously developed and validated a breast cancer (BCa) diagnostic test called mFISHseq on a retrospective cohort of 1,082 FFPE breast tissues. The test utilizes a multiplexed RNA-FISH panel consisting of estrogen (ESR1), progesterone (PGR), and HER2 (ERBB2) receptors and Ki67 (MKI67) to characterize regions of interest that are subsequently captured by laser capture microdissection for total RNA-SEQ. Here, we report the results from 53 patients that underwent a research use only (RUO) version of the test called Multiplex8+, which provides information about the four main BCa biomarkers, intrinsic molecular and TNBC subtypes, prognostic risk, and expression of 40 genes and 28 gene signatures that inform about cancer hallmark pathways and treatment response to chemotherapies as well as targeted therapies (e.g., immunotherapy, CDK4/6 inhibitors, and antibody drug-conugates (ADCs)). Three patients had two specimens (primary and metastatic tumor specimens) processed yielding a total of 56 patient specimens, which consisted of hormone receptor positive (HR+)/HER2− (n=11), HR+/HER2+ (n=9), HR−/HER2+ (n=4), and HR−/HER2− (n=30) as determined by IHC. Two patients had unavialable IHC data. The median age at surgery was 46 yrs and the patients comprised various node statuses (negative=17, positive=18, unavailable=21), tumor sizes (pT1=22, pT2-4=17, unavailable=17), and grades (G1=1, G2=16, G3=30, unavailable=9).
Multiplex8+ showed high agreement with 90.5% of IHC reported results. Agreement was highest for HER2-ERBB2 (96.4%) followed by PR-PGR (92.7%), ER-ESR1 (85.5%), and Ki67-MKI67 (85.3%). Most discordant results for hormone receptors were in gray zone areas for IHC (i.e., ER/PR low ≤10%) and Multiplex8+ classified them as negative. Notably, two patients were classified as basal-like by intrinsic subtyping (both mesenchymal (M) TNBC subtype) and had high expression of proliferation, immune, and/or angiogenesis signatures. Another patient was classified as HER2 overexpressed and had elevated HER2 amplicon signatures and low luminal pathway signatures. This suggests that Multiplex8+ reflected the underlying tumor biology better than IHC.
Follow up data for 20 patients revealed insights into the ultility of genes and gene signatures in predicting risk of relapse and response to treatment. Multiplex8+ was correct in predicting treatment sensitivity or resistance in 77% (17/22) of instances, which spanned chemotherapy, endocrine, anti-Her2, and novel targeted therapies. For example, one metastatic TNBC patient who had an excellent response to bevacizumab had two heterogeneous regions dissected using LCM and both regions had high levels of a hypoxia/inflammation/angiogenesis signature previously shown to predict sensitivity to bevacizumab. This patient also had high TACSTD2 and TOP1 the antigen and payload targets of the ADC, sacituzumab govitecan (SG). Notably, after progressing on bevacizumab, this patient responded well to SG, highlighting the value of this test in predicting response

to more than one line of therapy. Another patient with metastatic HR+/HER2− BCa had a low (neo)adjuvant chemotherapy response signature as well as a high E2F4 signature that was previously shown to predict resistance to aromatase inhibitors and sensitivity to CDK4/6 inhibitors. Indeed, this patient had a poor response to aromatase inhibitors and chemotherapy, relapsing 2 months after surgery, but then had a beneficial response to CDK4/6 inhibitors. These examples, and other patient vignettes that will be discussed, demonstrate the clinical potential of Multiplex8+ by providing unique insights for each patient that could identify potential treatments (including subsequent lines of therapy) and explain why prior treatments performed poorly.
The specimens in this observational study were obtained with the patients informed consent and the study was approved by the Ethics Committee of the Bratislava Self-Governing Region (Ref. No. 05320/2020/HF).

P1-05-24: Biomarkers and Spatial Determinants of SHR-A1811 Efficacy in Neoadjuvant Treatment for HER2-Positive Breast Cancer

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Background: The emergence of next-generation anti-HER2 antibody-drug conjugates (ADCs), such as T-DXd and SHR-A1811, represents a breakthrough in HER2+ breast cancer treatment. However, identifying factors that influence treatment response and pinpoint suitable candidates for these ADCs remains challenging.
Methods: We conducted a translational study on the neoadjuvant SHR-A1811 monotherapy arm in phase II FASCINATE-N trial (NCT05582499) to identify potential biomarkers for SHR-A1811 in HER2+ breast cancer. The first-stage analysis included the first 60 patients completing neoadjuvant treatment and surgery, of whom all were included in this translational study.
We performed multiomics profiling using baseline biopsy samples if they were adequate. A total of 48 patients had genomic data, 39 had transcriptomic data, 60 had computational pathology (CP) data based on whole slide images of paired H&E-stained and HER2 IHC-stained slides, and 21 had Xenium single-cell in situ spatial imaging.
We also developed an interpretable SHR-A1811 efficacy prediction model using multi-model integration and a voting classifier based on clinicopathological and CP features.
Results: In 60 patients included in the first stage analyses, 37 (61.7%) achieved pathological complete response(pCR). When stratified by hormone receptor (HR) status, the pCR rate was 48.10% (13/27) for HR+ patients and 72.72% (24/33) for HR- ones.
No difference in mutation or copy number were found between tumors of different efficacy. Transcriptomic analysis hinted that in the HR- subgroup, immune microenvironmental factors were associated with efficacy (upregulation of HLA molecules in pCR), whereas in the HR+ subgroup, tumor cell-associated factors played a more significant role (i.e., higher estrogen-related genes in non-pCR, including PGR, AREG, and GREB1). This hypothesis was

supported by further analysis.
Specifically, in the HR- subgroup, CP analysis showed significantly higher proportion of immune cells in pCR patients (P=0.0046), which were not observed in the HR+ subgroup (P=0.38). Xenium data further identified specific immune cell types and revealed that the T cells, particularly cytotoxic T cells, were the vital cell group that showed largest difference (P=0.0058), as is verified by higher CD8 IHC intensity (P=0.00031). Spatially, CP analysis found that pCR patients also showed higher mixture of immune cells and tumor cells, exhibiting a more immune infiltrated landscape. Xenium further found stronger antigen presentation between tumor cells and cytotoxic T cells in pCR patients, while non-pCR ones showed higher PD-L2–PD1 interaction.
In the HR+ subgroup, non-pCR diseases showed a clustering rather than even distribution of HER2-strong-positive tumor cells (P=0.016). This might result from the luminal-like molecular feature in non-pCR patients, as is supported by higher PGR and CCDN1 in non-pCR tumor cells in Xenium data and stronger PR staining in IHC tests.
In addition, we developed a practical model capable of predicting neoadjuvant treatment responses of both SHR-A1811 and based on clinicopathological features and pathological images (training set AUC = 0.95, 95% CI: 0.89-1.00; test set AUC = 0.86, 95% CI: 0.76-1.00).
Conclusions: Overall, our findings suggested the potential mechanism underlying tumor's response to SHR-A811. The investigation into biomarkers, particularly their spatial distribution, may enhance the prediction and identification of patients who are more likely to respond favorably to the treatment of SHR-A1811 and the related ADCs. Our study also indicated potential strategies to promote the efficacy of SHR-A811 with the combination of immunotherapy or endocrine therapy.

P1-05-25: Improving the Specificity and Sensitivity of Digital 3D Mammography using serum, plasma and saliva derived proteomes and mathematical modeling

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Digital 3D mammography is currently the most utilized screening tool used to identify early-stage breast cancer. Mammograms are scored using the BI-RADS categories (Breast Imaging Reporting and Data System). Categories IV and V mammograms are associated with a significant risk of breast cancer, mandating a biopsy and a pathologic diagnosis. Image guided percutaneous needle biopsy diagnoses cancer in about 25% of these BIRADS IV, V abnormalities emphasizing the lack of specificity and sensitivity of 3d Digital Mammography in diagnosing early-stage breast cancer. The poor positive predictive score subjects 75% of these patients with benign radiographic abnormalities to the potential anxiety, cost and harms of an unnecessary tissue biopsy. We studied 150 patients with BIRADS IV, and V mammographic abnormalities with serum, plasma and saliva obtained just prior to image guided biopsy. Hydrogel affinity nanoparticles were used to harvest and concentrate low abundance proteins, and the proteins were then identified by mass

spectrometry. Candidate peptides were identified, and these markers were verified in blinded confirmation. We developed and validated a statistical model to differentiate between cancer and non-cancer cases using a combination of proteomic and microbial biomarkers. Biomarkers were identified through bivariate analysis to find those with significant differences in abundance between cancer cases and controls. Additionally, lasso feature selection was employed to pinpoint the biomarkers most strongly associated with the cancer outcome. The model was constructed by comparing three distinct models using maximum likelihood estimates and Akaike information criterion, with the most parsimonious model selected for final analysis. K-fold cross-validation was used to ensure the model's robustness, and a receiver operating characteristic (ROC) curve analysis yielded an area under the curve (AUC) of 0.82, indicating strong overall predictive accuracy. To determine risk categories, we identified thresholds using spline analysis and calibration models. Splines were applied to the predicted probabilities to detect significant changes in cancer likelihood, and calibration curves were used to fine-tune the threshold. This process identified a 0.5 probability cut-off, which was used to categorize predictions into low and high likelihood of cancer. Confusion matrices were then employed to evaluate model performance at this threshold. The model demonstrated high sensitivity (92%), effectively identifying true positive cases, but had a lower specificity (45%), indicating a higher rate of false positives. Despite challenges such as handling factor levels and missing values, the model, which incorporated nine significant biomarkers (seven proteomes and two microbes), proved valuable for identifying high-risk patients. The model's high sensitivity potentially makes it a useful tool to guide the clinical decision to biopsy a mammographic abnormality or follow it clinically with additional interval follow-up imaging. An accurate risk marker can potentially reduce unnecessary invasive biopsies. To enhance clinical utility, future work should focus on improving specificity to reduce false positives and validating the model with larger, more diverse datasets to increase its generalizability and practical application.

P1-05-26: MammaPrint Genomic Signature, Molecular Subtype Characteristics and Outcomes of HER2-Low Early Breast Cancer (EORTC10041 / BIG 03-04 MINDACT Trial)

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Background: HER2-low breast cancers (BC) are heterogeneous with different degrees of HER2 expression and hormone receptor (HR) status. Recent developments in anti-HER2 antibody–drug conjugates (ADCs) open new therapeutic options for HER-low BC. We aim to further characterize HER2-low BC using genomic assay and molecular subtype and its association with clinical outcome.
Methods: In this exploratory analysis of the MINDACT trial (EORTC 10041/BIG 3-04) we selected HER2-low tumors (i.e. HER2 1+ or 2+ non-amplified) tested by immunohistochemistry (IHC)/-ISH and with available HR status (Estrogen and

Progesterone receptor) both accessed by central pathology review. HER2 expression was evaluated with the HercepTest kit (Dako polyclonal). Molecular subtype by BluePrint classified patients in Luminal A (blueprint luminal AND MammaPrint (MP) low risk), Luminal B (blueprint luminal AND MP high risk), HER2-enriched and Basal. Genomic risk was defined using 70-gene signature using MP. Association of molecular subtype and genomic risk with distant metastasis free survival (DMFS) was evaluated. DMFS were adjusted for chemotherapy and endocrine therapy administration.
Results: From a total of 5929 enrolled patients in MINDACT with central pathology review, 2320 (39.1%) patients were classified as HER2 low, 1505 (64.9%) were HER2 1+ and 815 (35.1%) had HER2 2+/-ISH negative tumors. Median age at diagnosis was 55 years, 1675 (72.2%) of tumors had ≤ 2cm, 1400 (60.4%) were grade 2, 1830 (78.9%) had lymph-node negative disease and 2186 (94.2%) were HR (ER and/or PgR) positive. HER2-low tumors molecular subtype distribution was: 1574 (67.8%) Luminal A, 554 (23.9%) Luminal B, 25 (1.1%) HER2 enriched and Basal 167 (7.2%). Genomic risk classified HER2-low as MP low risk 1589 (68.4%) vs. 731 (31.5%) high risk (p<.0001). Molecular BC subtype distribution according to HER2-low score 1+ vs. 2+ were: Luminal A 1116 (74.2%) vs 458 (56.2%), Luminal B 272 (18.1%) vs 282 (34.6%), HER2 enriched 14 (0.9%) vs 11 (1.3%) and Basal 103 (6.8%) vs. 64 (7.9%), respectively (p<.0001). Genomic risk distribution according to HER2-low score 1+ vs. 2+ were: low risk 1125 (74.8%) vs 464 (56.9%) and high risk 380 (25.2%) vs. 351 (43.1%), respectively (p<.0001). Median follow-up was 108.5 months (95% CI, 107.8 - 109.3). The HER2-low 5-year DMFS according to genomic risk was 96.5% (95% CI, 95.6-97.4) in MP low vs. 92.5% (95% CI, 90.5-94.4) in MP high risk (HR 1.9 [95% CI, 1.4-2.5], p<.0001). The HER2-low 5-year DMFS according to molecular subtype was 96.5% (95% CI, 95.5-97.4) for Luminal A, 92.8% (95% CI, 90.7-95.1) for Luminal B, 88% (95% CI, 76.1-100) for HER2 enriched and 92.6% (95% CI, 88.7-96.7) for Basal (p<0.0001). In the multivariable analysis, age > 50 years (HR 1.34, [95% CI, 1.0-1.8], p=0.0490), lymph node positive (HR 1.75, [95% CI, 1.2-2.4], p=0.0003) and genomic high risk (HR 2.02, [95% CI, 1.5-2.7], p<0.0001) were independently associated with worse DMFS.
Conclusions: In the MINDACT trial exploratory analysis most HER2-low tumors were HR positive, Luminal A BC molecular subtype and Low MP genomic risk. However, HER2-low BC is not a uniform biological entity with an enrichment of Luminal B and High MP genomic risk in the HER2 2+ score subset. In addition, this analysis demonstrated that clinical outcome of HER2-low early BC is associated with MP genomic risk. The MINDACT trial exploratory analysis, using a central pathology review and long-term follow-up, shows that HER2-low BC has heterogeneous molecular profile, genomic risk and clinical outcomes and thus these results may influence future clinical trials with new agents for the treatment of HER2-low early BC.

P1-05-27: Analytical comparison of tissue-based next-generation sequencing assays for the detection of PIK3CA, AKT1, and PTEN tumor alterations in breast cancer

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Background: Next-generation sequencing (NGS) testing in patients with advanced breast cancer (ABC) enables genomic biomarker interrogation, potentially guiding clinical management. Results from the CAPItello-291 Phase 3 randomized trial led to the approval of the first-in-class pan-AKT inhibitor capivasertib in combination with fulvestrant as a treatment option in patients with hormone receptor (HR)-positive/human epidermal growth factor receptor 2 (HER2)-negative ABC, with one or more PIK3CA/AKT1/PTEN tumor alterations following disease progression on/after prior aromatase inhibitor therapy. PIK3CA/AKT1/PTEN alterations are present in approximately 50% of all HR-positive/HER2-negative breast cancers and also in around 30% of all triple-negative breast cancers (TNBC). This study aimed to analytically compare the ability of commercially available tissue-based NGS assays to detect PIK3CA/AKT1/PTEN tumor genomic alterations in breast cancer samples.
Methods: Formalin-fixed, paraffin-embedded tumor samples collected from patients with TNBC were analyzed at three different sites using the following commercial NGS assays in accordance with the manufacturers' instructions: AVENIO Tumor Tissue CGP (Roche), TruSight Oncology 500 (Illumina), oncoReveal Core LBx (Pillar Biosciences), Oncomine Comprehensive Assay v3 (ThermoFisher Scientific), AmoyDx HANDLE Classic (Amoy Diagnostics), and SOPHiA ExtHRS (SOPHiA GENETICS). Detection of single nucleotide variants, insertions/deletions, and copy number variants in PIK3CA, AKT1, and PTEN approved by the FDA as genomic alterations which determine eligibility for treatment with capivasertib in combination with fulvestrant in patients with HR-positive/HER2-negative ABC was recorded and compared. Positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) were calculated for all assays. AVENIO Tumor Tissue CGP was used as the reference assay, as the content is broadly comparable to the FDA-approved FoundationOne CDx assay.
Results: Overall, 45 samples were processed, and all samples were included in the final analysis. PPA, NPA, and OPA for any alteration(s) (PIK3CA, AKT1 or PTEN) versus AVENIO (reference) were: TruSight: 86.0%, 100.0%, and 86.7%; oncoReveal: 81.4%, 100.0%, and 82.2%; Oncomine: 81.4%, 100.0%, and 82.2%; AmoyDx: 86.0%, 100.0%, and 86.7%; SOPHiA: 88.4%, 100.0%, and 88.9%. There was 100.0% PPA, NPA, and OPA for both PIK3CA and AKT1 between TruSight, Oncomine, AmoyDx, SOPHiA and AVENIO. For oncoReveal, PPA, NPA, and OPA were 100.0%, 94.1%, and 97.8% for PIK3CA and 100.0%, 100.0%, and 100.0% for AKT1 alterations, respectively. For PTEN alterations, PPA, NPA, and OPA per assay versus reference were: TruSight: 58.8%, 96.4%, and 82.2%; oncoReveal: 17.6%, 100.0%, and 68.9%; Oncomine: 35.3%, 100.0%, and 75.6%; AmoyDx: 47.1%, 100.0%, and 80.0%; SOPHiA: 58.8%, 100.0%, and 84.4%. Lower agreement for PTEN alterations was due to differences in gene coverage and ability of some assays to detect complex PTEN genomic

alterations, such as large rearrangements and copy number variations.
Conclusions: All assays evaluated demonstrated good concordance with the AVENIO Tumor Tissue CGP test, especially for detecting capivasertib treatment eligible alterations in AKT1 and PIK3CA. Further improvement on detection of PTEN structural and copy number alterations is needed for some assays in order to maximise patient identification for capivasertib in combination with fulvestrant. These data can help clinicians make informed decisions regarding suitable diagnostic tests to determine patient eligibility for breast cancer therapies.

P1-05-28: Immune related adverse events are associated with better event free survival in a Phase I/II Clinical Trial of Durvalumab Concomitant with Neoadjuvant Chemotherapy in Early-Stage TNBC

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Immune-related adverse events (irAE) during neoadjuvant immune checkpoint inhibitor therapy and chemotherapy are associated with improved outcomes in triple negative breast cancer. This analysis included patients from a phase I/II single arm clinical trial at Yale Cancer Center and its regional care centers was conducted from December 2015 to December 2020. Eligible patients were adults 18 years and older with clinical stages I–III, TNBC for whom systemic chemotherapy was indicated. Patients received durvalumab concomitant with nab-paclitaxel and dose dense doxorubicin-cyclophosphamide. Durvalumab was not administered postoperatively. We examined the association between developing an irAE with pathologic complete response (pCR=ypT0/is, ypN0), residual cancer burden (RCB), event-free survival (EFS) and overall survival (OS). Sixty-seven patients were eligible for toxicity and efficacy analysis, 27 had irAEs of any grade, 13 had multiple irAEs. Median follow up was 61 months (range 6.8-94.03 months). The most frequent irAEs were dermatologic (n=14), endocrine (n=13), and gastrointestinal (n=5). Patients who experienced irAEs achieved a pCR or RCB 0-1 rate of 58% and 73%, respectively, compared to 42% and 55% in those without irAEs (p=0.309 and 0.19). Development of irAE was also associated with significantly improved EFS (HR: 0.25; 95% CI 0.09-0.66; p=0.024), and a trend for improved OS (HR: 0.42; 95% CI 0.14-1.27; p=0.17). Patients with more than one irAE had no EFS events. Development of irAE was associated with a numerically improved pCR rates, lower RCB, and significantly higher EFS in patients treated with neoadjuvant immune checkpoint therapy plus chemotherapy.

P1-05-29: The Nanomechanical Phenotype of Lobular Breast Cancer

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Zaugg, Papa Diogop Ndiaye, Philipp Oertle, Vittorio Cristini, Alastair M. Thompson, Marija Plodinec

Background: Recent research has spotlighted lobular cancer as a distinct histological phenotype, potentially revolutionizing treatment paradigms. Invasive lobular carcinoma (ILC) is identified by a specific morphological feature, notably the "Indian file" arrangement of discohesive breast cancer cells. Frequently, these cancers are estrogen receptor positive, though they can appear within any molecular subtype of breast cancer. Emerging evidence indicates that, within the same molecular subtype, ILC leads to less favorable outcomes compared to invasive ductal carcinoma (IDC). These outcomes include higher rates of metastatic disease, lower response rates to both neoadjuvant and adjuvant treatments, and decreased overall and disease-free survival. Such findings strongly support reclassifying ILC as a distinct entity, advocating for a paradigm shift in therapeutic approaches. Mechanistic Insights into ILC The development of ILC is mechanistically linked to the loss of E-cadherin, a crucial epithelial cell-cell adhesion molecule. This loss is directly associated with changes in cell nanomechanical compliance and tumor microenvironment nanomechanical remodeling. This study presents the first clinically integrated, functional characterization of ILC tissues at the nanomechanical level, providing new insights into the physical properties of ILC and offering novel avenues for clinical management.

Methods: This investigation utilized data from a single-center, blinded, prospective study aimed at measuring the multiparameter nanomechanical signature of breast cancer. Conducted between 2016 and 2019, the clinical study included 588 fresh breast biopsy samples from 545 suspected breast cancer patients. Samples were measured using the AFM-based Automated and Reliable Tissue Diagnostics (ARTIDIS) investigational device within a routine clinical setting at the Breast Clinic, University Hospital Basel (Switzerland). Patients who underwent clinically indicated breast biopsy and consented to participate were eligible for the study. Biopsies were collected before treatment, and patients are being followed up to collect long-term responses for up to 10 years. Core needle biopsies from 125 invasive breast cancer patients were selected for nanomechanical profiling of ILC and IDC, including 12 pure ILC and 113 pure IDC cases. The nanomechanical signature integrated analysis was performed using the proprietary ARTIDISNet software platform.

Results: This study presents the first comprehensive nanomechanical signature of ILC compared to IDC, including stiffness, adhesion, and dissipation profiles and their association with other known clinico-histopathological aggressive features. The specific mechanical nature of ILC was confirmed through spatial analysis of multiplex imaging from several lobular breast cancer patients. This characterization supports the investigation and definition of ILC as a unique nanomechanical subtype of breast cancer.

Conclusion: The loss of E-cadherin in ILC is directly related to the loss of cell-cell adhesions, epithelial-mesenchymal transition, cell invasion, migration, and metastasis, all of which are mechanical aspects of ILC pathogenesis. This study presents the first integrated characterization of the nanomechanical phenotype of ILC, which will be further validated in the ongoing multicenter ANGEL clinical trial, currently enrolling over 2700 patients in the

USA over the next three years. Understanding and fully characterizing the mechanical nature of ILC has the potential to revolutionize clinical management of this unique disease.

P1-05-30: A statistical model for integration of on-treatment circulating tumor DNA dynamics and prediction of outcomes in patients with ER+/HER2- metastatic breast cancer

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Background: There is growing evidence that on treatment circulating tumor DNA (ctDNA) dynamics are a sensitive measure of treatment response in metastatic ER+/HER2- breast cancer (mBC). Questions remain about the approach to interpreting serial ctDNA tumor fraction (TF) measurements and how temporal evolution relates to clinical outcomes. Herein, we demonstrate a statistical joint modeling (JM) approach that combines longitudinal ctDNA and time-to-event data. This approach generates dynamic predictions which continually re-assesses a patient's event risk as ctDNA dynamics evolve, providing adaptive information that could potentially aid in decision making around treatment efficacy and risk for progression.

Methods: JM was applied to a single institution cohort of ER+/HER2- patients with mBC who underwent prospective collection of plasma while receiving endocrine therapy (ET) and CDK4/6-inhibitors (CDK4/6i). Plasma samples were collected pre-treatment and regularly on-treatment (mostly around the time of restaging CT). Samples were analyzed using the Guardant Infinity assay, a tumor-agnostic genomic and epigenomic platform. Patients with at least three plasma samples available were included in the model. To meet model assumptions, ctDNA TF values were logit-transformed. A hierarchical cubic spline random effects sub-model was utilized to capture the longitudinal ctDNA data and a cox-proportional hazard sub-model was used for the time-to-event data. Dynamic predictions for patient-specific progression-free survival (PFS) and overall survival (OS) curves were generated, which were continually updated as additional longitudinal ctDNA information was added. Baseline covariates were incorporated into the JM and are used to directly inform patient-level predictions. All results are displayed graphically.

Results: In total, 49 ER+/HER2- mBC patients with 279 ctDNA time points were used in the analysis. Baseline covariates included: patient age (median=62 years), CDK4/6i drug used [Palbociclib (71.4%) vs Ribociclib or Abemaciclib], histology [ductal (81%) vs lobular or mixed histology], current line of therapy [1 (75.5%) vs 2 or more], and prior adjuvant treatment [no (57.1%) vs yes]. Interrogation of various JM indicated that the patient's estimated current TF has a significant relationship with both OS and PFS (respective p-values <0.0001). This result is directly observable in the dynamic predictions, where increasing TF trends are indicative of a poorer PFS while decreasing TF trends show

improved outcomes. Each patient's unique combination of baseline covariate values informs PFS or OS predictions.

Conclusions: Here we demonstrate that complex longitudinal genomic data can be modeled successfully and leveraged to provide patient-specific prognostic estimates. This approach is in contrast to prognostication based on biomarker analysis of a baseline sample or the examination of TF changes between two set time points, which do not account for the continual evolution of dynamic biomarkers, such as TF, and how this evolution modifies outcome. This proposed statistical approach provides an opportunity to integrate serial biomarker results with patient information to enhance prognostic accuracy. The adaptive patient-level dynamic predictions generated by this model has the potential to improve the clinical utility of TF and longitudinal monitoring for clinical decision-making and should be studied and validated in additional clinical cohorts.

P1-06-01: Efficacy and Safety of Camrelizumab Combination Therapy in Triple-Negative Breast Cancer: A Systematic Review and Meta-Analysis

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Background: Triple-negative breast cancer (TNBC) has an aggressive clinical course with a high recurrence rate and risk of metastasis. Camrelizumab, a humanized monoclonal anti-PD-1 antibody, has shown promising prognostic outcomes for patients with TNBC by restoring T cell activation. This systematic review and meta-analysis aimed to evaluate the efficacy and safety of Camrelizumab combination therapy in women with TNBC.
Methods: Following PRISMA guidelines, a literature search was carried out on PubMed, Embase, Scopus, Cochrane, and ClinicalTrials.gov from inception until March 2024 using the keywords "Camrelizumab", "Anti-PD1", "Immune Check Point Inhibitors", "Breast Neoplasms", "Breast Cancer", "Breast Carcinoma". The initial search yielded a total of 10,282 articles, out of which 9 studies comprising women with histologically confirmed TNBC who were administered Camrelizumab were included in this review. We performed a meta-analysis using the "meta" and "metasens" packages via RStudio. Proportions were pooled using a random-effects model. Between-study heterogeneity was assessed using Cochrane Q χ^2 statistics and Higgins I^2 statistics. The evaluation of study quality was carried out using the "National Heart, Lung, and Blood Institute (NHLBI) Study Quality Assessment Tool for Before-After (Pre-Post) Studies with No Control Group." The protocol of this systematic review is registered with PROSPERO under the identifier CRD42024521992.
Results: A total of 397 patients with TNBC were included in the study. 116 patients had a T0 - T2 and 29 patients had a T3-T4 breast cancer stage before the start of the intervention. Camrelizumab was combined with Nab-paclitaxel, Apatinib, and Famitinib in 4, 3, and 2 studies, respectively. The pooled overall response rate was 58% (199/334, $P<0.01$, $I^2=94\%$). The pooled one-year survival rate was 71% (114/161, $P=0.12$, $I^2=49\%$). The

pooled stable disease and progressive disease at the last reported follow-up were 25% (71/271, P<0.01, I^2=85%) and 11% (31/242, P<0.01, I^2=79%), respectively. The most frequently reported adverse event was neutropenia in 166/277 (66%), followed by leukopenia 207/381 (59%), fatigue 173/406 (51%), Aspartate aminotransferase (AST) elevation 137/363 (39%), Alanine aminotransferase (ALT) elevation 130/363 (37%), and asthenia 37/115 (32%).
Conclusion: Our meta-analysis affirms that Camrelizumab combination therapy has shown promising results in terms of overall response rate and the one-year survival rate. Further research with large-scale randomized clinical trials is warranted to generate robust results.

P1-06-02: Remote functional cognitive rehabilitation in Breast Cancer Patients, a single center retrospective study

Einav Nili Gal-Yam, Sharon Harel, Rachel Kizony, Maya Ben-Yakov, Beatrice Shaham, Galia Barkai

Background: The number of breast cancer survivors is increasing substantially. About 15-50% of them cope with cognitive and executive functions difficulties that are related to decreased daily participation in meaningful activities and quality of life. Initial results point to the feasibility and efficacy of rehabilitation programs which focus on enhancing self-management abilities and executive functions, aiming to improve breast cancer survivors' participation in meaningful activities. Nevertheless, programs which are delivered remotely to improve accessibility to care are scarce. We report the results of a remote functional-cognitive occupational therapy rehabilitation in a single center.
Methods: Twenty-eight breast cancer patients were treated upon referral from their treating oncologists. Baseline evaluations were conducted in the clinic utilizing Montreal Cognitive Assessment (MOCA) and Trail Making Test (TMT-A and TMT-B). Personal treatment goals (meaningful activities, e.g: "organize a weekly schedule") were chosen and rated by each patient for perceived performance and satisfaction on a scale of 1(low) to 10(high) before and after the intervention, with the Canadian Occupational Performance Measurement (COPM). Improvement of two points or more in the COPM is considered as clinical meaningful.
The intervention included 8-12 sessions using videoconferencing. The first session aimed at promoting knowledge on the effects of cancer and its treatment on functional cognition and encouraging self-efficacy. The functional cognitive treatment was comprised of bottom-up sessions which utilized digital worksheets and gamified activities to improve cognitive skills, such as memory and executive functions. Additionally, top-down sessions focused on meta-cognitive strategies to enhance self-management for coping with daily life challenges, identified in the COPM. Additionally, self-practice exercises were provided to the patients.
Results: Between12/2021 to 01/2024, 28 breast cancer patients underwent the intervention and were included in this analysis. Twenty-six were breast cancer survivors on active follow-up and 2 were metastatic breast cancer patients who were in remission. 19/28 (68%) patients previously underwent chemotherapy treatment and 17/28 (60%)

patients were on endocrine treatment. Mean age was 50.4±11.2 (range 34-75). Twenty five patients (90%) completed at least 8 remote sessions. Mean MOCA score at baseline was 27.5±1.9, indicating intact general cognition. Baseline mean TMT scores were 39.5±12.6 seconds for TMT-A (20 (71%) below 50 percentile) and 83.5±39.6 seconds (14 (50%) below 50 percentile) for TMT-B. These results reflect a decrease in visual attention and executive functioning respectively. Mean COPM scores post intervention were significantly higher compared to the scores pre intervention for both perceived performance (pre = 4.2±1.6; post=7.0±1.2; p<.001) and satisfaction (pre = 3.0±1.8; post=6.7±1.8; p<.001). Out of 28 patients 21(75%) reported improvement in at least 2 or more points in performance and satisfaction of 2 activities.

Conclusions: Remote functional cognitive rehabilitation is feasible and beneficial in breast cancer patients who report cognitive impairment following medical therapy and should be integrated into the standard care. Our study is limited by its retrospective nature and short term follow up. We are now conducting a prospective randomized controlled trial with extended evaluations and a longer follow up.

P1-06-03: Concurrent GLP1R-agonist use with chemoimmunotherapy for early-stage triple-negative breast cancer: A potential detrimental effect

Bethania Santos

Introduction: Glucagon-like peptide-1 receptor agonists (GLP-1RAs) have emerged as a key class of drugs for treating type 2 diabetes mellitus (T2DM) and obesity, two of the most prevalent metabolic disorders globally. These drugs act by stimulating the GLP-1 receptor (GLP-1R), which enhances insulin secretion and exerts multiple metabolic benefits. Endogenous GLP-1 is rapidly degraded by dipeptidyl peptidase 4 (DPP4), limiting its plasma half-life to approximately 2 minutes. This rapid degradation led to the development of DPP4 inhibitors (DPP4i), which prevent GLP-1 inactivation and prolong its effects, thereby improving glycemic control in T2DM patients. GLP-1R was initially identified in the endocrine pancreas, but subsequent research revealed its expression in intraepithelial lymphocytes and immune cells, suggesting potential implications in immune modulation. Despite their widespread use and benefits in metabolic diseases, recent evidence suggests that GLP-1 receptor agonists and DPP4 inhibitors may also influence cancer biology. Current research suggests that these drugs might have paradoxical effects on cancer therapy, potentially altering the efficacy of immunotherapeutic approaches such as anti-PD-1 and anti-PD-L1 treatments. Furthermore, GLP-1R activation in tumor cells activates key signaling pathways, such as PI3K-AKT and MEK-ERK1/2, which regulate processes including anti-apoptosis, inflammation suppression, cell growth, autophagy, angiogenesis, and proliferation. This suggests that GLP-1R activation may not only undermine the efficacy of immunotherapy but also promote tumor cell survival and expansion, potentially accelerating cancer progression. This study aims to investigate the impact of GLP-1RAs and DPP4i exposure on pathological complete response (pCR) rates for patients with early-stage triple negative breast cancer (TNBC) receiving neoadjuvant chemotherapy with immunotherapy.
Methods: Patients with early stage TNBC diagnosed between July 1, 2021, and December 31, 2023 who subsequently received the KEYNOTE-522 regimen were identified through three institutional databases. Patients taking GLP-1RAs and DPP4i at breast cancer diagnosis and throughout the neoadjuvant period, either alone or in combination with other diabetes or obesity medications, were identified. Patients who started or stopped GLP-1RAs/DPP4i therapy during neoadjuvant chemoimmunotherapy administration were excluded. Bivariate analyses were conducted to determine if there were significant differences in pCR rates between GLP-1RAs/DPP4i users and non-users. Group comparisons were made using the Chi-square test for categorical variables and the two-sample t-test for continuous variables. IHC staining for GLP1R was performed on human TNBCs.
Results: Among the 347 eligible patients identified, 25 (7.2%) were using GLP-1RAs or DPP4i. The pCR rate for those receiving GLP-1RAs or DPP4i was 28% (7/25) vs. 63.66% (205/322) between those using other classes of diabetes medication or no medications, respectively (p=0.0006). The pCR rate among those using other classes of diabetes medications was 60% (27/45). There was no significant difference between the two groups

(GLP-1RAs/DPP4i-exposed vs not) by age, body mass index (BMI), or T stage. In univariate analysis, age younger than 50 years, grade 3 disease, BMI of ≥30, and the absence of diabetes had improved pCR rates ($p<0.1$). In multivariate analysis, grade, age, BMI, and diabetes were significant predictors of pCR [OR (95% CI): 2.15 (1.05, 4.40), 2.02 (1.15, 3.56), 1.85 (1.08, 3.20), and 2.31 (1.13, 4.75), respectively; $p<0.05$]. GLP1R was detected in tumor cells and infiltrating immune cells in human TNBC specimens (n=100). Ongoing spatial transcriptomics of pre and post treatment tumor specimens from patients taking GLP1R-agonists with neoadjuvant chemotherapy will be presented.

Conclusion: We observed significantly lower pCR rate among patients taking GLP-1RA/DPP4i during neoadjuvant chemotherapy for TNBC. These effects were not observed with other diabetic medications. Detection of GLP1R expression in TNBC specimens indicates there are direct and indirect effects of agonists to the GLP1 pathway on tumor immunity and response to chemotherapy that detrimentally affect response rates. Use of GLP-1RA/DPP4i use may need to be carefully considered during breast cancer therapy.

P1-06-04: Psychological stress and its correlations to patients with acute lymphedema after breast cancer surgery

Li-Chen Tang,Li-Ping Ge

Introduction: Breast cancer patients are commonly affected by lymphedema and psychological problems. This study aimed to determine the incidence and determinants of anxiety and depression in patients with lymphedema after breast cancer surgery.
Materials and Methods
The study enrolled 1,613 patients who underwent curative breast cancer surgery during 2023 in Shanghai Cancer Hospital, Fudan University, Shanghai, China. Descriptive analysis and logistic regression were conducted in the study by using SPSS 26.
Results: Among all the patients, 363 (22.50%) cases were identified as acute lymphedema. Anxiety was identified in 500 (31.0%) breast cancer patients after surgery and depression was detected in 21.2% of patients in the overall cohort.
Acute lymphedema was the significant impact factor for anxiety in breast cancer patients (OR = 1.57, 95%CI: [1.04 to 2.38], P=0.033). Subgroup analysis observed that in the acute lymphedema group, a longer period of hospital stay (OR = 0.51, 95%CI: [0.30 - 0.88], P=0.017) and invasive disease (OR = 0.39, 95%CI: [0.19-0.78], P=0.0008) were related to less anxiety; lymph-vessel invasive disease was related to increased anxiety (OR= 5.97, 95%CI: [1.15 – 30.97], P=0.03). On the other hand, menopause (OR = 0.72, 95% CI=0.56 - 0.94, P=0.014), and the length of hospitalization (OR = 1.30, 95% CI=1.00-1,68, P=0.047) were the factors influencing depression in all breast cancer patients. Among acute lymphedema group, lymphnode surgery (OR = 8.52, 95%CI: [1.56 - 48.23], P=0.014) was the impact factor to depression.
Conclusion: Psychological stress and acute lymphedema may have the crucial impact on breast cancer patients after surgery.

P1-06-05: Efficacy of using "cold caps" or "scalp cooling systems" with the DigniCap system in Uruguayan patients receiving chemotherapy for the treatment of early-stage breast cancer (Digni-T-Uy: DigniCap Trial Uruguay)

Dahiana Amarillo, Melanie Garcia, Maria Clara Rodriguez, Maria Guerrina, Noelia Strazzarino, Bruno Borrelli, Valeria Lemes, Natalia Camejo, Mauricio Cuello, Cecilia Castillo, Gabriel Krygier

Background: Alopecia is a generally transient adverse effect of chemotherapy treatment in patients with early-stage breast cancer, causing significant emotional impact and occasionally rejection of therapies. Although it is usually reversible, it can take months to resolve, amplifying the psychological effect of the treatment. Strategies such as cold caps or scalp cooling systems have emerged in recent years, aiming to prevent or limit this adverse effect, and have shown promising results. This prospective observational study aimed to describe our experience with the DigniCap system (DCS).
Methods: This is a prospective observational study in patients who started scalp cooling with DCS while undergoing chemotherapy for early-stage breast cancer. From March 2022 to June 2024, 50 consecutive early-stage BC patients who received anthracycline and/or taxane-based treatment were enrolled, subject to local Ethics Committee approval. Efficacy was defined as the "successful prevention" of hair loss, with a maximum Dean score of ≤ 2 (hair loss ≤ 50%). Safety, tolerance, successful prevention of hair loss as determined by the patient's perception, and quality of life were also evaluated.
Results: Hair loss of 50% or less (Dean score of 0-2) was seen in 27 (67.5%) of 40 patients. Complete preservation of the hair (G0) was observed in 6 pts (15%), G1 in 14 pts (35%), and G2 in 7 pts (17.5%). The most frequent scalp cooling-related symptoms were headache (72.5%), scalp pruritus (62.5%), neck pain (50%), and coldness (45%). Overall, 20% (n = 8) of patients discontinued DCS because of unsatisfactory hair preservation (n = 4, 10%) and cold discomfort (n = 4; 10%). The quality of life of patients who had successful treatment was not significantly affected.
Conclusions: Our results confirmed and reinforced previous evidence that scalp cooling with DCS is safe and effective for reducing chemotherapy-induced alopecia during CT with anthracycline and/or a taxane-based regimen.

P1-06-06: Oral cyclophosphamide-induced hemorrhagic cystitis from metronomic Cyclophosphamide/Methotrexate/5-Fluorouracil for treatment of synchronous bilateral breast cancer – A case report

Jina Yun, Grace Baek, Arianne Duong, Dane Fritzsche, William Gwin

Introduction: Cyclophosphamide/methotrexate/5-fluororuracil (CMF) has been used for the treatment of early-stage breast cancer since the 1970s. Cyclophosphamide, a main component of the CMF regimen, is an alkylating agent with potential to cause hemorrhagic

cystitis. This side effect is thought to be dose-dependent, with incidence up to 12-41% reported with doses of 150 – 200 grams. The risk of cyclophosphamide-induced hemorrhagic cystitis is very low with CMF, due to the low dose used in this regimen. At these doses of oral cyclophosphamide in CMF, intravenous (IV) hyperhydration or supportive care with mesna is not recommended. At the Fred Hutchinson Cancer Center, metronomic CMF (mCMF) is utilized in the adjuvant setting, where smaller doses are administered weekly for patients who may not tolerate higher doses of chemotherapy in traditional CMF. Cho and colleagues demonstrated similar efficacy and more tolerable side effect profile when compared with traditional CMF as well. The following report presents a patient with cyclophosphamide-induced hemorrhagic cystitis while receiving therapy with mCMF for the treatment of her breast cancer.

Case Report: In April 2021, a 46-year-old female was diagnosed with cT2 cN0 cM0 estrogen receptor (ER)-/ progesterone receptor (PR)-positive, human epidermal growth factor receptor (HER)2-negative IDC of the right breast in addition to cT1b cN0 cM0 ER-positive, PR-negative, HER2-negative IDC of the left breast. Her past medical history included ulcerative colitis status post ileostomy, history of Stevens-Johnson syndrome with sulfa drugs, and mucinous cystadenoma of the ovary status post total abdominal hysterectomy and bilateral salpingo-oophorectomy. In June 2021, the patient presented to the Fred Hutchinson Cancer Center for initiation of adjuvant systemic therapy with mCMF. Her regimen consisted of cyclophosphamide 60 mg/m2 by mouth once daily, methotrexate 15 mg/m2 given intravenously once weekly, and 5-fluorouracil 300 mg/m2 given intravenously once weekly. She began treatment in July 2021, and her total dose of oral cyclophosphamide was 125 mg daily. Prior to week 11 of treatment, the patient reported persistent dysuria, urinary urgency, and hematuria. Despite several emergency department visits, antibiotic usage, and non-pharmacologic supportive care, she continued to have urinary symptoms. Ultimately, she was diagnosed with cyclophosphamide-induced hemorrhagic cystitis, confirmed by cystoscopy and cytopathology. The cyclophosphamide was discontinued at this time and the patient had received a total of 9.4 grams of oral cyclophosphamide over a 75-day period. With the resolution of her cystitis, adjuvant chemotherapy with only methotrexate and 5-fluorouracil was resumed 37 days after her last methotrexate and 5-fluouracil infusions and 30 days after her last dose of oral cyclophosphamide.

Discussion: To date, there are four case reports of cyclophosphamide-induced hemorrhagic cystitis in patients with breast cancer. Two patients received capecitabine and oral cyclophosphamide while two patients received docetaxel and intravenous cyclophosphamide, all receiving cyclophosphamide at higher doses than the patient described in this case report. This case is the first to describe cyclophosphamide-induced hemorrhagic cystitis with adjuvant mCMF with oral cyclophosphamide at the lower dose of 60 mg/m2. The exact etiology of this adverse effect in this patient remains unclear; some hypothesized patient risk factors include reported nightly administration technique and her history of ulcerative colitis requiring ileostomy leading to inadequate oral hydration and bladder voiding and reduced fluid absorption, respectively.

P1-06-07: APOE4 impairs the cardiac stress response to doxorubicin through defective M2 macrophage activation and tissue repair transcription pathways

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Background: The human apolipoprotein E (APOE) gene has three major allelic variants: APOE3, APOE4, and APOE2. Carriers of the APOE4 allele are at high risk for Alzheimer's and other neurodegenerative disorders and exhibit elevated oxidative stress in the central nervous system. Recently, we observed that mice expressing the human APOE4 allele are predisposed to cardiac damage induced by doxorubicin (DOX), a potent and widely used breast cancer therapeutic. How APOE4 contributes to oxidative stress-related tissue damage remains poorly understood.
Objective: To identify mechanisms of APOE4-mediated vulnerability to DOX cardiotoxicity.
Methods: C57Bl/6 mice (5-8 mo and 14-18 mo, male and female) with human APOE3 or APOE4 homozygous knock-in (=APOE3 and APOE4, respectively) received a single IP injection of saline (Control) or DOX (10 mg/kg) and monitored between 3-45 days afterward. Cardiac function was quantitated using echocardiography (Vevo 3100) at baseline and during the study. TUNEL assays were used to identify apoptotic cells. Collagen was imaged by Masson's Trichrome staining. Myocardial protein and RNA were extracted from the left ventricle and subjected to immunoblotting, IHC, real-time PCR, and RNASeq. Transcriptomic data were analyzed using Gene Set Enrichment Analysis (GSEA) for pathway analysis and CIBERSORTx for deconvolution of myocardial immune cell subtypes.
Results: At baseline, no difference in cardiac function was observed between the 2 mouse lines. Following DOX treatment, APOE4 mice had greater declines in left ventricular ejection fraction, heart weight, and more myocyte apoptosis compared with age-matched APOE3 mice (all $p<0.05$). APOE4 also conferred more age-associated myocardial collagen and cardiomyocyte apoptosis than APOE3, and higher myocardial levels of 4-HNE (4-hydroxy-2-nonenal), an oxidative stress byproduct. Surprisingly, APOE4 mice had a marked reduction in immunoglobulin production and age-dependent IgG deposition in the myocardium compared with both APOE3 and wt mice ($p= 0.002$, E4 vs. E3 at 17 mo). The transcriptomic analysis confirmed defective activation of TGF-beta, TNF-alpha, Myc, and p53 pathway genes in APOE4 mice after DOX, as well as a defective tissue repair response. In APOE3 myocardium, mRNAs encoding neuregulin (Nrg1), a key cardiac survival/repair factor, and multiple cardiac-specific contractile proteins were induced at d3 after DOX; this response was markedly attenuated in APOE4 mice. CIBERSORTx analysis for 22 distinct immune cell types showed an increase in activated M2 macrophages after DOX in APOE3, but not in APOE4 mice. Western and immunohistochemical analyses confirmed increased CD206, a specific M2 macrophage biomarker, in APOE3 but not in APOE4 mice after DOX ($p<0.05$.) Of note, M2 macrophages increased with age in both wt and APOE3 mice, but not in APOE4 mice ($p<0.05$, E3 vs. E4, 17 mo.).
Conclusion: Our results suggest that the APOE4 allele may confer increased vulnerability to DOX-induced and age-mediated cardiac damage through specific impairment of post-injury

repair responses involving M2 macrophage polarization and activation. Whether this is related to known APOE allelic differences in lipoprotein transport or innate immunity functions remains to be determined, as does potential relevance to chemotherapy and other types of cardiac injury in humans.

P1-06-08: Quantifying Treatment Tolerability in Real-world Breast Cancer Practice: A Pragmatic Approach Utilizing Patient-Reported Outcomes (PROs)

Emelly Rusli, Aaron Galaznik, Debra Wujcik

Background: Treatment tolerability in cancer care, historically assessed by the clinician, is moving towards incorporating patients' voices to capture how they feel and function during treatment1-3. Women undergoing breast cancer (BC) treatment often experience debilitating symptoms and decreased quality-of-life (QoL). Routine symptom monitoring and patient-reported outcomes (PROs) may enhance the understanding of tolerability from patients' perspectives. Measurement of patient-reported tolerability (PRT) for thyroid cancer was recently published by Brose et al (2024)4. This study aimed to adopt this method to explore PRT in breast cancer using PROs collected in real-world clinical practice.
Methods: Study participants included patients who enrolled in Carevive PROmpt®, a remote symptom monitoring (RSM) platform, and received BC treatment between 9/2020 and 5/2024. Patients completed at least one weekly PRO survey that included treatment bother, measured by a single item FACT-GP5 ("I am bothered by side effects of treatment"), and were followed from baseline survey completion until last survey completion or end of study period (whichever was earliest). Treatment data (name, start, and end dates) were sourced from the electronic medical record or entered directly into the platform by the care team. Treatment tolerability was defined as the degree of treatment bother and was classified into two categories: 1) high treatment bother (HTB), defined as response 3 ("Quite a bit") or 4 ("Very much") and 2) low treatment bother (LTB), defined as response 0 ("Not at all"), 1 ("A little bit"), or 2 ("Somewhat") to the single item FACT-GP5 on a given survey. PRT was calculated as the proportion of surveys with HTB per patient per regimen. Persistent HTB was defined as reporting HTB 76-100% of the time. Results were analyzed by stage (Early vs. Metastatic), biomarker (HER2-/HR+, HER2+, TNBC), therapy type, and PRO assessment time (weeks 1-4 vs. weeks 5+).
Results: A total of 299 patients were included in the study. Median age was 55, 85% White, 21% metastatic, 14% TNBC, with median follow-up of 10 weeks. Average number of treatment regimens per patient was 1.2 (SD=0.5, Median=1). About 44% of patients (n=132) reported HTB at least once. Incidence rate of HTB was 6.46 per 100 patients per week. Most patients (79%) experienced HTB </=50% of the time, yet 21% experienced HTB 51-100% of the time on treatment. More patients in the metastatic group appeared to report persistent HTB (13.8%) than in the early-stage group (7.9%). Similarly, more patients with TNBC type reported persistent HTB than other biomarkers. When examined by therapy type, the proportion of patients with persistent HTB ranged from 6-13%.

Prevalence by PRO assessment time showed a higher percentage of patients experiencing persistent HTB at week 5 or later than during weeks 1-4 (8.4% vs 6%, respectively). Further exploration by stage showed the proportion of persistent HTB appeared to increase from weeks 1-4 (5.1%) to week 5 or later (8.4%) in the early-stage group while the proportion was more consistent in the metastatic group (9.4% vs. 9.8%, respectively). Conclusions: This study utilized PROs to quantify treatment tolerability in women receiving BC therapy in the real-world clinical practice. Nearly half of women reported HTB at least once during treatment and 1 in 5 reported persistent HTB. A closer look showed more patients with metastatic disease and TNBC reported persistent HTB, and bother was higher at week 5+ versus week 1-4. PRT provides the means to quantify tolerability and track longitudinal changes, which gives important insights into BC treatment experience. Future studies should focus on factors that impact tolerability, symptoms that are most persistent over time, and healthcare resource utilization associated with HTB.

P1-06-09: Importance of Assessing Thyroid Dysfunction in Breast Cancer Patients Undergoing Pembrolizumab-based Neoadjuvant Chemotherapy

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Introduction: Pembrolizumab is a novel immunotherapy agent that improves oncological outcomes in various cancer types. Although there are minor immune-related adverse events (irAEs) when used alone, various irAEs have been reported when it is combined with chemotherapy. The aims of this study were to investigate irAEs that occurred after neoadjuvant chemotherapy (NAC) with pembrolizumab based on the KEYNOTE-522 regimen for triple-negative breast cancer (TNBC) and to identify fatal irAEs that should be corrected before surgery under general anesthesia.
Methods: Between 2022 and 2024, a total of, 71 cases from 81 patients who completed NAC with pembrolizumab based on KEYNOTE-522 regimen (wP/Cab and pembrolizumab followed by AC and pembrolizumab) followed by surgery were reviewed based on their medical records. All irAEs that occurred were investigated and classified into five categories: systemic, gastrointestinal, respiratory, musculoskeletal, and endocrine. Underlying diseases that existed before the diagnosis of breast cancer or complications that occurred after surgery were excluded.
Results: The mean age of the patients was 49.62 (SD, ±10.6) years, and the stages at diagnosis were as follows: IIA (n=23, 32.4%), IIB (n=22, 31.0%), IIIA (n=14, 16.2%), IIIB (n=1, 1.4%), and IIIC (n=11, 15.5%). After NAC with pembrolizumab, the pCR rate was 49.3% (35/71)
Fifty-three patients (62.2%) experienced adverse events, and the number of adverse events were as follows: 1 (n=11, 20.8%), 2 (n=20, 37.7%), and ≥3 (n=22, 41.5%). Gastrointestinal symptoms were the most common (n=46, 86.8%), and dermatologic symptoms were the second most common (n=31, 58.5%). Among fatal irAEs, including colitis and pneumonitis,

they occurred in 11 patients (20.8%) and 2 patients (3.8%), respectively. There was no case of hepatitis, myocarditis, or hypophysitis as fatal irAEs after NAC and pembrolizumab for TNBC. Among these irAEs, thyroid disorders (n=16, 22.5%) were the most common adverse event after skin rash and nausea. Of the patients with thyroid disorders, 3 experienced fatal dysfunction. Their surgeries were delayed for an average of 8.5 weeks to correct the thyroid imbalance before they could safely undergo general anesthesia. The remaining patients, who did not experience such complications, were able to undergo surgery after an average of 5.2 weeks.
Conclusion: The irAEs that occurred after NAC with pembrolizumab in TNBC were varied and included some that were severe enough to affect the timing of surgery under general anesthesia. Thyroid dysfunction, in particular, should be evaluated and corrected during the neoadjuvant chemotherapy to prevent delays in surgery.

P1-06-10: Long-term Cardiovascular Risks in Breast Cancer Survivors with Diabetes

Sixten Harborg, Martin Magnusson, Olle Melander, Jonas Manjer, Signe Borgquist

Purpose: Examine the association between diabetes and long-term cardiovascular outcomes in breast cancer survivors enrolled in the Malmö Diet and Cancer Study (MDCS).
Methods:The MDCS enrolled 17,035 Swedish women from 1991 to 1996. We identified female MDCS participants diagnosed with invasive breast cancer between 1991 and 2014 and followed them until the first occurrence of a cardiovascular event (CVE), death, emigration, or December 31, 2020. Participants with prevalent breast cancer (N=576), carcinoma in situ (N=105), bilateral breast cancer (N=21), or metastatic disease at diagnosis (N=15), were excluded. Cardiovascular outcomes were ascertained through the Swedish National Patient Register and cause of death registries. CVE was defined as having an event of myocardial infarction, heart failure, or stroke. Diabetes information was obtained from six national and regional registries and treated as a time-varying variable. Survivors with diabetes before breast cancer diagnosis were exposed from the date of diagnosis, while those diagnosed after breast cancer were exposed from the date of diabetes diagnosis. We fit Cox regression models to compute hazard ratios (HRs) with 95% confidence intervals (CI) for CVEs and cardiovascular mortality comparing breast cancer survivors with and without diabetes and stratified by adjuvant cancer therapies. Sensitivity analyses were conducted for the subgroup of survivors with incident diabetes after breast cancer diagnosis.
Results: Among the 1,099 breast cancer survivors followed for a median of 10.7 years, 87 had diabetes before breast cancer diagnosis, and 116 were diagnosed after breast cancer diagnosis. During follow-up, 255 CVEs occurred, and 92 survivors died from cardiovascular disease. Survival analysis showed that breast cancer survivors living with diabetes had an increased risk for CVEs (HR 1.44, 95% CI 1.05-1.99) compared with breast cancer survivors without diabetes. Stratifying by adjuvant chemotherapy, we found that survivors who received chemotherapy had a substantially higher risk for CVEs (HR 2.34, 95% CI 1.16-

4.72). When only considering survivors with incident diabetes after breast cancer diagnosis, the risk for CVEs was increased (HR 1.54, 95% CI 1.00-2.39) again specifically for chemotherapy-treated individuals (HR 3.46, 95% CI 1.24-9.66). Additionally, the risk of cardiovascular mortality was increased in survivors living with diabetes (HR 2.23, 95% CI 1.38-3.60). No differences were observed for the association with CVE when analyses were stratified for adjuvant endocrine therapy or radiation therapy.
Conclusion: Diabetes is associated with an increased risk of long-term cardiovascular events and cardiovascular mortality in breast cancer survivors. The risk is particularly pronounced in survivors with incident diabetes following breast cancer diagnosis and among survivors treated with chemotherapy. These findings underscore the importance of cardiovascular monitoring and management in breast cancer survivors, particularly those who develop diabetes during follow-up. Early detection and treatment of post-breast cancer diabetes could potentially mitigate these cardiovascular risks.

P1-06-11: Intravenous Iron in Early-Stage Breast Cancer Patients Receiving Neoadjuvant Chemotherapy

Jules Cohen, Meghana Rao

Background: Early-stage breast cancer patients frequently demonstrate iron deficiency with or without anemia because of recurrent menstrual losses during their premenopausal years. It is not uncommon to remain iron deficient after menopause due to failure to replete whole body iron stores with diet alone. Chemotherapy induces anemia through its suppressive effects on the bone marrow and hematologic recovery can be impeded by iron stores insufficient to manufacture new red blood cells. Available data suggests that intravenous (IV) iron supplementation is safe in cancer patients and that anemia in cancer patients is associated with an increase in all-cause mortality. We are conducting a retrospective study to understand the effect of IV iron supplementation on efficacy and toxicity in early-stage breast cancer patients receiving neoadjuvant chemotherapy. We hypothesize that IV iron supplementation can improve rates of pathologic complete response (pCR) and improve our ability to administer standard neoadjuvant chemotherapy regimens by maintaining patient quality of life and reducing need for dose modifications and treatment delays.
Objective: To identify the effect of IV iron supplementation on the efficacy and toxicity of neoadjuvant chemotherapy in early-stage breast cancer patients.
Methods: We are analyzing efficacy endpoints (pCR, 3 year invasive disease-free survival, etc.) and toxicity endpoints (dose reductions, dose delays, hemoglobin decreases, hemoglobin recovery, etc.) in a retrospective cohort of breast cancer patients receiving neoadjuvant chemotherapy. Patients will be divided into three major groups: (1) iron deficient (ferritin <100) receiving IV iron, (2) iron deficient not receiving IV iron and (3) not iron deficient (ferritin >100) not receiving IV iron. pCR rate will be evaluated across the entire cohort and subdivided by luminal (ER-positive), triple negative and HER2-positive subtypes. We will further analyze the absolute and relative changes in hemoglobin levels

over time; the incidence of dose reductions and dose delays; and the effects on patient quality of life as evidenced in the medical record before, during and after the course of neoadjuvant chemotherapy.
Results: We anticipate that iron deficient patients with or without IV iron supplementation will manifest a pCR rate no worse than non-iron deficient patients but that there will be better tolerability and fewer dose reductions or dose delays in patients receiving IV iron supplementation. At best, patients receiving IV iron will demonstrate a pCR rate superior to patients not receiving IV iron, possibly regardless of whether it is indicated (ferritin <100) or not (ferritin >100).
Conclusions: Iron deficiency, with or without anemia, is common in women with early-stage breast cancer receiving neoadjuvant chemotherapy. Available data suggests that IV iron is safe during cancer chemotherapy and may improve efficacy and minimize side effects to these toxic drugs commonly known to cause myelosuppression. If we demonstrate that patients receiving IV iron have improved pCR rates and decreased dose reductions and dose delays, this could have a practice-changing effect on supportive care interventions administered during neoadjuvant chemotherapy for early-stage breast cancer.

P1-06-12: Efficacy and safety of the addition of prophylactic atropine to patients with metastatic triple-negative breast cancer treated with sacituzumab govitecan: a Spanish multicenter real-world study

María José Echarri, Marta Santisteban, Juan David Cárdenas

Background: Sacituzumab govitecan (SG) is a first-in-class trophoblastic antigen-2 (Trop-2)-directed antibody–drug conjugate (ADC) composed of a humanized monoclonal antibody (hRS7 IgG1κ) that recognizes Trop-2 and SN-38, a topoisomerase I inhibitor which is covalently attached to the antibody by a hydrolysable linker and results in DNA damage leading to apoptosis and cell death.
SG showed a significant and clinically meaningful improvement in progression-free survival (PFS) and overall survival (OS) compared with single-agent chemotherapy among patients with pretreated advanced triple negative (TN) and luminal HER2 negative breast cancer (BC) in the ASCENT1 and TROPiCS-022 trials, respectively.
SN-38 can cause early-onset diarrhea through parasympathetic stimulation and delayed diarrhea via intestinal epithelial damage, inflammation, and dysbiosis. In clinical trials, the safety profile of SG was manageable, with febrile neutropenia (4.8%), diarrhea (3.9%), neutropenia (2.6%) and pneumonia (2%) being the most frequently reported serious adverse reactions3, causing dose reductions and treatment discontinuations.
Atropine is a muscarinic acetylcholine receptor antagonist that suppresses the parasympathetic effects of SN-38. Premedication with atropine sulfate in treatments such as SG could improve the management of severe diarrhea associated with cholinergic syndrome.
Objective: To evaluate the efficacy and safety of the use of prophylactic atropine to prevent diarrhea in advanced metastatic BC patients treated with SG.

Case series: Here, we describe 17 female patients with a median age of 50 years (29-72) and histologically confirmed TN (13) and HR+/HER2- unresectable locally advanced mBC (4) who were treated with SG and prophylactic atropine according to the approved indications in Spain. At baseline, most of the patients had ECOG score of 1 (52.9%) or 2 (35.3%), positive germline BRCA 1/2 mutations (11.8%), PDL1+ (82.1%), and visceral metastasis 41.2%, central nervous system (CNS) 17.6%, and both 64.7%.
According to the efficacy results, the median lines of therapy was 3 (range 1-9), with a median duration of response of 4 months (2-12). We observed a clinical benefit rate of 64.7%, defined as the percentage of advanced cancer patients who achieved complete remission (5.9%), partial remission (41.2%) and stable disease (17.6%). Median progression-free survival was 5 months.
The main treatment-related adverse events were any grade (64.7%) or grade 3-4 (23.5%) neutropenia, and diarrhea (58.8% grade 0; 35.3% grade 1; and 5.9% grade 2; without grade 3-4), 17.6% of the patients required a dose reduction. No discontinuations due to treatment-related adverse events were observed.
Conclusions: We observed a similar median progression-free survival to that observed in the ASCENT trial. Adding atropine to SG led to a higher proportion of subjects without diarrhea (58.8%) compared to ASCENT (30.2%), with no subjects presenting grade 3-4 diarrhea. These finding suggested that adding atropine to patients treated with SG might be beneficial for reducing diarrhea while maintaining the efficacy of the treatment.

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P1-06-13: Financial Toxicity and Quality of Life in Breast Cancer Patients at a Safety Net Hospital

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Introduction: Financial toxicity (FT) is the burden faced from out-of-pocket expenses related to cancer treatment, including direct cost of treatments, lost income, and travel expenses. Studies suggest increased FT is associated with decreased quality of life (QOL) and, possibly, worse survival. Patients from potentially marginalized groups including non-English speakers or patients with low socioeconomic status may be particularly vulnerable to FT. Maimonides Medical Center (MMC) is a safety net hospital in Brooklyn, NY, that treats many such patients. Here we present preliminary data from a prospective, longitudinal study of FT in breast cancer (BC) patients at MMC.
Methods: All patients undergoing curative-intent treatment for BC at MMC were eligible. We collected demographic and clinical data from patients' electronic medical records. FT was

assessed via the total score on the validated Comprehensive Score for Financial Toxicity (COST) version 2 questionnaire and QOL was assessed via the summary score of the EORTC Quality of Life Core-30 questionnaire and EORTC question 30: "How would you rank your overall quality of life in the past week?" The EORTC summary score was calculated according to the scoring manual and does not include question 30. EORTC summary score is out of a maximum of 100, with a higher score indicating better QOL. COST is scored out of a maximum of 44, with a higher score indicating better financial well-being (FWB).
In the study, patients complete questionnaires at baseline (i.e. prior to treatment start) and 6 months later, with optional collection at 3, 9, and 12 months. Preliminary data presented here were collected at baseline. Data analysis was performed using SPSS (version 29.0, IBM Corp.) and R (version 4.4.1, R Core Team). Descriptive statistics, Pearson correlation, ANOVA, and Spearman's rank correlation analyses were performed. All p values are reported from 2-sided tests, and the results were deemed statistically significant at $p < .05$.
Results: Thirty-seven patients were included in this analysis. Mean age at diagnosis was 57 (SD: 10). 13 patients (35%) did not speak English. Six patients (16%) identified as Asian, 18 (49%) as Black, 8 (22%) white, and 5 (13%) declined to answer. Twenty patients (54%) reported household income of less than $60k per year, 8 (22%) reported more than $60k, and 9 (24%) declined to answer. Fourteen patients (38%) had private insurance coverage, 9 (24%) Medicare, and 13 (35%) Medicaid. Six (16%) patients had DCIS, 21 (57%) had stage T1 BC, 5 (14%) had T2-3 BC, 4 (11%) had node-positive BC and 1 (3%) had bilateral BC.
At baseline, mean COST score among all participants was 22 (SD: 10). The mean EORTC summary score was 83 (SD: 17). For EORTC question 30, patients responded on a 7-point Likert scale where 1 indicates "very poor" QOL and 7 indicates "excellent" QOL. No patient marked 1 or 2, 2 (5%) marked 3, 10 (27%) marked 4, 7 (19%) marked 5, 9 (24%) marked 6, and 9 (24%) marked 7.
Better FWB measured via (higher) COST score positively correlated with better overall QOL via both EORTC summary score ($\rho = 0.47$; $p = 0.004$) and EORTC question 30 ($\rho = 0.39$; $p = 0.017$). There was a statistically significant association between higher COST score and patients with household incomes >$60k ($p = 0.002$). Early-stage (DCIS or T1) was also associated with higher COST score ($p = 0.047$). There was no difference between the COST scores of patients of differing age, insurance type, language, or ethnicity ($p > 0.05$).
Conclusion: Worse FT at baseline is associated with lower QOL in BC patients, underscoring the importance of financial burdens for cancer patients. Patients with lower household incomes and those with more advanced disease experience worse FT even before initiation of treatment. Further work including longitudinal data collection will help inform our understanding of how FT develops over the course of treatment and help develop future interventions to screen for and mitigate the effects of FT.

P1-06-14: The relevance of carbon footprint in enviro-logistical analysis in breast cancer trials

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Background: The consequences of environmental pollution in oncology are well known and should be addressed. Clinical trials in breast cancer typically compare different therapeutic strategies regarding their effect on survival endpoints such as overall survival, disease-free survival, or patient-reported outcomes like quality of life (QoL). Both endpoints relate to the patients' quantitative (time) and qualitative (QoL) benefits, but they do not reflect the patients' time burden caused by the treatment and the associated environmental burden that can ultimately negatively affect the achieved benefit.
Methods: In the ongoing MyTime study (DRKS00033577) during the neoadjuvant treatment of early breast cancer and the PADMA trial (NCT03355157) in the metastatic setting, an enviro-logistical analysis is included to evaluate the logistical burden on the patient and the consequent ecological burden on the environment. Therefore, we propose the clinically relevant key principles of carbon footprint and enviro-logistical analysis that could be incorporated in future breast cancer trials.
Results: First, according to different treatment schemes, the necessary number of contacts with the healthcare system should be recorded as a primary variable. Its relevance lies in the logistical analysis of the patients' burden from the treatment. The related parameters to the number of contacts include the time spent on necessary contacts (time burden of the patient due to the therapy) and the climate impact in CO2 equivalents caused by the means of transport. Second, a material evaluation based on the material and energy consumption necessary for the application of the therapy should be assessed using carbon footprinting or streamlined life-cycle assessment methodology. Consequently, the additional therapy-triggered CO2 equivalents are included, and the carbon footprinting is completed. By including the time burden on patients and the consequent environmental burden from transport, materials, and energy consumed, beneficial information for comparing treatment options holistically can be acquired.
Conclusions: In summary, while the primary goals will remain survival and QoL, for treatment options with equivalent effectiveness, the enviro-logistically optimal one could be selected to reduce patients' burden and environmental pollution, thereby avoiding secondary negative factors affecting outcomes. Therefore, the enviro-logistical approach should be included in clinical trials.

P1-06-15: Multi-Site Real World Data of Patient Reported Outcomes from Patients with Breast Cancer on Immunotherapy or Antibody Drug Conjugates using the Carevive PROMPT™ Web-Based Platform

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Introduction: Immunotherapy and antibody drug conjugates are quickly becoming the backbone of treatment regimens for breast cancer. However, these therapies do present with appreciable treatment emergent adverse events that are variable and difficult to predict in individual patients. Patient reported outcomes (PROs) are critical for

understanding the patient experience and kinetics of treatment emergent adverse event. Therefore, providing tools for patients to capture these events outside of a clinical visit is critical.
Methods: The Patient Reported Outcomes mobile web-based platform, Carevive PROmpt™ was used to collect symptom and wellness data once per week for at least 12 weeks using validated psychometric tools (PRO-CTCAE (symptoms), FACT-GP5 (treatment bother), PROMIS 4a (physical function), ECORTC QLQ C30 (quality of life)). Descriptive statistics are shared from 97 patients with breast cancer who were treated with immunotherapy (atezolizumab, pembrolizumab) and/or an antibody drug conjugate (ado-trastuzumab emtansine (T-DM1), fam-trastuzumab deruxtecan-nxki (T-Dxd), sacituzumab govitecan-hziy (sacituzumab)) as standard of care. The following 16 symptoms were measured: anxiety, constipation, cough, decreased appetite, diarrhea, fatigue, general pain, insomnia, mouth/throat sores, muscle pain, nausea, numbness/tingling, rash, sadness, shortness of breath, and vomiting. The number of patients reporting an individual symptom was divided by the total number of patients each week to generate a percentage positive value for each symptom for each week. The median percentage and interquartile range (IQR) of each symptom over the evaluation period is reported. Data was used from multiple centers.
Results. Fifty-two (54%) triple negative, 8 (8%) HR-HER+, 17 (17%) HR+HER2+, 19 (20%) HR+HER2-, and 1 (1%) unknown subtype were included in this analysis. The majority of patients, 56 (58%), were clinically early-stage. The remaining patients were metastatic (32 (32%)) or unknown stage (10 (10%)). Fifty-two (54%) patients were treated with immunotherapy plus chemotherapy regimens and 34 (35%) were treated with antibody drug conjugates, including 18 (18%) T-DM1, 15 (15%) T-Dxd, and 2 (2%) sacituzumab. Eleven (11%) patients were treated with an immunotherapy and antibody drug conjugate during the study period. Sixty-one (63%) were < 55 years of age, 29 (30%) were <45 years of age, and 18 (19%) were less than 40 years of age. The race and ethnicity distributions were 64 (66%) White, 19 (20%) Black or African American, 3 (3%) Asian, 2 (2%) American Indian or Alaska Native, 4 (4%) Hispanic/Latine, and 9 (9%) Other/Unknown. The highest reported symptoms over a 32-week period were fatigue (median 6.70% (IQR 21.65%)), muscle pain (median 4.12% (IQR 10.31%)), anxiety (median 3.09% (IQR 9.28%)), sadness (median 2.06% (IQR 6.09%)), and mouth/throat sores (median 2.06% (IQR 3.09%)). The highest reported symptoms were reported starting within the first week of reporting and were often reported in at least 50% of the timepoints for each patient that reported the symptom. There were no appreciable differences between reported symptoms from patients with metastatic versus early-stage disease.
Conclusions: The onset and duration of treatment emergent adverse events can be assessed for individual patients. This information will facilitate earlier interventions for symptom management of individual patients. However, a limitation is that although the questionnaires were sufficient to alert clinical care teams of potential toxicity in general, they were not specific to toxicities associated with immunotherapies or antibody drug conjugates. Therefore, an opportunity area for the field is to create more specific or tailored PROs for immune toxicities associated with immunotherapies, antibody drug conjugates, and cell-based therapies.

P1-06-16: Tumor Immune Micro-Environment (TIME) Effect of Cryoablation in Triple-Negative Breast Cancer - It was the best of TIMES…

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Background: Cryoablation of breast cancer (BC) is an out-patient procedure, less invasive than surgery, cost-efficient and may provide the added benefit of anti-tumor immunity. Cryoablation has been approved for small (≤1.5 cm) low-risk (hormone receptor positive) breast tumors and is currently being investigated for high-risk breast cancers (HER2+ and TNBC). Cryoablation uses ultrasound to guide a cryo-probe into the tumor and, through a series of freeze thaw cycles, kills the tumor and induces necrosis. The tumor remains in the patient and renders it "hot" promoting immune cell infiltration while preserving tumor associated antigens (TAA) to generate an immunogenic response. Our pre-clinical reports show cryoablation results in lower rates of tumor recurrence and metastasis with increased TILs at distant tumors. Using a murine model for TNBC cryoablation, we evaluated the immune response following cryoablation compared to surgical resection to identify early mechanism of the abscopal effect and potential biomarkers for cryoablation efficacy.
Methods: We used a syngeneic TNBC mouse model with an intact immune system to better understand cryoablation and the abscopal effect utilizing a distant tumor for immune response read out. BALB/c mice were orthotopically transplanted with 1x106 cells of the highly metastatic TNBC 4T1-12b-luciferase expressing cell line into the fourth and ninth mammary fat pad on left and right sides. Tumor growth and metastasis were monitored by palpation, caliper measurements and the in vivo imaging system (IVIS) for luminescence during the course of the experiments. At 2 weeks post-transplantation, the left tumor was treated by either resection or cryoablation. IVIS imaging showed complete tumor cryoablation at 24-hrs. One-week later the mice were sacrificed, necropsied for metastasis and tissues evaluated by flow cytometry for the anti-tumor immune response.
Results: The cryoablated tumor had significant infiltration of immune cells (naïve CD4+ T cells and myeloid cells) for tissue damage control and clean-up which allows for de novo TAA presentation. In addition, there was a significant increase in migratory cDC1s (CD103+/XCR1+) in the tumor draining lymph node (TDLN) as well as the spleen (secondary lymphoid tissue). In examining the distant abscopal tumors, cryoablation of the primary tumor resulted in significantly smaller abscopal tumors and changes in TIME with increased activated CD8+ ICOS+ T cells compared to resection. Differentially expressed gene analysis of bulk tumors (cryoablation abscopal vs. resection abscopal) showed increased anti-tumor changes with upregulation of tumor suppressor genes (Stat6 and Nlrp12) and T and NK cell cytotoxicity (Prf1 – perforin) and downregulation of metastatic (Cx3cr1) and angiogenesis and immunosuppression (Cxcl2) genes. STRING analysis for gene pathway ontology showed several immune processes to be involved including immune system process, cell activation, cytokine production, and cell surface receptor signaling pathways. GSEA (gene set enrichment analysis) plot analysis identified significant changes for

hallmark genes in the Inflammatory response, IL2-Stat5 Signaling, and IL6-Jak-Stat3 signaling.
Conclusion: Our results suggest that cryoablation enhances the myeloid response playing an early role in generating the anti-tumor immune response and potentially influences the TIME at distant tumors. We found increased infiltration of migratory cDC1 (CD103+/XCR1+), potent antigen presenting cells (APC) critical for effective anti-tumor CD8+ T cell priming, in the cryoablated TDLN and spleen. This dendritic cell population also has the ability to "cross-dress"/pass antigens to other APCs, making cDC1s a potential target for immune modulation to increase anti-tumor immunity enhancing the abscopal effect.

P1-06-17: Inhibition of oncogenic PTK6 kinase enhances immune response against triple negative breast cancer via EMT reversal

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Background/Rationale: PTK6/Brk, a non-receptor tyrosine kinase, is highly expressed in approximately 40% of triple negative breast cancers (TNBC). Higher levels of PTK6 expression are associated with worse patient outcomes. PTK6 is a driver of oncogenic growth, survival, invasion, epithelial-mesenchymal transition (EMT), chemotherapy resistance and metastasis. Recent studies have reported an association between PTK6 expression and an immunosuppressive microenvironment in patient breast cancers. Therefore, in addition to tumor cell-intrinsic oncogenic functions, PTK6 may play a critical role in shaping the tumor immune microenvironment which could modulate sensitivity to chemo/immunotherapy of TNBC. Inhibition of PTK6 could therefore suppress TNBC tumor growth and metastasis via dual (cell autonomous and microenvironmental) mechanisms.
Methods. Genetic and pharmacological approaches were used to downregulate/inhibit PTK6 in immunocompetent and immunodeficient mouse models of TNBC (MMTV-myc, 4T-1). The effects of PTK6 shRNA expression, as well as treatment with a validated PTK6 kinase inhibitor (P21d), on TNBC tumor growth were monitored. For studies using immunocompetent models, immune populations (tumor-infiltrating and systemic) were analyzed by flow cytometry and immunofluorescence.
Results: MMTV-myc or 4T-1 tumor-bearing, immunocompetent mice were treated with P21d, a validated small molecule inhibitor of PTK6 kinase activity. P21d treatment significantly inhibited tumor growth and increased tumoral infiltration by activated, cytotoxic immune cells (CD8+ T cells, NK cells). These effects were phenocopied by PTK6 shRNA or SNAIL shRNA expression, both of which also promote EMT reversal. Interestingly, the effects of P21d and PTK6 shRNA treatment on tumor growth were discordant when these same tumors were grown in immunodeficient mice; PTK6 shRNA, but not P21d treatment, inhibited TNBC growth. These results indicate that P21d's inhibitory effect on tumor growth is reliant on immune modulation and that this immune regulation is PTK6 kinase-activity dependent. These results also suggest that there are kinase activity-independent mechanisms that are important for tumor growth control by PTK6. The importance of immune modulation in P21d-dependent triple negative tumor

inhibition was reinforced by the fact that co-treatment of tumors with CD8 T cell or NK cell-depleting antibodies nearly completely abrogated P21d's effects on tumor growth. We identified CXCL10 as a mediator of P21d treatment-induced tumoral immune cell infiltration and tumor growth inhibition.
Summary/Conclusion: Our studies highlight novel tumor immune microenvironmental functions of PTK6 oncogene in TNBC. PTK6 inhibition leads to recruitment and activation of cytotoxic T and NK cells that are critical for TNBC growth inhbition. Ongoing studies will determine whether these functions can be leveraged to enhance efficacy of immunotherapies currently used in the care of patients with TNBC.

P1-06-18: Variation of CCR7 Immune Cell Receptor Expression by Nodal Burden in Patients with Invasive Breast Cancer

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Background: CCR7 is a G-protein coupled immune cell receptor differentially expressed on breast cancer cells to mediate trafficking toward lymphatic vessels. Current understanding of CCR7 expression profile in relation to patient and tumor characteristics is lacking. We aimed to characterize CCR7 expression and correlate expression patterns with clinicopathologic factors and outcomes in patients with invasive breast cancer.
Methods: Surgical resection samples were obtained from patients diagnosed with invasive breast cancer from 2006-2016. Tissue microarray (TMA) was prepared with immunohistochemical staining of samples in duplicates. CCR7 staining was scored by two expert breast pathologists for staining pattern (membranous vs. cytoplasmic), percentage (0-100% in 10% increments), and intensity after normalizing for background staining (range 1-3+). Chi-squared and one-way ANOVA tests were used to compare CCR7 staining patterns between groups. Pearson correlation coefficient was used to measure relationships between staining parameters and Kaplan Meier analysis was used for survival estimates
Results: In total, 217 patients were included. The median age at diagnosis was 56.0 (IQR 48.0-64.0) with 62.7% (136) White, 17.1% (37) Hispanic, 14.3% (31) Black, and 4.1% (9) Asian, and 4 (1.8%) Other patients. Up to 61.5% (88/143) of the cohort had clinical AJCC stage II-III disease and majority had ductal (85.7%, 186), grade 2 or higher (87.1%, 189), HER2-negative disease (81.1%, 176). Receptor subtypes consisted of 56.2% (122) HR+/HER2-, 24.4% (53) triple negative, 15.7% (34) HR+/HER2+, and 2.8% (6) HR-/HER2+. LVI was present in 30.4% (66) of patients and 28.6% (62) of patients received neoadjuvant chemotherapy prior to surgical resection. Overall, majority of patients had diffuse (100%) membranous and cytoplasmic expression of CCR7 (90.3%, 196). Up to 6.9% (15) had mixed membranous and cytoplasmic expression (< 100%) while 1.4% (3) had only cytoplasmic expression and 1.4% (3) had no CCR7 expression altogether. Cytoplasmic CCR7 staining positively correlated with membranous CCR7 for both percentage and intensity ($p < 0.01$ for both, $r = 0.647$ and 0.503, respectively). Staining intensity tended to be higher for membranous (median 3+) compared to cytoplasmic CCR7 expression (median 2+). Nodal burden was a key contributor to both membranous and cytoplasmic CCR7 staining

intensity. Patients with higher clinical and pathologic nodal burden demonstrated significantly increased membranous CCR7 staining intensity (3+: 75% for cN3c vs. 44.1% for cN1, p = 0.021 and 80% for pN3 vs. 55.3% for pN0, p = 0.015). Similarly, higher pathologic nodal status, greater clinical and pathologic tumor size, and higher overall pathologic stage were significantly associated with increased cytoplasmic CCR7 staining intensity (all p < 0.05). There were otherwise no differences in CCR7 staining pattern and intensity by age, race, tumor grade, histology, presence of lymphovascular invasion, clinical nodal status, and receptor subtype. Overall survival and progression-free survival did not vary by cytoplasmic and membranous CCR7 staining intensity (OS: p = 0.241 and 0.369, PFS: p = 0.898 and 0.147, respectively).
Conclusion: In this cohort of patients with invasive breast cancer, CCR7 is highly prevalent and expression pattern varied significantly with extent of nodal disease. Given its association with nodal burden, CCR7 may serve as a promising therapeutic target for patients with invasive breast cancer, particularly among those with locally advanced disease.

P1-06-19: Circulating Cytokine Profiles Associated with Differential Stromal Tumor Infiltrating Lymphocyte (sTIL) Levels in ER-positive Early Breast Cancer

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Background: Luminal breast cancer (BC) is associated with a lower level of tumor immune response, but recent results from trials with neoadjuvant immunotherapy (IT) and translational studies suggest that some subgroups of luminal BC might be sensitive to IT. The significance of the relation between the immune peripheral and the immune tumor compartments is not yet well understood and might be relevant for the search of new predictive immune biomarkers. Blood levels of cytokines partially reflect immune and inflammatory activation and might be related to differential immune response levels in the tumor. The aim of this study was to determine if certain profiles of blood cytokines are related to stromal lymphocytic infiltration (sTIL) in early luminal BC treated with neoadjuvant chemotherapy (NCT).
Methods: We analyzed pre-treatment plasma levels of 29 cytokines in a prospective single-center cohort of women with estrogen receptor (ER)-positive BC treated with NCT (2012-2017). Cytokine levels were analyzed using bead-based multiplex assays and Luminex technology. Pre-treatment sTIL (percentage) were measured in the diagnostic core biopsy following validated standard methods. For statistical analysis, preprocessing of plasma cytokine values included log transformation, assignment of half the limit of detection to non-detectable values, treatment of outliers and imputation of missing values with an iterated random forest algorithm. The correlation between cytokine levels and sTIL was

analyzed with Spearman's Rho, and those cytokines with stronger correlations were included in a multilinear regression model. Partial least squared-discriminant analysis (PLS-DA) was additionally performed to identify the association of cytokine levels with high sTIL infiltration (dichotomous sTIL with a 10% cut-off); performance was assessed with AUC (area under the cuve) of the ROC (receiver-operating characteristic) curve. R version 4.2.3 was used for statistical analysis and data visualization.
Results: A total of 63 patients with ER-positive breast cancer were studied. Median age: 49 (range: 31-76); 39.7% grade III; 33.4% stage III; 39 patients ER+/HER2- (61.9%) and 24 ER+/HER2+ (38.1%). The mean sTIL infiltration was 15% (SD, 16.6); 32 patients were categorized as sTIL-low (≤ 10%) and 22 as sTIL-high (>10%). A higher correlation with sTIL was found for pre-NCT levels of IL-15, TNFα, MICA, MICB, CD27, HVEM, and PDL2. A multilinear regression model for sTIL as a continuous variable only included plasma PD-L2 (coefficient 19.72; 95%CI: 5.81, 33.62; p=0.006) and BTLA (coefficient: -7.66; 95%CI:-15.85, 0.53; p=0.06). In the optimized model obtained by PLS-DA for sTIL, two principal components (PCs) explained 52% of cytokine variation and had an AUC=0.82 for classification between sTIL-low and sTIL-high. The first PC was exclusively formed by pre-treatment PD-L2, further confirming its association with tumor lymphocytic infiltration.
Conclusions: In ER-positive early BC, baseline levels of circulating PD-L2 are associated with stromal TIL, exemplifying the interactions between the peripheral immune compartment and the immune response in the tumor microenvironment. Further research is needed to better understand the potential role of PD-L2, a PD-1 ligand, in modulating immune-related mechanisms of response to neoadjuvant treatment.

P1-06-21: The Ral GTPases regulate the TNBC secretome to drive growth and metastasis.

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Breast Cancer (BC) is the most common cancer and leading cause of cancer associated mortality in women worldwide, with TNBC patients have the highest mortality compared to other subtypes. Ras-Like Protooncogene A (RalA) and Ras-Like Protooncogene B (RalB) are small GTPases that are known to regulate growth and metastasis in several cancers. Our group and others have identified the Rals as molecular drivers of TNBC, however the roles of these GTPases in BC is poorly understood. The goal of this study was to investigate the contributions of RalA and RalB in TNBC.
We found that silencing RalA or RalB in the MDA-MB-468 (468) TNBC cells reduced primary tumor growth in NSG mice. To better recapitulate human disease, we utilized two genetically engineered mouse models of TNBC. First, we found in the MMTV-Cre; MMTV-PyMT spontaneous TNBC tumor model that loss of Rala significantly reduces primary tumor growth while loss of Ralb increases growth, with no changes in tumor initiation or mammary gland development. Then we tested loss of Rals in the Brca1; Trp53 mouse model, which form TNBC tumors phenotypically similar to human TNBC when given and

intraductal injection of adenoviral-Cre. Here we find that loss of Ralb significantly increases primary tumor growth with no changes in tumor initiation.
To understand how the Rals regulate tumorigenesis, we looked closer at the 468 tumors and cells. In the 468 model we found no differences in proliferation or apoptosis, leading us to test the tumor-microenvironment (TME) to explain the reduction in tumor growth. Using Masson's Trichrome staining, we found increased collagen deposition in the 468 tumors upon depletion of either Ral. Staining for blood vessels using CD31 revealed depletion of RalB, and not RalA, reduced stromal and intertumoral blood vessel density. We then tested for macrophage infiltration and find a reduction of either Ral increases overall macrophage infiltration and specifically M1-like macrophages. Depletion of RalA also reduced recruitment of M2-like macrophages compared to control or RalB depleted cells. To find what factors lead to the differences in the TME, we performed a human secreted protein array on conditioned media from the 468 shRal cells and confirmed significant results via ELISAs. We found the Rals promote secretion of the angiogenic factor ANGPT1 and interleukins CXCL1-3. Western blot on intracellular protein showed no changes in expression of ANGPT1, revealing that changes in secreted protein are not due to altered expression from sh depletion.
These results show that the Rals influence protein secretion that alters tumor angiogenesis and collagen dynamics to promote tumor microenvironment changes that aid TNBC growth and metastasis.

P1-06-22: Challenging the paradigm for NK cell activation

Isabella Terrazas, Isaac S. Chan

Breast cancer (BC) is the most common cancer in women and metastatic disease accounts for most BC-related deaths. Immunotherapy, which is currently focused on activating T cells, has revolutionized cancer care but results have been mixed for patients with metastatic BC. Natural killer (NK) cells are critical members of the innate immune system with potent anti-cancer and anti-metastatic properties and provide a potential new immunotherapy for BC patients. However, these therapies are quite nascent and improving them requires a mechanistic understanding of how NK cell cytotoxicity is regulated. NK cell cytotoxic response is typically described as being regulated by a variety of activating and inhibitory receptors whose activity depends on specific ligands on cancer cells; yet we still do not fully understand what causes NK cells to lose their anti-cancer properties in BC. Many studies characterizing NK activating or inactivating receptors in the context of cancer describe the activity of each receptor independently. However, we and others have found that these activating and inactivating receptors are not exclusively expressed. In a recent study we found intratumoral NKs have high gene and protein expression of a prominent inhibitory receptor, NKG2A, in breast tumors. We found as many as 95% of BC patients' NK cells were NKG2A+. We further sought to characterize the NKG2 receptor family by measuring expression of activating receptor NKG2D because BC patients with higher NKG2D+ NK cells have improved outcomes. Surprisingly, we found 100% of NKG2A+ NKs from healthy donors were also NKG2D+. The temporal and spatial regulation of the co-

expression of these receptors has not been defined in the context of BC.
NK cells were characterized through flow cytometry from whole blood samples from patients with BC. Using a dataset we previously published, which contains single-cell transcriptomic profiling of a very large cohort of breast tumors, we validated which BC cell lines are most resistant to NK cell killing. Healthy human NKs were cocultured with these lines and BC death and NK cell receptors were measured via flow cytometry. To test the function of NK cell receptors, during coculture, an antibody blockade for both NKG2A and NKG2D or their known ligands were added. This included MICA/B and ULBP1-6 for NKG2D and HLA-E for NKG2A. Post coculture, IFNg from the media were measured using an ELISA. Using a coculture system with NKs and cancer cells, we characterized the phenotypic and functional significance of NKG2D and NKG2A, an activating and inhibitory receptor, respectively. Phenotypically, in BC patients we have found that the receptor expression varies based on disease status. For example, NKs from a patient with ductal carcinoma were 48% double positive while NKs from a patient with invasive lobular carcinoma is 98% double positive. Next, we defined the 24-hour sensitivity or resistance of BC cell lines to NK cell cytotoxicity using primary NK cells isolated from healthy human donors. Compared to an established NK-sensitive erythroblast cell line, K562s, BT474 in coculture with NK cells had ~25% death, whereas K562 cells in coculture had 80% death, confirming that BT474s are more resistant to NK cell killing. We then tested functional blockade of both NKG2D and NKG2A on NK cells in coculture with BT474s and found IFNg secreted in the media increased by 2.5-fold compared to blocking receptors individually. When testing double positive NK function in coculture with NK cells from healthy donors, NKG2A+NKG2D+ cells had an 8.9-fold IFNg increase when cocultured with BT474, compared to NKG2D+ NK cells. Adding HLA-E, the natural ligand for NKG2A did not significantly change IFNg production. These results suggest NK cell function requires both NKG2D and NKG2A signaling. Identifying how these receptors function in tandem will clarify how NK cells respond specifically to BC. Ultimately, this could aid development of targeted BC NK cell immunotherapies.

P1-06-23: Extracellular matrix in Young Onset Breast Cancer – A dynamic niche as a therapeutic target

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Background: Breast cancer (BC) incidence in young women is on the rise as reported over the last decade and presents with aggressive features and high metastatic rates. Young Breast Cancer (YBC), as defined by ESMO would be <=40 years of age, has worse prognosis and is postulated to represent a distinct pathological entity compared to older breast cancer (OBC). In our prior analysis with YBC, we had analyzed TCGA breast cancer data for differential gene expression between the young and older breast cancers (>60y), and the most significant set of genes pointed to those coding for the Extra Cellular Matrix (ECM)

(n=98 for <=40 and n=493 for >60 FDR <0.05). The ECM is a highly dynamic 3D network of structural and bioactive macromolecules secreted in part by cancer associated stromal cells such as fibroblasts (CAFs), known to prime the pre-metastatic niche in the breast environment. We thus hypothesized that progression of YBC could be associated with age-related expression differences in ECM associated genes due to remodeling and reactivation of the paracrine pathways, highlighting its relevance in metastasis of BC.
The aim of this study was to analyze gene expression differences of ECM degrading and ECM processing genes which seem to be associated with YBC. We also aim to examine the role of ECM remodeling in YBC using patient-derived primary culture models of tumour-cells and cancer associated fibroblasts (CAFS) to understand the metastasis pattern.
Methods: This study was conducted at Sri Shankara Cancer Hospital and Research Center, Bangalore after obtaining Institutional Ethical Committee approval. In-silico analysis of TCGA-BRCA, RNAseqV2 was conducted with clinical data. A set of ECM associated 17 gene panel of collagens, laminins and matrix metalloproteinases was established using differentially expressed genes from the TCGA RNA seq findings. This gene set was tested on a 55 primary retrospective FFPE specimens of YBC and a control group of 35 OBC using q-RT-PCR. A patient-derived primary culture was established and differentially separated for CAFs.
Results: 55 YBC (≤40years) and 32 OBC (≥60 years) with complete clinical information were included in the study. Median age of YBC and OBC was 37 and 67 years respectively. Although discontinuous in series, 44% were hormone receptor positive, 34% were Triple negative and 22% were HER2 expressing in the YBC. In OBC they were 75%, 12.5% and 12.5% respectively. Median tumour size was 3cm in both groups. Interestingly we found expression levels of ECM degrading genes to be significantly higher in YBC ($p<.05$) A comprehensive score generated based on combined score of 4 genes showed a significant association with an event of distant metastasis at a median follow up of 24 months. We also found that this score was not significantly associated with molecular subtypes of breast cancer showing age as an independent factor ($p=0.21$) Patient derived primary culture from biopsy of YBC and an OBC is established, and CAFs isolated. Identification of CAF secretome and validation of select markers with Immunohistochemistry to support our findings is underway

Conclusion: Our work suggests ECM changes in YBC play a significant role in creating a supportive microenvironment for cancer cell aggressiveness. More investigations of YBC tumour cell and CAF secretome using primary culture models are underway. This could pave ways to understand role of ECM in progression to metastasis leading to possibilities for novel therapeutic interventions. A large validation study with collaborators at Mayo Clinic is underway.

P1-06-24: Single-cell RNA sequencing reveals the role of HSPA1B+ immune cells in breast cancer microenvironment remodeling during neoadjuvant chemotherapy

Zihan Zhai

Background: Advanced breast cancer is still a major problem of cancer treatment in the world, and the overall prognosis is poor. The application of neoadjuvant chemotherapy (NAC) provides a new strategy for the treatment of locally advanced breast cancer and chemotherapy sensitive tumors. However, some patients are still facing the problem of NAC resistance. Studying the mechanism of NAC immune regulation may solve the problem of drug resistance. In this study, we aimed to clarify the characteristics of TME in primary tumor specimens before and after NAC. By analyzing the potential mechanisms of NAC resistance, we tried to find potential targets to prevent breast cancer progression and overcome NAC resistance.
Methods: Thirteen patients initially diagnosed with primary invasive breast cancer were recruited for this study. The standard neoadjuvant chemotherapy strategy was performed for 6 cycles. Tumor tissues were collected using core needle biopsies before NAC and through surgical excision after NAC. Single-cell RNA sequencing was used to analyze the tumor microenvironment in PCR and Non-PCR samples. The scRNA-seq data were used to analyze ligand-receptor-mediated intercellular communication at the molecular level via the CellPhoneDB tool. Moreover, paired formalin-fixed paraffin-embedded (FFPE) blocks were obtained before and after NAC and subjected to immunohistochemical staining for further analysis.
Results: Using a scRNA-seq protocol with unique transcript counting, cells were classified into breast epithelial cells, smooth muscle cells, T/NK cells, mast cells, fibroblasts, plasma cells, endothelial cells, myeloid cells, and B cells. Given that NAC can promote the reshaping of specific immune cell subpopulations, B cells were reclustered and annotated as 6 subgroups. The results showed that HSPA1B+ immune cells increased in the Non-PCR group, consistent with the immunohistochemical staining results. Interestingly, the TNF pathway was identified as a common pathway in HSPA1B+ immune cells, thus promoting inflammatory responses.
Conclusions: HSPA1B is upregulated in breast cancer tissue and is associated with poor prognosis in breast cancer patients. HSPA1B+ immune cells act on endothelial cells and cancer-associated fibroblasts (CAFs) by secreting pro-inflammatory cytokines (TNF), promoting chronic inflammation in the tumor microenvironment, and affecting the efficacy of NAC. Targeting HSPA1B may be a new strategy to prevent breast cancer progression and overcome treatment resistance.

P1-06-25: B7-H3 (CD276): A Metabolic Switch for the Survival of Breast Cancer Brain Metastasis

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Introduction: Up to 30% of cancer patients will have brain metastasis (BrM) at the time of their death, particularly those with lung cancer, breast cancer (BC), and melanoma. The prognosis for BCBrM patients is poor, with limited treatment options, highlighting the need to understand how cancer cells develop BCBrM. High immune cell infiltration in BrM suggested immunotherapies as a treatment promise, but they have been largely unsuccessful in BCBrM. This failure could be due to other immune checkpoints, such as B7-H3 (CD276), and the deranged metabolism that metastasized BC cells adapt to survive in the brain. These cells rely on oxidative phosphorylation (OXPHOS) and need more energy than normal brain cells, which are growth restricted and rely on glucose as their main source of energy. We hypothesize that targeting CD276-mediated metabolic reprogramming in BCBrM will mitigate BCBrM and improve therapeutic outcomes.
Methods: We performed in-silico dataset analysis and RNA sequence profiling on BCBrM CD276 Scr and knockout (KO) cells. Analysis was done using in-vitro and in-vivo experiments to ascertain the significance of CD276 in causing BCBrM. Metabolic perturbations were studied using metabolomics and Seahorse assays to unravel the mechanism of CD276 driving BCBrM. Furthermore, to evaluate CD276 as a therapeutic target, we used a CD276 antibody-drug conjugate (ADC), m276-SL-PBD, with a pyrrolobenzodiazepine (PBD) payload, which was administered intraperitoneally against macro-metastases implanted intracranially in mice and studied for tumor progression and survival.
Results: In-silico data analysis revealed B7-H3 (CD276) as a potential candidate target in BCBrM. CD276 was overexpressed in cancer cells, tumor-associated stromal cells, and tumor vasculature in most solid cancers, but was almost undetectable in normal tissues. CD276 expression was consistently elevated in BrM tissues and in BC cell lines metastasizing to the brain. Consequently, we observed that CD276 KO BCBrM cells lost migration potential, as assessed by trans-well migration ($p<.05$) and failed to create BrM lesions in NSG mice intracardially injected with MDA-MB231Br CD276 KO cells (BrM variant of MDA-MB231; $p<.001$). The immune-centric role of CD276 has been previously recognized, but lately its role in regulating metabolism is emerging, so we investigated if CD276-mediated metabolic changes could be driving BrM. Transcriptomic analysis revealed that CD276 KO cells have decreased expression of asparaginase synthetase (ASNS), among the top three downregulated genes, including its transcription factor ATF4 as well. We postulated that their downregulation could be due to decreased glutamine (Gln) metabolism. Effectively, we found that CD276 could be regulating glycolytic and OXPHOS. Seahorse analysis of oxygen consumption rate (OCR) in CD276-KO BCBrM cells, showcased their shift from OXPHOS ($p<.0001$). CD276 KO also significantly decreased glucose uptake by cancer cells leading to energy crisis and colony formation assay showed decreased

proliferation of CD276 KO cells. The reduced glucose uptake by cancer cells will be available to the immune cells, that showed increased infiltration in fully immunocompetent C57BL/6 mice injected with syngeneic E0771Br CD276 KO cells as compared to E0771Br CD276 Scr. Therefore, targeting Gln metabolism and, subsequently, OXPHOS through CD276 in BrM cells will be novel and could attenuate BCBrM by creating a metabolic crisis that can be leveraged to enhance immunotherapy. Finally and expectantly, we observed that m276-SL-PBD ADC treatment decreased BCBrM and improve survival in preclinical mouse models. Conclusions: Data suggest CD276 is critically involved in BrM and make cancer cells switch towards OXPHOS metabolism for survival and growth in a glucose-restricted brain microenvironment. Our results identify m276-SL-PBD ADC has the potential to enter early phase clinical trials for BCBrM patients.

P1-06-26: The optimal cut-off point for Tumor-Infiltrating Lymphocytes (TILs) in Triple-Negative Breast Cancer (TNBC)

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Introduction: TILs are promising, inexpensive biomarker with prognostic and predictive potential in triple-negative breast cancer. There is no consensus on the appropriate cutoff point to define high and low TILs. Therefore, we aimed to evaluate the prognostic value of TILs in an independent TNBC cohort and to determine an appropriate cut-off point by which to stratify TILs scores into prognostically significant categories.
Methods: The study included information on 140 patients with I-III stage TNBC and estrogen receptors <10%. Tumor tissue samples at baseline biopsies were evaluated the histological type, HER2 expression, estrogen expression levels, Ki-67 and TILs. The pathological response was evaluated according to the ypTNM, Miller—Payne, and RCB classifications. According to the criteria of the International Working Group, the classification of TILs into low (< 10%), intermediate (≥ 10% to ≤ 40%), and high (> 40%) levels was performed. When analyzing TILs in the main subgroups, we used a binary division of TILs into high level (TILs > 40%) and low (TILs ≤ 40%). The differences between groups were assessed using the Chi-square test. The correlation was analyzed with Spearman correlation test. Event-free survival (EFS) was estimated by the Kaplan-Meier method and Cox regression analysis.
Results: The average level of TILs in biopsy specimens before NACT was 29.3±23.1%. Low levels of TILs (<10%) were defined in 21% of cases, intermediate levels (≥10% to ≤40%) in 55% of cases, and high levels (>40%) in 24% of cases. Using the two-tiered system, low TILs (≤40%) were defined in 76% and high TILs (>40%) in 24% of cases. The level of TILs was correlated with histological grade (R=0.187; p=0.027) and estrogen receptor expression level (R=0.211; p=0.012). There were no significant differences depending on the level of TILs and other pathological parameters. When assessing EFS depending on the level of TILs across three cohorts, the 3-year rates were 64%, 65%, and 95% for low, intermediate, and

high levels of TILs respectively. No significant differences in EFS were found between the low and intermediate levels of TILs (HR=0.386, 95% 0.10- 1.44, p=0.156). Using the two-tiered system, 3-year EFS in patients with high TILs levels was 95% versus 65% in the low TILs group (HR=0.119, 95% 0.02 - 0.88, p=0.037).
Conclusion: Stromal TILs are an important prognostic biomarker in TNBC. Using a cutoff of 40%, high TILs are significantly associated with longer EFS.

P1-06-27: Role of MARCKS in regulating the tumor microenvironment in inflammatory breast cancer

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Background: Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer, accounting for 1-3% of cases and more frequent in North of Africa (7-11%). A major challenge in treating IBC is the lack of specific therapeutic targets. Myristoylated Alanine-Rich C Kinase Substrate (MARCKS) was found overexpressed and associated to poor prognosis in IBC vs. non-IBC (nIBC). As a an activator of inflammation in different types of cancer, our study aimed to evaluate in vitro and in clinical samples the role of MARCKS in inflammation and tumor microenvironment in IBC samples compared to nIBC. Methods: Using siRNA; we knocked-down MARCKS expression in IBC (SUM149) and nIBC (MDA-MB-231) cell lines, then evaluated the inhibitory effect on migration, invasion and mechanism of action in both single and co-culture with M2 macrophages. Inflammation was induced by LPS in vitro, and the clinical relevance of protein expression of MARCKS, CD163, CD68, LPS and NFkB was assessed in a large series of IBC vs. nIBC patients using immunohistochemistry (IHC).

Results: Using IHC, our results showed that in IBC samples only, the presence of M2 macrophages (CD163) was associated with obesity and that obese patients presenting M2 macrophage infiltration had a poorer 5-years overall survival. We also found an overexpression of NFκB in IBC patients correlated to MARCKS and M2 macrophages infiltration. We also showed in vitro that MARCKS-knockdown impaired cell migration and invasion in SUM149 (IBC) compared to MD-MB-231 (nIBC) cells. Furthermore, using western blot, only in IBC, MARCKS inhibition regulated several proteins involved in IBC inflammation and tumor microenvironment (TME) particularly NFκB and EGFR known to be directly implicated in IBC aggressiveness with better regulation of the different pathways in co-culture with macrophages M2.

Conclusions: Our study suggests an important role of MARCKS as a potential target modulating the TME and inflammation in IBC that needs further investigation using in vivo models.

P1-06-28: Assessment of the immune microenvironment of the breast cancer PAM50 Subtypes – a deconvolution approach

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Background: Breast cancer is a heterogeneous disease with many drivers involved I disease progression. Diagnosis and treatment decisions are guided by clinical, pathological, and molecular markers. The PAM50 subtypes which include Luminal A, Luminal B, Normal-like, Basal-like and HER2-enriched, are associated with different tumour phenotypes and overall survival.
In breast cancer, immune infiltration influences treatment response and disease outcome. In estrogen receptor negative and basal-like breast cancers a higher degree of immune cell infiltration has been associated with improved survival and enhanced responses to treatment. The recruitment of different immune cell types has different effect on the tumor growth and sensitivity to treatment Understanding the function and phenotypes of immune cell types in breast tumors may allow to better map which immune cell types may explain subtype specific tumor pathogenesis.
Aim and method:
Using two deconvolution algorithms, CIBERSORT and xCell, we aim at investigating whether the PAM50 subtypes have different immune microenvironment landscapes. We used 15 independent publicly available breast cancer cohorts with transcriptomics available for which, we inferred tumor immune cell infiltration. To experimentally assess the association between breast cancer subtypes and immune microenvironment, stained was performed on 80 tumors spanning the PAM50 subtypes for marker genes defining 6 minor immune cell types.
Results: Unsupervised clustering of the CIBERSORT scores inferring for the quantity of 22 immune cell types in the tumor and xCell scores predicting for the presence of 64 cell types showed that the immune microenvironment was strongly correlated with the molecular PAM50 subtypes. We also found differences in the immune microenvironment of Luminal A and B subtypes, which are considered close in lineage, with the Luminal A subtype showing higher infiltration of mast cells while and Luminal B‘s higher infiltration of regulatory T cells. The HER2-enriched exhibits a microenvironment with high amount of plasma cells and neutrophils. Finally, Basal-like tumors have in general higher immune infiltration characterized by of natural killer cells, cytotoxic T cells and dendritic cells.
Conclusion: Our findings show that the PAM50 subtypes may have overall differences in their immune microenvironment landscape which could be taken in consideration when explaining response to therapy or prognosis.

P1-06-29: Targeting NRP1 reduces triple-negative breast cancer lung metastasis and improves immunotherapy efficacy

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Metastasis is the leading cause of death in breast cancer, with approximately 90% of breast cancer-related deaths attributed to complications arising from metastasis. Lung metastasis is with a significantly poor prognosis and a 5-year overall survival (OS) rate of only 16.8% in breast cancer. Among all subtypes, triple-negative breast cancer (TNBC) shows the highest propensity for lung metastasis, accounting for 36.9% of all metastatic TNBC cases and contributing substantially to TNBC mortality. A key challenge in clinical practice is the inability to accurately predict lung metastasis at an early stage, leading to delayed treatment. In this study, single-cell RNA sequencing (scRNA-seq) was conducted on samples from TNBC patients without distant metastasis (n=3) and those with lung metastasis (n=3). scRNA-seq guided both in vivo and in vitro experiments, identifying NRP1 as a critical gene promoting lung metastasis. Mechanistically, NRP1 binds to the CLH1 protein, facilitating cell secretion via vesicles. The oxygen-rich lung environment inhibits CLH1-mediated vesicle secretion, thereby stabilizing membrane-bound NRP1 expression and promoting breast cancer lung metastasis.Furthermore, lung-tropic subcutaneous TNBC immunosufficient models displayed a significant increase in immunosuppressive cells (such as Treg cells and M2 macrophages) and PD-1+CD8+ T cells within the lung microenvironment. Combination therapy with anti-PD-1 and anti-NRP1 was found to reduce lung metastases and improve immune function. To screen the target population for this combined therapy, we integrated scRNA-seq data with The Cancer Genome Atlas (TCGA) breast cancer prognosis data and constructed a 6-gene predictive model for TNBC patients. This model facilitates the identification of individuals with lung-tropic metastasis potential who are likely to benefit from combined anti-NRP1 and anti-PD-1 treatments. In conclusion, our study demonstrates that screening for populations likely to benefit from and targeting NRP1 for treatment could effectively mitigate breast cancer lung metastasis, enhance the breast cancer immune microenvironment, and improve the efficacy of immunotherapy.

P1-06-30: Use of decellularized tumor and adipose tissue to model triple negative breast cancer

Marcus Moody

Triple-negative breast cancer (TNBC) constitutes 10-15% of all breast cancers, is associated with poor patient prognosis, and has a higher propensity for metastasis compared to other breast cancer subtypes. It is characterized by the absence of estrogen and progesterone receptors and the expression of human epidermal growth factor receptor 2 (HER2). Due to the lack of actionable markers, there is a need to develop novel targeted therapies for TNBC. While traditional cell culture techniques offer insights, current preclinical models inadequately replicate the native tumor and tumor microenvironment (TME), leading to loss of preclinical validation in clinical trials. Thus, there is a demand for more sophisticated 3D models capable of mimicking mechanical and biochemical signals present in the TME. The studies performed here aim to determine the translational potential of decellularized tumor and breast adipose tissue for use as 3D in vitro tumor models.
To assess the effects of tumor-specific decellularized ECM (dECM) on tumor growth in vivo,

a TNBC cell line was seeded on dECM derived from a pre-neoadjuvant chemotherapy TNBC tumor and implanted in SCID/beige mice. This was compared to TNBC cells injected with VitroGel. Tumors were detected at 4 weeks in the VitroGel injected TNBC cells and at 8 weeks in the dECM seeded group. The dECM seeded TNBC cells demonstrated slower tumorigenesis than the VitroGel injected TNBC cells. Upon tumor and organ harvesting, TNBC cells were isolated from tumors in both groups and RNA-Sequencing was performed.

Results: demonstrated that TNBC cells had changes in gene expression associated with ECM regulation, epithelial-mesenchymal transition (EMT) and cellular attachment between the injected control and dECM tumors. Specifically, ECM associated collagens and integrins COL6, COL8, ITGA7 and ITGA9 were significantly upregulated in the dECM group compared to the injected control. Cell attachment and immune response genes, CD6, CD8, HLA-DMB and HLA-DOA, were also significantly upregulated in the dECM seeded TNBC cells.
Due to the suggested impact of tumor-derived dECM on TNBC transcriptome, dECM tumor scaffolds were next optimized breast dECM for in vitro use. For this, dECM scaffolds derived from breast adipose tissue were lyophilized and homogenized into a powder. The resulting dECM powder was then used to generate tumor spheroids with different dECM concentrations, which were evaluated for spheroid formation, shape and cell death. Whereas control spheroids self-assembled within 24 hours without the need for centrifugation, the dECM spheres needed additional manipulation for aggregation and spheroid formation was observed at 48 hours. Preliminary histology showed that cells in dECM spheroids with higher amounts (50ug) of dECM were heavily concentrated on the outer edge of the sphere and had more irregular circularity than lower (10ug) dECM concentrations and control spheres. However, all groups showed significant compactness over a 7 day period. These observations suggest that dECM powder affects sphere formation and growth, and further characterization of dECM spheroid formation and structure is in progress. These initial findings support the use of breast tumor dECM as appropriate models for medium throughput models of TNBC in vitro.

P1-07-01: The Semi-Dry Dot-Blot Kit Can Detect Lymph Node Metastases in Breast Cancer Regardless of Neoadjuvant Chemotherapeutic Effects

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Background: The accurate diagnosis of sentinel lymph node (SLN) metastasis is important in breast cancer. We developed a new detection kit for lymph node (LN) metastases using the semi-dry dot-blot (SDB) method, which visualizes the presence of cancer cells by washing sectioned LNs with cytokeratin (CK)-19. Compared with the permanent histological diagnosis of 924 LNs to distinguish macrometastases that are larger than 2.0 mm in diameter, the SDB kit demonstrated 94.4% sensitivity, 96.2% specificity, and 95.6% accuracy in identifying macrometastases in patients who did not receive neoadjuvant chemotherapy (NAC). Recently, adjuvant treatment strategies considering efficacy of NAC, known as the residual disease-guided approach, have been used in human epidermal

growth factor receptor 2 (HER2)-positive and triple-negative (TN) breast cancer. However, it is unclear whether NAC affects the diagnostic ability of SDB kits for LN metastases with cancer cell degeneration. This study aimed to determine whether the SDB kit could correctly diagnose LN metastases in patients receiving NAC.
Methods: Sixty patients (101 LNs) who received NAC and underwent surgery between May 2022 and March 2024 at Nagasaki University Hospital were enrolled. The target number of patients was assumed to be 60 (120 LNs). Eligible LNs were all excised SLNs in patients without clinical LN metastasis, and two dissected axillary LNs in patients with pre-chemotherapeutic clinical LN metastasis. Micrometastases and isolated tumor cells (ITCs) were diagnosed when the tumor cells were larger than 0.2 mm but not 2.0 mm, and not more than 0.2 mm in the greatest extent. Non-macrometastasis was defined as LN status, including micrometastases, ITCs, and negative status. Diagnoses based on the SDB kit were compared with the permanent pathological diagnoses. Primary endpoints were the sensitivity, specificity, and accuracy of the SDB kit for distinguishing macrometastases from non-macrometastases.
Results: Among the 60 patients, the subtypes were hormone receptor-positive/HER2-negative (luminal) in 15 (25.0%), HER2 in 29 (48.3%), and TN in 16 (26.7%). The pathological complete response rates were 20% (3/15) for luminal, 48.3% (14/29) for HER2, and 43.8% (7/16) for TN. Twelve (11.9%) and 89 (88.1%) LNs were assessed as macrometastases and non-macrometastases, respectively, using permanent section examination with hematoxylin and eosin staining. Non-macrometastases included three micrometastases (3.0%) and one case of ITCs (1.0%). The SDB kit correctly diagnosed 11 of the 12 LN macrometastases and all of the LN non-macrometastases. The sensitivity, specificity, and accuracy of the SDB kit were 91.7%, 100%, and 99.0%, respectively. One false-negative case was caused by sparse residual swollen tumor cells with a long diameter of 2.1 mm.
Conclusion: This study revealed that the SDB kit could detect LN metastases in breast cancer, regardless of NAC effects. Therefore, we plan to conduct a multicenter prospective study to expand the use of the SDB kits in the diagnosis of LN metastasis in patients receiving NAC.

P1-07-02: Alteration of HER2 status following neoadjuvant chemotherapy in breast cancer: a clinicopathological analysis focusing on HER2-low status

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Background: Human epidermal growth factor receptor 2 (HER2) status can undergo alteration following neoadjuvant chemotherapy (NAC) in breast cancer. This study aimed to investigate the alteration of HER2 status after NAC in breast cancer and its impact on clinical outcomes of patients, focusing on HER2-low status.
Methods: We retrospectively reviewed 1,063 breast cancer patients who received NAC

followed by surgery between 2013 and 2020. Using paired samples of 670 patients with residual disease, HER2 discordance rate between pre- and post-NAC samples, the relationships between HER2 discordance and clinicopathological characteristics, and clinical outcomes of the patients were analyzed.
Results: As a whole, HER2-low status before NAC was associated with a lower pathological complete response rate and higher Residual Cancer Burden (RCB) class, compared with HER2-zero and HER2-positive status. However, in subgroup analysis by hormone receptor (HR) status, no statistical differences were found in chemo-responsiveness between HER2-low and HER2-zero breast cancers. Following NAC, the overall HER2 discordance rate was 21.2% ($\kappa = 0.676$). The most common type of alteration was zero-to-low (10.8%) conversion, followed by low-to-positive (3.4%) conversion. HER2 discordance was significantly associated with lower HER2 levels and HR positivity before NAC, as well as lymphovascular invasion, higher ypT stage, lymph node metastasis, and higher RCB class in residual disease after NAC. In further analyses, HER2-zero-to-low conversion showed an association with HR positivity and low histologic grade. In multivariate logistic regression analyses, HR positivity and higher RCB class were identified as independent predictive factors for HER2 discordance. In survival analyses, HER2 discordance revealed a worse prognostic impact on disease-free survival of the patients, particularly within HR-positive subgroup, which remained statistically significant on multivariate Cox regression analysis. However, no survival differences were found between patients with HER2-zero-concordant and those with zero-to-low conversion.
Conclusion: Given the prognostic implications of HER2 discordance, which primarily involves zero-to-low conversion, and the therapeutic benefits of newly developed antibody-drug conjugates in HER2-low breast cancer, HER2 status should be re-evaluated in surgical resection specimens following NAC, especially in cases showing HR positivity and high RCB class.

P1-07-03: Improved Standardization and Accuracy of HER2 Score with AI support in Breast Cancer: Large Multicenter Study

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Objective: HER2 expression is a key prognostic and treatment-influencing factor in breast cancer and is assessed for all invasive breast carcinoma (BC). As with all immunohistochemistry (IHC) staining, visual interpretation of HER2 expression is subjective, which leads to intra- and inter-pathologist variability. This study aims to evaluate the clinical utility (concordance, accuracy, and user feedback) of artificial intelligence (AI)-aided HER2 scoring solution on whole slide digital images of HER2 IHCs of

breast samples.
Methods: The cohort included biopsies and excisions from 1,997 patients from 12 US, EU, and UK clinical laboratories, including academic medical centers and reference/private laboratories. HER2 slides of diverse BC subtypes from primary and metastatic tumors were stained with anti-HER2 antibody (4B5, VENTANA) at each laboratory and scanned with different scanners (Leica GT450DX, Philips UFS, Aperio AT2). This observational two-arm multi-reader study compared the performance of 26 pathologists ("readers") on HER2 scoring (each reviewed 50-200 slides) unassisted vs. aided by AI HER2 solution (Ibex Breast HER2®), which detects the invasive tumor area and on slide control, classifies tumor cells based on their staining pattern, and derives a slide-level HER2 score by applying 2018 ASCO/CAP guidelines. Both study arms were compared to ground truth (GT), established as majority score of three breast pathologists ("experts") who reviewed the slides manually.
Results: Experts' overall inter-observer agreement on all HER2 scores was 73.9% (95%CI: 72.6%,75.2%) and for 0/1+/2+/3+ was 80.8%/ 66.2%/ 63.7%/ 94.3%, respectively. Readers' overall inter-observer agreement was significantly higher when assisted by AI, 87.5% (85.9%,89.0%) vs. 74.3% (72.3%,76.3%) without AI, $p < 0.05$. Moreover, reader's accuracy for all HER2 scores (agreement with GT) was significantly higher with AI 80.9% (79.7%,82.2%) vs without AI, 76.6% (75.2%,77.9%) ($p < 0.05$).
For 0/1+ vs 2+/3+ cutoff, readers with AI showed significantly higher inter-observer agreement 93.1% (91.8%,94.2%) vs. without AI 86.8% (85.2%,88.3%), $p < 0.05$, and significantly higher accuracy, 91.9% (91.0%,92.8%) with AI vs 88.8% (87.7%,89.8%) ($p < 0.05$) without AI.
For the HER2-low cutoff of 0 vs. 1+/2+/3+, readers with AI showed significantly higher inter-observer agreement of 95.0% (93.9%,95.9%) vs. 88.8% (87.3%,90.2%), $p < 0.05$, with slightly higher accuracy (89.8% (88.8%,90.7%) with AI vs 88.5% (87.4%,89.4%) without AI).
The standalone automatic AI solution demonstrated high accuracy for HER2 scoring of 89.4% (88.0%,90.7%), and 91.2% (89.8%,92.3%) for the respective clinical cutoffs of 0 vs. 1+/2+/3+, 0/1+ vs. 2+/3+.
Feedback from reader pathologists' user survey indicates an increased confidence in their HER2 scoring accuracy and consistency. Additionally, 77% of the pathologists expressed a preference for HER2 scoring supported by AI over manual scoring.
Conclusions: This study reports a large multi-site validation of a fully automated AI solution for HER2 scoring in BC. Pathologists supported by AI showed significant improvements in HER2 scoring consistency, evidenced by inter-reader agreement, and accuracy overall and for other clinical cutoffs. The AI solution demonstrated high accuracy and generalizability to multiple different laboratories (pre-analytics and staining protocols) and scanners. AI solutions, such as the one investigated here, could be used as decision-support tools for pathologists in routine clinical practice, enhancing the reproducibility and consistency of HER2 scoring, thus enabling optimal treatment pathways and improved patient outcomes.

P1-07-04: Re-evaluation of a novel diagnostic kit which uses the semi-dry dot-blot method combined with an automatic reader to detect breast cancer metastases in sentinel lymph nodes: A multi-center prospective study

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Background: The semi-dry dot-blot (SDB) method, a diagnostic procedure for detecting lymph node (LN) metastases using an anti-cytokeratin (CK) antibody, is based on the theory that epithelial components, such as CK, are not found in normal LNs. Thus, metastases are diagnosed based on the presence of CK in the lavage fluid of sectioned LNs. We developed a prototype kit which uses the SDB method and a newly created anti-CK19 antibody with a borderline CK 19 absorbance of 60 milliabsorbance (mABS) to distinguish macrometastases. We then prospectively evaluated 924 sentinel LNs and 94 macrometastases using a mass-produced SDB kit which was equivalent to the prototype kit. In a prospective clinical study, the sensitivity and specificity of the kit for detecting macrometastasis was 68.1% and 99.9%, respectively, with a borderline CK19 absorbance of 60 mABS. This was not sufficient for clinical application. However, when the analysis was conducted using a borderline CK19 absorbance of 11.9 mABS, the sensitivity and specificity was 94.7% and 98.3%, respectively. Therefore, we established a new borderline for the mass-produced SDB kit and conducted a multicenter prospective study to evaluate its performance.
Methods: We obtained 527 sentinel LNs dissected between August 2023 and May 2024 at six institutions in Japan. Patients who received neoadjuvant chemotherapy or endocrine therapy were excluded. The LNs were sliced at 2-mm intervals and washed with phosphate-buffered saline. Cells suspended in the lavage fluid of sliced LNs were centrifuged and lysed. The extracted protein was applied to the SDB kit to diagnose LN metastasis using absorbance as evaluated by an automatic reader. The washed LNs were blindly examined using intraoperative and permanent histological examinations. Diagnoses based on the SDB kit were compared with those made by permanent histological examination of the LNs. The primary endpoints were sensitivity, specificity, and overall agreement of the SDB kit for distinguishing macrometastasis from non-macrometastasis.
Results: Forty-seven of the 527 LNs were assessed as macrometastases on histological examination, 14 as micrometastases, and 466 as negative. Using a borderline CK absorbance of 11.9 mABS for detecting macrometastasis, the sensitivity and specificity of the SDB kit and its overall agreement with permanent histological examination was 89.4%, 94.6%, and 94.1%, respectively. Furthermore, the kit with automatic reader yielded a diagnosis within approximately 20 minutes at a cost of less than $30 for the SDB kit and less than $3,000 for the automatic reader.
Conclusions: Using the kit to diagnose LN metastasis was fast, accurate, and cost-effective.

The kit was also useful for distinguishing macrometastases and will be sufficient for clinical application.

P1-07-05: Association of BMI variations with response to neoadjuvant chemoimmunotherapy for early-stage triple-negative breast cancer

Bethania Santos

Introduction: Increased body mass index (BMI) has been associated with improved response to immune checkpoint inhibitors (ICIs) in multiple cancer types. This phenomenon has been attributed in part to obesity, higher adipose tissue composition in the breast and/or metabolic syndrome leading to T-cell dysfunction. However, BMI may vary during neoadjuvant therapy due to dietary changes, toxicity of therapy or psychosocial factors including anxiety and stress. This study aims to investigate the impact of BMI variation on pathological complete response (pCR) for patients with early-stage triple negative breast cancer (TNBC) receiving neoadjuvant chemotherapy with immunotherapy.
Methods: Patients with early-stage TNBC diagnosed between July 1, 2021, and December 31, 2023, were identified through two institutional databases. All patients received the KEYNOTE-522 regimen. Based on their baseline BMI, patients were categorized as obese (BMI ≥30) or non-obese (BMI <30). The chi-square test of independence was used to assess whether a significant association exists between BMI variation categories, greater than 1 point vs. 1 point or less (7lb/3Kg) and pCR status (achieved vs. not achieved). Univariate and multivariate analyses were performed using logistic regression to identify factors associated with pCR.
Results: Among the 264 patients identified, the median age was 52.9 years, the average BMI was 28.3, and 119 (45.1%) were obese. In univariate analysis, a BMI of ≥30 at baseline was associated with an improved rate of pCR ($p < 0.1$). In multivariate analysis, patients with a BMI ≥30 had improved pCR rates [OR (95% CI): 1.85 (1.08, 3.20), $p < 0.05$]. An unbiased selection of 38 patients from our cohort was analyzed to determine if BMI variation between the beginning and end of neoadjuvant treatment impacts outcomes. Among patients with a BMI variation > 1 point, 78.95% (15 out of 19) achieved a pCR, while among patients with a BMI variation < 1, only 21.05% (4 out of 19) achieved a pCR. A chi-square test of independence indicated a statistically significant difference between the two groups ($\chi^2 = 12.9240$, $p = 0.002$).
Conclusion: These findings highlight the importance of considering BMI variation as a factor in treatment outcomes. Nutritional status, changes in diet and physical activity during treatment can influence BMI variation. Intrinsic differences in tumor biology might influence both BMI variation and response to treatment. More aggressive tumors might cause more weight loss, which could be linked to the high activity of the immune response. Further understanding these factors should help to interpret the results and improve treatment outcomes. Larger, prospective studies are needed to confirm these results.

P1-07-06: Adjunctive statistical standardization of quantitated adjuvant HER2 and very low statistically standardized HER2 in CCTG MA.27

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Background: We proposed adjunctive statistical standardization of quantitated ER and PgR to improve inter-laboratory comparability of biomarker results and therapeutic management of breast cancer. Adjunctive statistical standardization of quantitated HER2 is used here; we also examined the effects on outcome of ultra-low HER2 and very low statistically standardized HER2.
Methods: We utilized CCTG MA.27 (NCT00066573), an adjuvant phase III trial of exemestane versus anastrozole in postmenopausal women with ER+ and/or PgR+ tumors. IHC HER2 HSCORE and % positivity (%+) were centrally assessed by machine image quantitation, and statistically standardized to mean of 0, standard deviationn (SD) of 1 followig Box-Cox variance stabilization transformations of 1.) natural logarithm (ln with addition of 0.1 to 0 HSCOREs and 0 %+), 2. square root. Additionally, centrally assessed FISH HER2 and CEP17 values were used to define ASCO/CAP categorizatiion. Post hoc, the effects of ultra-low HER2 , IHC 0 with (0,10%] 1+ stain, were examined. The primary endpoint was distant disease-free survival (DDFS) at the longest trial follow-up of median 4.1 years. Survival was described with Kaplan-Meier plots and tested with the univariate Wilcoxon (Peto-Prentice) test statistic. We examined cut-points at standard deviations about mean of 0 (<-1; (-1,0]; (0,1]; >1). Cox multivariant regressions were adjusted for age, T and N stage, grade, lymphovascular invasion, treatment, baseline patient demographics, ER and PgR; 2-sided Wald tests had nominal significance if $p<0.05$.
Results: Of 7576 women accrued to MA.27, 2900 women had ER, 2726 had PgR, and 2680 had HER2 results; 2325 had all three biomarkers for multivariant investigations. Twenty-five women received received herceptin with only one experiencng a DDFS event. ASCO/CAP categorization significantly differentiated univariate DDFS (p=0.01). Image analysis identified 57% of IHC 0 to have ultra-low HER2. Five-year DDFS for IHC 0 without stain was 92% [95% CI (90,95); N=864] which was similar to that for ultra-low HER2 of 96% [95% CI (94,97); N=1143]. Statistical standardization did not significantly differentiate univariate DDFS (p=0.08-0.27). DDFS for ln standardized values <-1.0 (HSCORE, or %+ <0.1) was similar to that with standardized values >1.0 (HSCORE >19, or %+ >14): for HSCORE <1.0, 5-year DDFS was 92% [95% CI (85.98); N=88] vs for >1.0, 92% [95% CI (89,95); N=577]; for %+, 5-year DDFS was 91% [95% CI (85,98); N=102] vs >1.0, 92% [95% CI (89,95); N=613].
In multivariant assessments with ASCO/CAP guideline and statistically standardized data, both ER (p=0.65-0.94) and HER2 (p=0.20-0.97) were not significantly associated with the DDFS primary endpoint in models with PgR; while higher PgR had significantly better DDFS (p<.003) in models with ER and HER2.

Conclusions: ASCO/CAP HER2 guidelines significantly differentiated univariate DDFS although not values of IHC 0 and ultra-low HER2, with <10% weak stain. Statistical standardization did not differentiate univariate DDFS. Image quantitation identified very small numbers of 1+/2+/3+ intensity stained nuclei. DDFS was similar for any intensity of low ln(HER2) stain (<1 SD below the mean) compared to any intensity of higher HER2 stain (>1 SD above the mean), although we offer caution in assessment of ultra-low, or very low, HER2 stain due to the dynamic range of the HER2 assay. Neither ASCO/CAP nor standardized HER2 had multivariant significance in these hormone receptor rich patient tumors. The adjunctive statistical standardization of ER, PgR, and HER2 performed here is similar to that mandated for clinical practice by the World Health Organization for BMD.

P1-07-07: Secretory Carcinoma: Clinical, Pathological and Molecular Insights into a Rare Breast Cancer Subtype

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Introduction: Secretory carcinoma of the breast (SCB) is an exceptionally rare subtype of breast cancer , accounting for only 0.02% to 1.5% of all breast cancers. Initially described as juvenile breast carcinoma, most cases are now also described in adults. Diagnosis of SCB requires an experienced breast pathologist due to its rarity and histological overlap with other subtypes. Although SCB generally has a favorable prognosis, some cases result in fatal metastatic disease. This study aims to elucidate the clinical, pathological, and molecular characteristics of SCB. Methods: This study includes 9 patients diagnosed with SCB from 2015 to 2022 at the Institut Catala d'Oncologia (ICO)-Hospital Universitari de Bellvitge (HUB) and 5 additional Spanish centers. Each diagnosis was reviewed by two expert breast pathologists. Comprehensive analysis was conducted on clinical data, histopathological diagnoses, immunohistochemical profiles, NTRK positivity (assessed with EPR17341 clone) and translocation status by fluorescence in situ hybridization (FISH), and PAM50 molecular subtype using the nCounter platform. Results: A total of 15 SCB tumors from 9 patients were analyzed, with 4 patients providing paired primary and relapsed tumor samples. The median age at diagnosis was 64 years (range 45-78). Median tumor size was 1.7 cm (range 0.6-9.5 cm). Estrogen receptor (ER) positivity was detected in 44.4% of tumors, albeit with weak intensity; all tumors were HER2-negative. The mean Ki67 index was 7.15% (range 1-22%). Histologically, four patients had moderately differentiated (G2) tumors, and five had well-differentiated (G1) tumors. Positive regional lymph nodes were found in 23.23% of cases. All tumors exhibited NTRK positivity and rearrangements by FISH. During a median follow-up period of 172 months (range 48-480), 5 patients experienced recurrences, with one patient succumbing to pleural metastasis. PAM50 molecular subtype analysis revealed that 6 of 9 primary cases (66.6%) were normal-like, while 4 cases (44.4%) were basal-like. All recurrence samples (3/3, 100%) were basal-like. Review of normal-like cases ruled out

normal breast tissue involvement. Conclusion and Future Directions: This comprehensive analysis of SCB advances our understanding of this rare breast cancer subtype and suggests new hypotheses for targeted molecular approaches and disease-agnostic tools. The indolent nature and low proliferation rates of SCB likely contribute to its normal-like molecular classification. However, specific tailored molecular techniques are still warranted to accurately detect SCB cases with aggressive behavior. Additionally, a next-generation sequencing (NGS) mutational analysis (TS0500) will be conducted to identify specific genetic markers in such cases. Keywords: Secretory carcinoma of the breast, NTRK, PAM50 molecular subtype.

P1-07-08: LUMINAL BREAST CANCER HER 2 AMPLIFIED IS A DIFFERENT

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Background: Breast cancer with overexpression of Her 2 represents approximately 15-20% of alll breast cancers diagnosed in women worldwide. The group of patients with HR(+) and Her2(+) overexpressed (triple positive breast cancer) represent 65% of cases that belong to the HER2 tumor group. In Latin America as in the rest of the world the management of this luminal Her2(+) tumors based on anti-hormonal therapy and anti-Her2 therapy as neoadjuvant, adjuvant and metastatic disease. we aimed to identify differences between Luminal BC patients with Her2 IHC 2+ vs 3+.
Methods: We identified medical records of patients with luminal Her2(+) BC diagnosed at INSTITUTO NACIONAL DE ENFERMEDADES NEOPLASICAS (LIMA-PERU) and ONCOBOLIVIA (SANTA CRUZ - BOLIVIA) between2015-2022. we only included patients with confirmed diagnosis of breast cancer ER(+) PgR(+) and Her2overexpressed. The exclusion criteria were: Patients without confirmation of HER2 status by immunohistochemistry of FISH techniques; patients who have discotinued therapy whitout any reason or those who have not received anti-HER2 therapy. Statistical analysis comparisons between HER 2 IHC 2(+) vs 3(+) groups were made using chi-squared Fisher`s exact, or Wilcoxon rank-sum tests as appropiate univariate logistic regression assessed the association between HER 2 status and pathological complete response (pCR). Kaplan- Meier survival analysis was performed to evaluate overall survival (OS) by HER 2 IHC scorea whit survival curves compared using the long-rank test. All analyses used R; eith statistical significance set al $p<0.05$
Results: We found 58 patients who met the study criteria; 87.9% reported with HER2+ IHC(3+). Overall median age was 44.5 years (IQR 40.0-47.0). Most patient had T2 tumors (n=27,46.6%) and cN1 nodal involvement (n=43,74.1%). Tumors classified as G3 (n=29,59.2%) were the most frequent. HR status showed that 89.7% of patients were ER+ and PgR+ and Ki 67 proliferation index had a median of 40% (IQR 25-50). Most common chemotherapy treatment was AC - TH (n=25,42.1%). Regarding treatment response 25.5% of the HER2 + IHC 3+ group achieved pCR; However most patients in HER 2 IHC 2+ and IHC 3+ had a partial response (66.7% and 74.4% respectively). In our cohort we did not find

Her2 scores as a predictive variable for pCR(IHC 2+ vs IHC 3+, OR=0.69, 95% CI=0.12-5.39, p=0.7) and for OS (p=0.3)
CONCLUSION. In conclusions we found no significant differences in pCR or OS between LUMINAL HER 2+ / IHC2+ (amplified) vs IHC 3+ patients. Larger studies are needed to further explore the potential prediction of treatment response in these subgroups and the importance of the luminal component in the response to anti-Her2 treatments.

P1-07-09: Development and validation of Residual tumor Burden calculator specific for Inflammatory breast carcinoma (IBC) to predict outcome (R-IBC)

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Background: Inflammatory breast carcinoma (IBC), a rare and aggressive variant of breast carcinoma, is a distinct clinicopathological entity characterized by lymphovascular tumor emboli in skin of the breast. Residual cancer burden (RCB) trained and validated in non-IBC is not optimal for using in IBC because it does not take into consideration pathologic features of IBC such as skin involvement and lymphovascular invasion or important features such as proportion of lymph nodes involved and extranodal extension. We sought to develop and validate a residual tumor burden calculator specific for IBC (R-IBC) using pathological features after neoadjuvant chemotherapy in combination with clinical features to predict progression-free survival (PFS) and overall survival (OS) .
Methods: We used 415 IBC patients (training set, 332 [80%]; test set, 83 [20%]) to develop and validate R-IBC score. A univariate Cox proportional hazard regression analysis was used to evaluate pathological features- tumor size, residual tumor cellularity (RTC), proportion of lymph nodes (PLN) with metastatic tumor, presence of residual lymphovascular invasion (LVI) in skin/breast, skin involvement, extranodal extension (ENE), and largest size of lymph node metastasis, for predicting each survival outcome separately. The significant pathological features were included in a multivariate model to identify factors to be utilized for building the R-IBC score. For PFS, the raw R-IBC score was calculated as R-IBCraw = (0.8668 x RTC) + (1.0646 x PLN) + (0.5894 x ENE). The score was then scaled from 0 to 10 as follows: R-IBC = 10 x R-IBCraw/max(R-IBCraw). Clinical features- age, race, clinical stage, clinical N category, ER/PR/HER2 status, were evaluated using univariate followed by multivariate analysis to identify significant factors to be combined with R-IBC for predicting outcome. Performance of R-IBC score alone and a final model including R-IBC score combined with clinical features was assessed by implementing the area under the receiver operating characteristic curve (AUC-ROC). The process was repeated for OS.
Results: Clinical features for training and test sets were: mean ages 59/58 years, clinical stage III 77%/73%, clinical stage IV 22.9%/25.9%, clinical T4d 100%, ER+ and PR+ 54.2%/54.5%, HER2+ 26.5%/38.7%, and triple-negative status 19.3%/6.8%. Pathological

features in the training and test sets were: residual tumor size 4.07 cm/4.1 cm, RTC 10.9%/11.8%, PLN 0.3/0.2, ENE 35.7/41.5%, lymphovascular invasion 40.1%/45%, skin involvement 26.6%/28.9%, pathologic complete response (PCR) 27.7%/29.8%, and non-PCR 72.3%/70.2%. The univariate Cox regression analysis identified all pathological features evaluated in the training dataset to be significant for inclusion into R-IBC score. Multivariate analysis identified RTC, PLN, and ENE alone to be significant for calculating R-IBC score (0-10). The calculated R-IBC score showed strong correlation with increasing risk of death or disease progression in patients with higher scores. Clinical nodal (N) category and triple-negative status were identified as significant clinical features that could be combined with R-IBC score for developing the final model. The AUC from the full model for the test set was 0.780 and 0.783 for PFS and OS ; the AUC of R-IBC score alone was 0.717 and 0.710 for PFS and OS .
Conclusion: The developed and validated R-IBC is the first IBC prediction calculator. R-IBC uses RTC, PLN, and ENE as key pathological features after NC in predicting outcome of IBC. Clinical N category and triple negative status could be combined with R-IBC for added predictive ability. Large-scale validation of R-IBC is warranted to enable consideration of incorporating the IBC specific calculator for predicting outcome of IBC patients in clinical practice and research.

P1-07-10: Clinicopathologic Characterization of Hormone-Receptor Positive, HER2 Null, Ultralow, and Low Breast Carcinoma in the Metastatic Setting

Raza Hoda, Patrick J McIntire

Background: The landscape of HER2-directed therapy is evolving at a rapid rate. Patients with hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2) Low (IHC 1+ or 2+/ISH-) and HER2 Ultralow advanced (IHC 0 with membranous staining in <10%) demonstrate clinically meaningful progression-free survival improvement with HER2-directed antibody drug conjugates (e.g. trastuzumab deruxtecan). Studies are limited on the clinical and pathological features of patients with HR+ /HER2 Ultralow and Low metastatic breast carcinoma. Herein, we provide the prevalence of HER2 Low, HER2 Ultralow, and HER2 Null status across MBC and characterize eligible patients and their tumors of a one-year retrospective review from our institution.

Methods: We retrospectively assessed rates of HER2 Null, HER2 Ultralow, and HER2 Low in patients with MBC who underwent biopsy at Cleveland Clinic from 1/1/2023 to 12/31/2023. Clinical and pathologic variables were recorded for patients with HR+/HER2 Ultralow; limited clinical and pathological parameters for patients with HR+/HER2 Low, HR+/HER2 Null, HR+/HER2 amplified, HR-/HER2-amplified, and HR-/HER2- breast carcinoma [‘triple-negative’ breast carcinoma (TNBC)] were recorded with detailed review forthcoming. Results of ER, PR, and HER2 testing on primary and metastatic tumor samples were reviewed in the electronic medical record. Patients without ER, PR, and HER2 testing

performed on metastatic tumor sample were excluded. HR+ disease was considered immunoreactive for estrogen or progesterone receptor in ≥1% of invasive tumor-cell nuclei. Glass slides of all HER2 IHC 0 cases were jointly assessed by two expert breast pathologists and scored as "HER2 Ultralow" (incomplete and is faint/barely perceptible and membranous staining in <10%) or "HER2 Null" (absence of any membranous staining) by consensus.

Results: A total of 130 patients were included in the study. All patients were women, and median age at time of MBC diagnosis was 67 years (mean, 65 years, range: 47-86 years). Common sites of MBC sampled included bone (n=73; 56.2%), liver (n=24;18.5%), lung (n=10; 7.7%), and soft tissue (n=8; 6.2%). Seventy (53.9%) patients had HR+/HER2 Low MBC, 21 (16.2%) had HR+/HER2 Ultralow MBC, and 8 (6.1%) had HR+/HER2 Null MBC. Twenty (15.4%) patients had metastatic TNBC, 8 (6.1%) had HR+/HER2+ MBC, and 3 (2.3%) had HR-/HER2+ MBC. Amongst the 21 patients with HR+/HER2 Ultralow MBC, the median age at time of MBC diagnosis was 68 years (mean, 68 years; range, 46-86 years). A majority of these cases (n = 9 of 17 primary cases available; 53%) demonstrated invasive carcinoma of no special type histology. Oncotype DX recurrence score (RS) was obtained in 5 cases, with 4 cases showing RS of 11-25 and 1 case showing of RS >25. Sixteen (76%) of 21 patients with HR+/HER2 Ultralow MBC received cytotoxic chemotherapy, and all patients received endocrine therapy. The median number of lines of therapy for MBC was 2 (range, 1-5). Median clinical follow-up time since time of primary breast carcinoma diagnosis was 89 months (mean, 94 months; range, 3-317 months). Twelve (57%) of 21 patients with HR+/HER2 Ultralow MBC had partial response or stable disease on therapeutic regimen, while 3 (14%) showed progression of disease and 6 (29%) died of disease.

Conclusions: Our one-year single-institution retrospective study shows a majority of MBC are HR+/HER2 Low, followed by a smaller proportion of HR+/HER2 Ultralow, indicating a large population of patients who may show benefit with HER2-directed antibody drug conjugates. Further comparison between patient cohorts will be performed to identify potential differences in clinical characteristics and pathologic variables, including HR and HER2 status of primary tumor, primary tumor histology and grade, and Oncotype DX RS.

P1-07-11: Evaluating the Correlation Between FISH and NGS in Assessing ERBB2 Alterations in Breast Cancer

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Introduction: Fluorescence in situ hybridization (FISH) is the gold standard for evaluating ERBB2 amplification in breast cancer (BC) when HER2 status is equivocal by immunohistochemistry, per ASCO guidelines (15–20% of cases). Next-generation sequencing (NGS) can confirm the HER2 status of BC samples and, in turn, help reduce the

incidence of equivocal samples. Here, we examined the correlation between FISH and NGS in annotating HER2 status of BC samples and evaluated the clinical utility of NGS as a support tool for improving analysis outcomes.
Methods: The BostonGene internal breast cancer (total n=358, FISH annotation=124) and TCGA-BRCA (total n=972, FISH annotation n=285) cohorts were assessed together for ERBB2 expression via RNA sequencing and ERBB2 mutation and amplification via DNA sequencing. High ERBB2 gene expression was set at >8.5 log2(TPM+1)1. For NGS, Gain was defined as an increase of one gene copy relative to sample ploidy, while Amplification referred to an increase of two or more gene copies compared to sample ploidy. Samples were assessed for FISH positivity (+) per ASCO/CAP guidelines.
Results: There was substantial agreement between FISH and NGS analysis for both cohorts combined (Cohen's kappa=0.57). All FISH+ samples had significantly higher ERBB2 expression (n=71, median (med)=8.8) than all FISH- samples (n=338, med=6.6, U-statistics (stats), p<0.001). Among the FISH+ samples, 53% (n=37) had high ERBB2 expression. Samples with amplification as shown by NGS had significantly higher ERBB2 expression (n=35, med=9.6) than non-amplified samples (n=335, med=6.6, U-stats, p<0.001). High ERBB2 expression was observed in 31% (n=23 of 74) of samples with ERBB2 gain or amplification. FISH+ samples with ERBB2 gain had significantly higher ERBB2 expression (n=14, med=10.5) than FISH- samples (n=13, med=7.9, U-stats, p<0.001).
NGS also detected 23 unique ERBB2 mutations, among which 18 were classified as missense, 4 as frameshift, and 9 as gain-of-function. Interestingly, 20 non-amplified samples with ERBB2 mutation exhibited significantly higher ERBB2 expression (med=7.4) than non-amplified samples with wildtype ERBB2 (n=999, med=6.9, U-stats, p=0.004).
Conclusion: Our study demonstrated substantial agreement between FISH and NGS (Cohen's kappa=0.57) in evaluating HER2 status in BC, highlighting NGS as a viable support tool for assessing HER2 status, particularly in equivocal cases. First, NGS reliably detected significantly higher ERBB2 expression in FISH+ samples than in FISH- samples. Next, it detected ERBB2 mutations in non-amplified samples that were not covered by FISH. Since these mutations may be associated with increased ERBB2 expression, they are clinically relevant and warrant further study. Last but not least, NGS detected focal ERBB2 amplifications and gains at a higher resolution than FISH. While associated with varied FISH results, these events consistently concurred with higher ERBB2 expression, reflecting the heterogeneous nature of HER2 amplification in BC that FISH does not take into account. The ability of NGS to identify these nuanced genomic alterations yields valuable insights into the tumor biology and potential resistance mechanisms of BC that are crucial for designing personalized treatment strategies and improving patient outcomes.

P1-07-12: Comprehensive characterization of invasive mammary carcinoma with lobular features: Integrating morphology and E-cadherin immunochemistry pattern

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Introduction: Breast cancer treatment prioritizes molecular subtypes over histologic types. However, considering the unique biologic behavior of invasive lobular carcinoma (ILC), its diagnosis is crucial for patient management. In this study, we reviewed breast cancer, with a particular focus on investigating the E-cadherin staining patterns and lobular morphology of cases that were misclassified in the original reports.
Materials and Methods: A comprehensive review was conducted on 481 cases diagnosed with invasive breast carcinoma of no special type (IBC - NST) or ILC through biopsy, in which E-cadherin staining was also performed. Subsequently, these cases were categorized into six groups based on a combination of tumor morphology (ductal / lobular) and E-cadherin expression pattern (membranous / loss / aberrant).
Results: In 211 cases (43.8%), an E-cadherin pattern indicating ILC (loss & aberrant) was observed alongside lobular morphology, representing 5.52% of all breast cancer biopsies during the relevant period. 181 cases (37.6%) showed membranous pattern with ductal morphology, 4 (0.8%) were mixed IBC-NST and ILC, and 85 (17.7%) exhibited discordance between morphology and E-cadherin. Notably, of 58 cases reviewed as ILC due to aberrant pattern, only 15 (25.9%) had initial diagnosis of ILC. Among 58 cases showing membranous pattern with lobular morphology, only 2 were diagnosed as ILC in the original reports. Similarly, despite the presence of ductal morphology, 17 cases (63%) exhibiting either loss or aberrant pattern were initially diagnosed as ILC.
Conclusion: Despite the E-cadherin expression pattern being recognized as a 'desirable' diagnostic criterion for ILC in the WHO blue book, real-world practice tends to depend on E-cadherin results, even in the presence of evident lobular morphology. Especially, aberrant pattern was often interpreted as membranous pattern, leading to misdiagnoses of IBC-NST. Additionally, cases showing discordance between morphology and E-cadherin patterns were observed in 85 cases (17.7%), highlighting the need for molecular clarification of these discrepancies.

P1-07-13: Adipocyte size in relation to clinical factors, mammographic density and quantitative histologic metrics in ductal carcinoma in situ (DCIS).

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Background: Ductal carcinoma in situ (DCIS) is a non-obligate precursor to invasive breast cancer, but distinguishing patients with harmless from potentially hazardous ductal carcinoma in situ (DCIS) remains a challenge. Consequently, a cornerstone of DCIS research is finding prognostic biomarkers. One such recent biomarker is adipocyte hypertrophy, which has been shown to be predictive of iIBC in post-menopausal women with primary DCIS 1. Little is known about the correlation between adipocyte size and clinical factors, mammographic breast density and other tissue composition metrics conferring risk of breast cancer.
Methods: Using the digital pathology software program HALO®, we automated the

adipocyte detection pipeline by building a machine learning algorithm for tissue segmentation, whereafter individual adipocytes were characterized using the vacuole module version 3.2.2. The correlation between the automated adipocyte measurement and the pathologist's generated measurement was assessed for 249 patients. Thereafter, archival hematoxylin and eosin- stained breast biopsy and excision specimens were retrieved from 700 women diagnosed with primary DCIS between 2000 and 2020 treated at the Netherlands Cancer Institute. These slides were digitized whereafter the machine-learning algorithms were applied to retrieve adipocyte size, proportion of epithelial cells, stromal cells and fibroglandular tissue. Radiology reports were obtained to extract mammographic BI-RADS density. Age at diagnosis, weight, length and menopausal status were extracted from electronic patient records. Associations between adipocyte size and clinical factors, BI-RADS density and tissue metrics were investigated using multivariable linear regression models.
Results: The median age at primary DCIS diagnosis was 55 years (interquartile range (IQR): 49.0 -63.0) and most DCIS lesions were grade 3 (40.9%). The median BMI was 24.1 (IQR: 21.9 - 27.1). The majority of women were post-menopausal (47.8%) and had dense breasts, whereby 40.6% had heterogeneously dense breasts and 13.7% had extremely dense breasts 2. Furthermore, there was good concordance between the automated adipocyte measurements in comparison to the pathologist's measurement with an intraclass correlation coefficient of 0.96 (95% CI = 0.95-0.97). In December, we will present the associations between adipocyte size and the clinical factors, mammographic breast density and tissue composition metrics.
Conclusion: Adipocyte hypertrophy has been shown to be predictive of iIBC in post-menopausal women with primary DCIS. This biomarker can be confidently measured with machine learning algorithms. This study will elucidate the associations between adipocyte size and clinical factors, mammographic breast density and other tissue composition metrics.
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P1-07-14: MutL loss in ER+ breast cancer promotes pro-tumorigenic macrophage polarization that aids migration capacity

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The Mismatch Repair (MMR) pathway, a crucial tumor suppressor mechanism, is dysregulated across cancer types. We previously identified the loss of the MutL complex of the MMR pathway, as a key factor inducing resistance to endocrine therapy in Estrogen Receptor positive (ER+) breast cancer patients. Preliminary data previously generated in

the lab supports a role for MutL loss in promoting metastasis of ER+ breast cancer. A mammary-specific knockout mouse model for MLH1, a principal component of MutL, created in the Haricharan lab develops aggressive mammary tumors that metastasize to multiple organs. To unveil the mechanism by which MLH1 loss triggers metastasis, an unbiased RPPA and RNA-Seq was conducted which revealed cytokine secretion in the immune system as a distinctive signature of MLH1- ER+ breast cancer cells. The tumor secretome, particularly cytokines, can manipulate macrophage plasticity to skew polarization states towards either an anti- or pro- metastatic phenotype. Secreted cytokine levels altered by MLH1 loss were identified in multiple ER+ breast cancer cells lines from concentrated conditioned media. Additionally, in vivo experiments with xenograft MLH1- ER+ tumors in nude mice, revealed an elevated number of CD206 expressing tumor-promoting M2 macrophages in the tumor microenvironment. Furthermore, MLH1- ER+ breast cancer cells grown in the presence of macrophages exhibit an increase in their migratory capacity. Our preliminary data strongly indicate that loss of MLH1 alters the ER+ breast cancer secretome and alters macrophage maturation, suggesting a role for MLH1 in immunomodulation supporting metastatic progression. Investigation of this new role will uncover key tumor-intrinsic drivers of “cold” tumor immune microenvironment profiles and present unique opportunities for therapeutic interventions to convert them into “hot” profiles, thereby improving ER+ breast cancer patient outcomes.

P1-07-15: Tumor size reduction and overall survival improvement in HER2-overexpressing cancer patients vaccinated with HER-Vaxx, a B-cell peptide-based vaccine, plus standard-of-care chemotherapy

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Purpose: Overexpression of HER2 has been observed in several types of cancers, including in 10-30% of breast and gastric/gastroesophageal tumors. We have previously identified three peptides representing B cell epitopes on HER2 extracellular domains, comprising trastuzumab’s binding site. In a Phase I study including patients with metastatic breast cancer, vaccination with the single peptides conjugated to virosome particles induced humoral and cellular immune responses, as well as an excellent safety profile. For the clinical efficacy evaluation, a hybrid peptide based on the three single peptides was generated and conjugated to the carrier protein CRM197 (toxoid of diphtheria toxin). In conjunction with the Th1/Th2-deriving adjuvant Montanide, the formulated vaccine compound, HER-Vaxx, was evaluated in HER2-overexpressing gastric cancer patients from countries where trastuzumab is not the first line of treatment. In a dose escalation Phase Ib trial, HER-Vaxx was shown to be safe, and the higher doses were associated with increased antitumor effect and progression-free survival. Thus, the recently completed randomized open-label, multicenter phase 2 HERIZON trial (NCT02795988) was conducted to compare

vaccination with HER-Vaxx plus chemotherapy, and chemotherapy alone.
Methods and Patients: Patients (n=36) with HER2-overexpressing gastric/gastroesophageal junction (GEJ) cancer naïve to anti HER2 therapy, were vaccinated with HER-Vaxx plus chemotherapy (n=19) using the recommended Phase 2 dose (50µg), or treated with standard-of-care chemotherapy (n=17). The study evaluated overall survival (OS; primary endpoint), safety, progression-free survival (PFS) and clinical response (secondary endpoints), and vaccine-induced, HER2-specific antibody levels in serum and their correlation with tumor response rates (exploratory endpoints).
Results: A 40% OS benefit (hazard ratio [HR]: 0.60; median OS: 13.9 months; 80% (2-sided) confidence interval [CI]: 7.52-14.32) was observed in vaccinated patients compared to OS of 8.31 months (80% CI: 6.01-9.59) in chemotherapy-alone patients. No additional toxicity due to HER-Vaxx was observed. A 20% PFS difference was obtained for the vaccination arm (HR: 0.80; 2-sided 80% CI:0.467, 1.381). Highly significant HER2-specific IgG and IgG1 antibody responses, particularly after 3 or more doses of HER-Vaxx were induced (P<0.001 vs. controls), which significantly associated with tumor reduction (IgG, P=0.001; IgG1, P=0.016), inhibition of phosphorylation of the intracellular HER2-signalling pathways, and mediation of antibody-dependent cellular cytotoxicity. An association between increased vaccine-induced antibodies and decreased levels of immunosuppressive FOXP3+ Tregs was also shown.
Conclusions: In gastric/GEJ cancer patients, HER-Vaxx plus standard chemotherapy improves OS, the vaccine-induced immune responses are significantly associated with tumor size reduction, and a favorable safety profile compared to standard-of-care chemotherapy alone is exhibited. This study suggests a possible inclusion of HER-Vaxx as first line treatment modality in HER2-overexpressing cancers.

P1-07-16: Real-World Duration of Sacituzumab Govitecan-hziy Treatment in Patients with Metastatic Triple-Negative Breast Cancer

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Background: The objective of this study was to determine the duration of Sacituzumab Govitecan-hziy (SG) treatment in patients with Metastatic Triple-Negative Breast Cancer (mTNBC) stratified by granulocyte colony-stimulating factor (GCSF) treatment.
Methods: This retrospective, observational study included US adult patients with mTNBC who received SG treatment (any line) between January 1, 2020 and July 1, 2023 in the community oncology setting. Patients were followed until January 4, 2024. The study utilized real-world structured electronic health data from the Integra Connect PrecisionQ de-identified database. Primary prophylactic GCSF usage was defined as GCSF being received on or after the initial date of SG administration but before neutropenia diagnosis. Secondary prophylactic/treatment GCSF usage was defined as GCSF received on or after the initial date of SG administration and on or after the date of neutropenia diagnosis.

Neutropenia was defined as neutrophils <1500 cells/mL. Duration of treatment was calculated as absolute duration utilizing start and stop dates documented in the data.
Results: A total of 337 patients were included in this study. The median (IQR) age at first treatment was 58.3 (49, 67) years. Of these patients, 219 (65%) were White, 57 (17%) were Black/African American, and 6 (2%) were Asian. The median (IQR) time from initial breast cancer diagnosis to the first SG administration was 3.3 (1, 6) years. ECOG status at SG initiation was unknown in 35 (10%) patients. Of the patients with ECOG status data at SG initiation, 93 (28%) were ECOG 0, 164 (49%) were ECOG 1, 38 (11%) were ECOG 2, 5 (1%) were ECOG 3, and 2 (1%) were ECOG 4.
Overall, 202 (60%) mTNBC patients treated with SG received a concomitant GCSF agent. Of patients who received GCSF, 98 (49%) received a long-acting GCSF agent, 102 (50%) received a short-acting GCSF agent, and 2 (1%) received both a long-acting and short-acting GCSF agent. Of the 202 patients who received GCSF, 92 (46%) received primary prophylactic GCSF and 110 (54%) received secondary prophylactic/treatment GCSF. Among the 92 providers who used a primary prophylactic GCSF, 34% treated with a short-acting GCSF and 65% treated with a long-acting GCSF; the remaining 1% treated with both short- and long-acting GCSF agents. In contrast, among the 110 providers who used a secondary prophylactic/treatment GCSF, 65% used a short-acting GCSF, 35% used a long-acting GCSF, and 1% used both short- and long-acting GCSF agents.
The median (IQR) duration of SG therapy was 137 (71, 239) days for those who received GCSF and 85 (44, 188) days for those who did not receive GCSF. The median (IQR) duration of SG treatment was similar among patients who received primary prophylactic GCSF (134 [65, 241] days) and secondary prophylactic/treatment GCSF (139 [71, 239] days). The median (IQR) duration of SG treatment was 155 (72, 246) days for patients who received long-acting GCSF agents and 134 (71, 224) days for those who received short-acting GCSF agents.
Conclusions: This descriptive study found that more patients with mTNBC on SG treatment received secondary prophylactic/treatment GCSF rather than primary prophylactic GCSF. Patients who received a GCSF appeared to have a longer median duration of SG treatment than those who did not. Further research, including both structured and curated data, is needed to better understand the reasons behind these differences.

P1-07-17: Mitotically-associated long noncoding RNA (MANCR): A novel long noncoding RNA that promotes genomic stability and cellular proliferation in Triple Negative Breast Cancer

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Background: While recent advances have improved outcomes for Triple Negative Breast Cancer (TNBC), it continues to have a poor prognosis. Long non-coding RNAs are among a recent class of epigenetic regulators that function in the nucleus to support the stability of cells and maintain the fidelity of chromatin interactions. Our laboratory discovered the long

noncoding RNA MANCR (LINC00704) as being upregulated in human breast cancer. Furthermore, our work demonstrated that MANCR is enriched in TCGA breast cancer patient samples that are not estrogen or progesterone receptor positive. We also demonstrated that the 10-year survival in this TCGA analysis is substantially worse in patients with high MANCR expression. More recently, we have found MANCR to be aberrantly expressed in TNBC cells, and these cells are highly dependent on MANCR to retain their tumorigenic characteristics.
Methods: Functional in vitro studies in MDA-MB-231 TNBC cells used short antisense nucleic acids (GapmerRs) to knockdown MANCR. For in vivo studies, TNBC cells were injected into the mammary fat pad of mice, allowing tumor formation, and subsequent treatment with 2 nmol/g of a negative control or MANCR targeting GapmeR. We also identified genome interaction sites at single nucleotide resolution by chromatin isolation by RNA purification sequencing (ChIRP-seq) to determine the mechanism of MANCR activity in TNBC cells.
Results: We now demonstrate that MANCR knockdown promotes DNA damage and decreases cell proliferation, migration, anchorage-independent colony formation, transwell invasion, and cellular survival. Additionally, in vivo targeting of MANCR drastically inhibited tumor growth over time and the end-point tumor mass. The MANCR GapmeR treatment also inhibited the ability of TNBC cells to circulate and disseminate to distant organs in vivo. After performing ChIRP-seq in the MDA-MB-231 cells, we identified 1206 genome-wide binding sites that exhibit MANCR interactions, of which 48% are intergenic and 52% are in genic regions. Furthermore, many MANCR ChIRP peaks were found to overlap with fragile sites in the genome, indicating MANCR provides stability to these sites.
Conclusions: These data suggest that targeting MANCR has therapeutic potential for patients with “MANCR-high” TNBC tumors by disrupting genome stability. Indeed, our in vivo studies demonstrate that “MANCR-high” TNBC tumors require MANCR to rapidly grow and promote disease progression. Significantly, many of the MANCR-chromatin interactions identified were found in intergenic regions and overlap with fragile sites within the genome. Collectively, these data strongly indicate that MANCR stabilizes the TNBC genome, and disrupting genome stability by targeting MANCR has therapeutic potential.

P1-07-18: Testing the Rac/Cdc42 inhibitor MBQ-167 in ex vivo cultures of Hispanic TNBC tissue

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Triple-negative breast cancer (TNBC) is a deadly form of breast cancer that is a health disparity in the US because the incidence of TNBC is tripled in African American women compared to Caucasian women. In Puerto Rico, where the admixture consists of Spanish, native Caribbean (Taino), and African American, more younger women present with TNBC at an advanced stage, when the tumor has already metastasized to distant sites, thus reducing their survival to ~10%. Since the absence of estrogen/progesterone (ER/PR)

receptors and human epidermal growth factor receptor (HER2) limits targeted therapy for TNBC, novel targeted therapies are required. To fill this critical need for targeted therapy for advanced cancer, our group developed MBQ-167 as a specific inhibitor of the homologous metastasis drivers Rac and Cdc42, which are risk factors for advanced breast cancer patient survival. MBQ-167 inhibits Rac/Cdc42 activation by GTP loading and the activation of their downstream effector P21-activated kinase (PAK) by phosphorylation at an IC50 of ~100nM. Consequently, MBQ-167 reduces TNBC tumor growth and abolishes metastasis without toxicity in animal models. In addition to inhibiting cancer cell survival and migration, MBQ-167 reduces immunosuppressive myeloid cells and upregulates cytotoxic T cells in the tumor microenvironment (TME). Therefore, we have initiated a Phase 1 clinical trial to determine the safety of MBQ-167 in advanced Puerto Rican breast cancer patients (NCT06075810). Immunohistochemistry (IHC) of breast cancer tissues from Puerto Rican breast cancer patients demonstrate that Rac and Cdc42 are expressed at all stages of the disease and that the levels of phospho (P)-PAK are high in advanced breast cancer. The objective of this study is to determine the efficacy of MBQ-167 in Puerto Rican TNBC patient tissues using an ex vivo drug testing platform. Treatment naïve TNBC patient tissues (>2cm lesion) were collected immediately following surgical resection from the Auxilio Mutuo Cancer Center Hospital, San Juan PR, under IRB 1487543-6. Tissue slices (2mm) were incubated in 0-1000nM MBQ-167 for 24-48h in ex vivo culture. Tissue slices were fixed for H&E staining and IHC, immunostained for KI-67 and Cyclin D for proliferation, and p-PAK for MBQ-167 efficacy. Results (N=5) show that hematoxylin-stained invasive ductal carcinoma cells were reduced upon MBQ-167 treatment. When tissues were stained with antibodies to p-PAK (1,2,3)T423 or Cyclin D, positive p-PAK and Cyclin D staining were prominent in the vehicle but not in the MBQ-167 treated tissue. Therefore, MBQ-167 is effective as a targeted therapeutic in advanced breast cancer tissues. The results of this study promise to directly address the dynamics of Rac/Cdc42 inhibitor therapy in Puerto Rican TNBC patients and forward Phase 2 trials for this novel therapeutic in advanced breast cancer patients. This study was supported by the US Army Breast Cancer Research Program (BCRP) HT9425-23-1-0166 (to SD), 5R25GM061151-21 (ATS), and NIH NCI 5R25CA240120-05 (MDT).

P1-07-19: Old Medicine, New Application: Therapeutic Potential of the Second-Generation Antihistamine Aerius as a N-WASP Inhibitor in Breast Cancer Metastasis

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Background: Aerius (desloratadine) is a second-generation antihistamine commonly prescribed to alleviate allergy symptoms. Recently, there has been growing interest in repurposing existing drugs for cancer therapy, leveraging their known safety profiles and mechanisms of action. Thus, Aerius has emerged as a candidate for targeting metastatic pathways in cancer. N-WASP, a key protein involved in actin polymerisation, plays a crucial role in cell motility and invasion. By facilitating cytoskeletal reorganisation, N-WASP

enables cancer cells to migrate and invade distant tissues, making it a significant target for therapeutic intervention. Understanding and disrupting the pathways that contribute to metastasis can lead to improved treatment strategies and better clinical outcomes for patients with advanced breast cancer.
Methods: Utilizing two breast cancer cell lines, MDA-MB-231 and MCF-7, along with HECV, an endothelial cell line, comprehensive in vitro biological assays included cytotoxicity tests, cell growth assay, Electric Cell-substrate Impedance Sensing (ECIS), wound scratch assay, immunofluorescence, qPCR and western blotting were conducted to discern Aerius's effects on cell proliferation, adhesion, motility, and its specific impact on N-WASP protein expression at both the mRNA and protein levels and its downstream events. Molecular docking was carried out by computer-assisted modelling to model the interaction between N-WASP and Aerius at the atomic level.

Results: Aerius demonstrated a significant inhibitory effect on cell proliferation in all three cell lines tested. When Aerius (at ? concentration for ? hours) was used in MDA-MB-231 cells, proliferation decreased by 51.76% ($P<0.0001$), while in MCF-7 cells, it decreased by 39.23% ($P<0.0001$), and in HECV cells, it reduced by 16.78% ($P=0.0004$). Furthermore, Aerius markedly impaired cell adhesion and migration in the ECIS assay. Wound healing assay revealed a 10.73% reduction in migration rate in MDA-MB-231 cells ($P<0.0001$), 35.89% reduction in MCF-7 cells ($P= 0.0014$), and 10.73% reduction in HECV cell lines ($P= 0.2367$) under the experimental conditions, indicating diminished motility.
Importantly, Aerius treatment led to a significant downregulation of N-WASP protein expression. Immunofluorescence assays and Western blot analysis confirmed these results. Additionally, qPCR results indicated the treatment of Aerius did not regulate the N-WASP mRNA expression, as it did not show any significant change compared with the untreated group, this suggests that the mechanism of action likely occurs at the post-transcriptional, translational or post-translational level. Finally, molecular docking revealed that Aerius had a binding profile with multiple amino acids of key domains of N-WASP protein.
These results highlight Aerius' multi-faceted inhibitory effects on key processes involved in breast cancer metastasis, including cell proliferation, adhesion, and migration, primarily through the downregulation of N-WASP protein expression.
Conclusion: Aerius shows significant promise in inhibiting N-WASP protein expression, thereby disrupting critical processes necessary for breast cancer metastasis. These results propose a new therapeutic application for Aerius, beyond its conventional use as an antihistamine, potentially offering a dual-purpose treatment strategy for managing metastatic breast cancer. Further clinical exploration is warranted to validate these findings and assess their therapeutic potential in a clinical setting.

P1-07-20: Mechanism of miR-221-3p/PARP1 regulating the progression of triple-negative breast cancer through the NF-κB pathway

Qianqian Lei, Ye Hong, Kegui Weng, Yanyan Long, Min Ying, Jing Chen, Mengyu Hu, Deqing Liu

Purpose: The underlying mechanisms driving the occurrence and progression of triple-negative breast cancer (TNBC) necessitate comprehensive elucidation. Our previous findings have demonstrated a significant association between low expression of miR-221-3p and unfavorable prognosis in TNBC patients, wherein it exerts inhibitory effects on TNBC cell proliferation and metastasis by negatively regulating PARP1. Prior studies have suggested that PARP1 facilitates TNBC progression through modulation of the NF-κB pathway. This study aims to elucidate the role of the miR-221-3p/PARP1/NF-κB axis in TNBC progression.
Materials and Methods: We established MDA-MB-231 cell lines with either overexpression or downregulation of PARP1 by plasmid transfection. Transcriptome sequencing and KEGG(Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis were employed to identify differentially expressed genes and associated signaling pathways in the various cell lines. The effect of PARP1 on the expression of NF-κB pathway-related molecules was validated through analysis using the TCGA (The Cancer Genome Atlas) database, Real-time PCR(Polymerase Chain Reaction), and Western blot techniques. Additionally, miR-221-3p modulation by miRNA mimic or inhibitor was performed to elucidate its role in regulating the NF-κB pathway via targeting PARP1. Cell proliferation and migration abilities in TNBC cells were assessed using CCK8 assay and Transwell chamber.
Results: KEGG pathway enrichment analysis revealed significant alterations in the expression of molecules related to the NF-κB signaling pathway between cell lines with differential PARP1 expression. Subsequent analysis of the TCGA database demonstrated that high expression of NF-κB signaling pathway-related molecules, particularly the NFKB1 gene, was predominantly enriched in cells overexpressing PARP1. In vitro experiments confirmed a substantial increase in both NFKB1 gene and protein levels in PARP1-overexpressing cells compared to the control group. In contrast, the knockdown of PARP1 exerted an opposite effect, thus establishing a positive correlation between PARP1 and NFKB1. Additionally, miR-221-3p exhibited a negative correlation with NFKB1. Furthermore, we observed that downregulation of PARP1 attenuated the regulatory effect of miR-221-3p on NFKB1 and improved outcomes for TNBC. In contrast, the upregulation of PARP1 exerted an opposite effect.
Conclusion: PARP1 exhibits a positive correlation with NFKB1, while miR-221-3p demonstrates a negative correlation with NFKB1. The regulatory role of miR-221-3p in TNBC proliferation and migration, as well as the modulation of NFKB1 expression, is mediated through its targeting of PARP1. The miR-221-3p/PARP1/NFKB1 axis plays a crucial role in regulating the progression of TNBC cells.
Keywords: triple-negative breast cancer, miR-221-3p, PARP1, NF-κB signaling pathway

P1-07-21: Efficacy of CDK4/6 Inhibitor Abemaciclib in Metastatic Triple-Negative Breast Cancer

Chuling Zhuang

Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype with the lowest 5-year survival rate among different subtypes. Although TNBC is less frequently diagnosed in U.S. women compared to the HR+/HER2- subtype, it remains as the second most common breast cancer subtype (13.6%) in the most recent five years. However, there is still an unmet need to develop effective treatment for TNBC due to the lack of actionable drug targets and high resistance rate. Cell cycle dysregulation is a common cancer hallmark. By targeting key regulators of cell cycle progression, multiple cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors have achieved clinical benefits with FDA approval to treat HR+/HER2- advanced and metastatic breast cancer. Interestingly, our datamining of two public breast cancer patient datasets (TCGA and GEO) consistently showed that the CDK4/6 signaling pathway was significantly more enriched in TNBC patients, compared to luminal and HER2-enriched breast cancer patients. After stratifying patients based on CDK4/6 gene activation signature scores into high (CDK4/6-high) and low (CDK4/6-low), we observed that high CDK4/6 activation signature is significantly correlated with a shorter time to develop overall metastasis, as well as, site-specific metastasis to bone, lung, or brain. It is noteworthy that CDK4/6-high TNBC patients have significantly shorter metastasis-free survival, with a median of 20 months, while that of CDK4/6-low TNBC patients is substantially extended with a median of 51 months. Immunohistochemistry staining of tissue microarray of 46 pairs of human primary and metastatic breast tumor samples validated our findings in datamining as CDK4/6 positivity is increased in lymph node metastases compared to matched primary tumors, and it is the highest in TNBC patients in both primary (6/9, 67%) and lymph node metastasis (7/9, 78%) tissue samples. Western blot analysis of a panel of breast cancer cell lines showed that the CDK4/6 signaling pathway is activated in the majority of TNBC cell lines. Next, we investigated the efficacy of Abemaciclib, an FDA-approved CDK4/6 inhibitor, and demonstrated that it is effective in inhibiting the proliferation of TNBC cell lines, with a half maximal inhibitory concentration (IC50) of 5.6-9.6 μM. Abemaciclib also effectively induced apoptosis of TNBC cells as indicated by increased PARP cleavage and Annexin V staining positivity. Additionally, TNBC cells treated with Abemaciclib exhibited significantly inhibited migration, invasion, and mammosphere formation abilities. Using an intracardiac inoculation mouse model, we found that systemic administration of Abemaciclib treatment significantly reduced overall TNBC metastatic burden and brain metastases. Collectively, these findings suggest a promising utility of CDK4/6-targeted therapy in treating metastatic TNBCs.

P1-07-22: Phase I Study of B7-H3 Specific Chimeric Antigen Receptor (CAR) T Cell Therapy in Patients with Triple-Negative Breast Cancer

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Background: Triple-negative breast Cancer (TNBC) accounts for 15-20% of all breast cancers, among which it has the worst prognosis and limited therapeutic options. B7-H3, a type I transmembrane protein, is expressed in 75% to 85% of breast cancer, with the

highest expression found in TNBC. Preclinical studies have demonstrated significant anti-tumor activity of B7-H3 CAR-T cells in cell lines and patient-derived xenograft (PDX) models. Preliminary data from an ongoing phase 1 clinical trial of B7-H3 CAR-T cells for patients with relapsed ovarian cancer (NCT04670068) at our institution has shown a tolerable safety profile. Additionally, the implementation of an inducible Caspase 9 safety switch aims to mitigate the risk of severe complications such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), enhancing the clinical application potential of B7-H3 targeted CAR-T cell therapy in TNBC. Based on these premises, we will conduct the first phase 1 study of B7-H3 CAR-T cells in patients with previously treated metastatic TNBC to establish the safety of this novel treatment modality and identify a recommended dose for further study in this population of patients with poor prognosis and unmet clinical need.
Methods: This is a Phase I, single-center, open-label study designed to evaluate the safety of escalating doses of CAR T cells targeting the B7-H3 antigen, incorporating an inducible caspase 9 safety switch (iC9-CAR.B7-H3 T cells) in patients with previously treated metastatic TNBC. The study utilizes a modified 3+3 dose-escalation design, starting with a dose of 1 × 106 transduced cells/kg. Patients with TNBC who meet eligibility criteria will undergo leukapheresis for the collection of cells to manufacture iC9-CAR.B7-H3 T cells. During the time period necessary to manufacture the iC9-CAR.B7-H3 T cells, patients will be allowed to receive standard-of-care bridging therapy at the discretion of their local physician. Eligible patients will then receive lymphodepletion chemotherapy with cyclophosphamide (300 mg/m2) and fludarabine (30 mg/m2) administered intravenously for three consecutive days, followed by the infusion of iC9-CAR.B7-H3 T cells. The primary objective is to evaluate the safety and tolerability of iC9-CAR.B7-H3 T cell administration. Safety assessments will include monitoring for adverse events, graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Secondary objectives include identifying the recommended Phase II dose (RP2D), determining the objective response rate (ORR), duration of response (DOR), progression-free survival (PFS), and overall survival (OS). Patients will be monitored for 15 years post-treatment completion in accordance with guidelines for gene transfer studies. This extended follow-up will evaluate the long-term safety and persistence of the genetically modified T cells. The trial is currently enrolling patients, NCT06347068.

P1-07-23: Matrix Metalloproteinase-7 regulates the process of pulmonary metastasis in triple-negative breast cancer cells

Ji Su Kim

Background: Organ-specific metastasis is a phenomenon where certain types of cancer preferentially metastasize to specific organs. This process is determined by a variety of factors such as the molecular traits of tumor cells, the microenvironment of target organs, and the interactions between tumor cells and the immune system. Understanding the molecular mechanisms regulating the organ-specific metastasis is crucial for developing

targeted therapies for metastatic breast cancers.
Methods: We established patient-derived xenograft (PDX) models of triple-negative breast cancer (TNBC), which exhibited the repetitive metastasis patterns either to lung or liver. Four TNBC PDX models, which repeatedly developed lung metastasis, were analyzed by performing RNA sequencing of their primary tumor and lung metastatic tumor tissues to characterize the gene expression features associated with lung metastasis. Control and MMP7 overexpression vectors were transfected into MDA-MB-231 and MDA-MB-468 cells, and transwell assay was performed to assess the role of MMP7 in cell migration and invasion in vitro. Furthermore, an in vivo orthotopic mouse model was established by injecting control and MMP7-overexpressed MDA-MB-231 cells into the fourth mammary fat pad. Experimental metastasis models were established via spleen injection to induce liver metastasis and tail vein injection to induce lung metastasis.
Results: Matrix metalloprotease-7 (MMP7) is one of the genes found to be consistently upregulated in lung metastatic tumors compared to primary tumors, based on RNA sequencing data. MMP7 overexpression in TNBC cell lines increased the migration and invasion in vitro. In addition, MMP7-overexpressed MDA-MB-231 cells showed significantly increased tumor growth in vivo in terms of tumor weight and volume. Moreover, there was no statistically significant difference in an experimental liver metastasis model while MMP7 significantly increased the metastasis in an experimental lung metastasis model.
Conclusion: In our TNBC PDX models, MMP7 was significantly upregulated in the lung metastasis tissues when compared to that of primary tumors. MMP7 upregulation was associated with increased cancer cell migration, invasion, and tumor growth, and MMP7 selectively promotes lung metastasis in TNBC. Further understanding of the biologic functions of MMP7 in TNBC can provide insights into the organ-specific metastasis to the lung.

P1-07-24: Induced Electric Fields Restrict Breast Cancer progression and metastasis via Immune Microenvironment Regulation

Manish Charan, Travis Jones, Nandini Acharya, Vish V. Subramaniam, Ramesh Ganju, Jonathan W Song

Breast cancer is the leading cause of cancer-related deaths in women across the globe. TNBC is a significant cause of mortality among breast cancer subtypes due to its aggressive nature and limited treatment options compared to other subtypes. Therefore, the development of safer and more effective therapies is urgently required. We found that alternating (100 kHz), and low intensity (<1 mV/cm) induced electric field (iEF) inhibits tumor growth and metastasis in an orthotopic TNBC model. Non-contact iEF treatment can be delivered safely and non-invasively in vivo via a hollow, rectangular solenoid coil. Furthermore, we discovered that iEF treatment significantly enhanced the anti-tumor immune responses. In particular, it enhances anti-tumor immune activity in both the primary tumor and distant lung metastases. Notably, iEF treatment decreases the exhaustion of CD8+ T cells and reduces the infiltration of immunosuppressive immune cells

in the tumor microenvironment, creating a less hospitable environment for cancer progression. Moreover, iEF treatment reduces the formation of lung metastases by promoting the enrichment of CD8+ T cells while suppressing immunosuppressive Gr1+ neutrophils in the lung microenvironment. We further observed that iEFs inhibit the ability of cancer cells to undergo epithelial-to-mesenchymal transition, thereby decreasing their metastatic potential. Furthermore, these findings suggest that iEFs could be a promising strategy to enhance overall anti-tumor immunity against aggressive and metastatic breast cancers. Taken together, the utilization of iEF technology could revolutionize current treatment paradigms for metastatic breast cancer.

P1-07-25: E3 ubiquitin-protein ligase RNF40 promotes triple-negative breast cancer progression and metastasis by promoting TRIM28 expression and stabilizing TWIST1 protein

Junjiang Fu, Chunli Wei, Jingliang Cheng, Jiewen Fu

Breast cancer (BC) is the common tumor in women globally and were approximately 2.3 million new cases of breast cancer and approximately 685,000 dead patients worldwide in 2020. In China, approximately 416,371 new female BC patients were reported in 2020, constituting 18.41% of new female cases in women worldwide. By 2040, the burden of BC is predicted to increase to over 3 million new patients and over 1 million dead patients each year. Triple-negative breast cancer (TNBC), a pathological subtype of BC defined by the lack of receptor expression for estrogen, progesterone, or epidermal growth factor receptor 2 accounts for 15~ 20% of all types in BC, is a metastatic and intractable cancer with limited treatment options. RNF40 is a RING finger E3 ubiquitin ligase containing multiple coiled-coil domains and a C-terminal RING finger motif engaging in protein-DNA and protein-protein interactions. RNF40 acts as a tumor suppressor or oncogene to play major epigenetic roles in cancer development, progression, and metastasis, highlighting the essential functions. However, the role and mechanism for RNF40 in TNBC is unclear. In this study, we found that RNF40 promotes TNBC progression and metastasis both in vitro and in vivo. Mechanistically, RNF40 epigenetically upregulated TRIM28 expression via histone modification including H3K4me3 in the TRIM28 promoter region, thereby further stabilized TWIST1 protein, highlighting its regulatory effects on EMT-inducing transcription factors (EMT-TFs) and TNBC progression and metastasis. Moreover, TRIM28 upregulated RNF40 expression in TNBC cells in a feedback loop manner. Clinically, RNF40 expression is positively associated with both TRIM28 and TWIST1 protein levels in most of tumor tissues compared with matched healthy tissues. Altogether, we identified novel RNF40/TRIM28/TWIST1 and EMT pathways as potentially valuable molecular targets for TNBC progression.

P1-07-26: Annexin A6 modulates the secretion of pro-inflammatory cytokines and exosomes via membrane fusion with SNAP 23 in triple negative breast cancer

Nobelle Sakwe, Portia Thomas, Josiah Ochieng, Amos M. Sakwe

The role of Annexin A6 (AnxA6) in the secretion of pro-inflammatory cytokines (PICs) and exosomes in triple negative breast cancer cells remains to be fully elucidated. However, understanding how AnxA6 influences or whether it is involved in the secretion of PICs and exosomes is still debatable. PICs are largely known to be secreted into the cancer microenvironment through exocytosis. Moreover, previous studies have reported the involvement of PICs in drug resistance and metastasis during exocytosis. We had previously shown that AnxA6 is secreted as a component of extracellular vesicles and that breast cancer cells secrete annexins via the exosomal pathway. Here, we examined the secretory function of AnxA6 in monocyte chemoattractant protein 1 (MCP-1) and interleukin 8 (IL-8) cytokines that contribute to triple negative breast cancer (TNBC) progression and we show that the secretion of cytokines and exosomes is AnxA6-dependent. AnxA6 upregulation in TNBC cells promotes increased extracellular vesicles (EVs) secretion. We equally show that cholesterol loading into EVs may also be AnxA6-dependent and that lapatinib-induced upregulation of AnxA6 leads to increased cytokine secretion in MDA-MB-468 lapatinib-resistant (LAP-R) cells. Furthermore, we demonstrate that lapatinib-induced upregulation of AnxA6 expression in EVs in MDA-468 LAP-R cells also leads to increased cholesterol accumulation. SNAP 23 interacts with AnxA6 for increased secretion through fusion of vesicles with plasma membrane. Together, these data not only suggest that PIC, exosome secretion, and cholesterol enrichment is AnxA6-dependent, but also provide evidence that induced AnxA6 upregulation triggered by chronic lapatinib treatment will lead to increased PIC secretion in the cells, which is bad for TNBC progression and poor patient outcome.

P1-07-27: Triple-negative breast cancer with bone marrow involvement and response after use of antibody-drug conjugate (ADC)

Sophia Freitas, Rafael Silva, Thales Silva, Bianca Carnevalli, Danillo Souza, Aumilto Junior, João Victor Oliveira

Introduction: Breast cancer is the most common malignancy in women. Among the histological profiles, the triple-negative subtype is the most aggressive and has the worst prognosis. It has high recurrence rates and frequently spreads to the brain and viscera. Bone marrow infiltration is a rare type of metastasis. In this context, antibody-drug conjugates (ADC) were developed in the attempt to offer a more stable and less toxic treatment, and have been demonstrating significant therapeutic improvement in these patients.
Case report: R. V. B., female, 60-year-old patient, previously healthy, ECOG 0, was diagnosed in 2018 with luminal breast carcinoma (HER-2 0, negative PDL-1, negative BRCA), and was treated with neoadjuvant AC-T regimen (doxorubicin and cyclophosphamide followed by

paclitaxel), with pathological partial response. In January 2024, the patient developed bone recurrence (S1, T12 e L5) and underwent a bone biopsy with diagnosis of a triple-negative breast carcinoma (TNBC), which led to the beginning of treatment with two weekly cycles of paclitaxel. In April 2024, the patient was admitted again to the hospital due to severe pancytopenia (hemoglobin 4,0/leukocytes 1900/platelets 48.000) and underwent blood transfusion, followed by the interruption of chemotherapy. Hematological evaluation recommended the execution of myelogram, which confirmed the bone marrow neoplastic involvement. Therefore, the antibody-drug conjugate (ADC) sacituzumab-govitecan (SG) was prescribed on April 12th, 2024. Surveillance CT scanning performed in June 2024 showed partial response in bone and bone marrow; laboratory results showed complete recovery of hematological series. The patient is currently using SG, with good clinical evolution.

Discussion: Bone marrow involvement in breast cancer is rare and is linked to a worse prognosis, generally associated with the occurrence of bone metastases. Conventional chemotherapy has been usually chosen as the main treatment for TNBC, but it is less effective and significantly reduces quality of life due to its adverse effects, making ADCs an excellent option. ADCs are composed of a monoclonal antibody, a cytotoxic agent and a linker between them. After the binding to a specific antigen on the surface of malignant cells and subsequent internalization, the conjugate releases its cytotoxic payload to kill them. The selective delivery of the drug to a specific target cell reduces the minimum effective dose, because it increases the amount of drug that reaches the tumor, while reducing the amount of drug that reaches non neoplastic tissues. More than 50 ADCs are being studied, such as SG and glembatumumab-vedotin, which have already been approved for breast cancer.

In the case reported, the patient was diagnosed with triple-negative breast carcinoma, with diffuse bone marrow involvement, and started the use of ADC (SG). This treatment was based on ASCENT trial, which demonstrated the increase of progression-free survival (PFS) (5,6 months, compared with PFS of 1,7 months with chemotherapy of physician choice) and of overall survival. The patient showed satisfactory response in radiological and laboratory tests, as well as improvement in the hematimetric levels and quality of life.

Other similar cases were reported by the literature, with positive responses to the use of SG and disitamab-vedotin (RC48) in patients with advanced breast cancer and bone marrow infiltration, resulting in the increase of hemoglobin levels without need of further blood transfusions.

Conclusion: Although the metastatic TNBC has a median overall survival of less than two years, the use of ADCs is a good therapeutic option for patients with bone marrow involvement and other visceral metastases. Additional randomized trials are needed to prove this benefit with a satisfactory sample.

P1-07-28: Real-World Study on Efficacy and Safety of SG Alone and Combined with Immunotherapy in Metastatic Triple Negative Breast Cancer Patients

Jingze Liu, Linxiaoxiao Ding, Tianzhu Long, Zouxiang Chen, Yangyang Cai, Simin Luo, Hongna Lai, Xiujuan Gui, Jie Chai, Ying Wang, Jianli Zhao

Introduction: Triple negative breast cancer (TNBC), characterized by the absence of estrogen receptor, progesterone receptor, and HER2 expression, is known for its aggressive behavior, high recurrence rate, and challenging prognosis. Sacituzumab Govitecan (SG), an antibody-drug conjugate targeting human trophoblastic cell surface antigen 2 (Trop-2), has shown promising efficacy in treating metastatic TNBC (mTNBC). Despite these advancements, there remains a significant lack of clinical research comparing SG alone versus SG combined with immunotherapy. This study seeks to address this gap by evaluating the efficacy and safety profile of SG plus immunotherapy compared to SG alone in mTNBC patients.
Methods: Retrospective data from metastatic triple negative breast cancer (mTNBC) patients treated with Sacituzumab Govitecan (SG)-based therapies were collected to assess both efficacy and safety outcomes. Clinicopathological data, premedication details, treatment efficacy metrics, and incidences of treatment-related adverse events (AEs) were rigorously analyzed across three distinct treatment cohorts: SG alone, SG combined with immunotherapy, and SG combined with immunotherapy in conjunction with anti-angiogenic therapy. Statistical methods were employed to evaluate and compare outcomes among these groups.
Results: Between January 8, 2023, and May 6, 2024, a cohort of 39 patients with metastatic triple-negative breast cancer (mTNBC) underwent treatment with SG-based therapies at the Breast Cancer Center of Sun Yat-sen Memorial Hospital. Among them, 28 patients received SG monotherapy, while 6 patients were treated with a combination of SG and PD1 monoclonal antibody (pembrolizumab/carrilizumab). Additionally, 5 patients received SG in combination with PD1 monoclonal antibody and anti-angiogenic therapy (Apatinib). Our findings indicate that compared to SG monotherapy, SG combined with immunotherapy showed a higher objective response rate (41% vs. 54%). Notably, the combination of SG with PD1 monoclonal antibody and anti-angiogenic therapy exhibited a trend towards even higher ORR (60%). Similarly, SG combined with Immunotherapy achieved a superior disease control rate compared to SG alone (62% vs. 81%). Moreover, the combination therapy group demonstrated improved progression-free survival rates compared to SG monotherapy, with a 3-month PFS rate of 73% versus 40% and a 6-month PFS rate of 34% versus 21%. Importantly, the incidence of treatment-related adverse events of grade 3 or higher did not increase with the addition of immunotherapy to SG compared to SG alone ($P < 0.05$). Interestingly, in contrast to adverse events reported in the ASCENT study, our data showed a reduced incidence of grade 3 or higher neutropenia (8%) with premedication using rhG-SF prior to SG treatment, with no occurrences of febrile neutropenia.
Conclusion: Our findings suggest that combining SG with immunotherapy enhances efficacy compared to SG alone in mTNBC patients, with minimal increase in treatment-related

adverse events. This combination therapeutic approach holds promise for future clinical applications, pending validation through well-designed clinical trials. Additionally, our data indicate that implementing preconditioning measures like rhG-SF may effectively reduce neutropenia incidence, offering a potential strategy for managing adverse events associated with SG in mTNBC patients.

P1-07-29: Single cell RNA sequencing of triple negative breast cancer patient-derived xenograft model identifies AK1 as a key regulator of breast cancer metastasis

Woohang Heo, Sieun Yang, Dakyung Lee, Rokhyun Kim, Jong-Il Kim, Hyeong-Gon Moon

Triple-negative breast cancer (TNBC) is a subtype of breast cancer that carries a higher risk of distant metastasis, particularly to the lung and liver. However, cancer cells within a tumor mass are heterogeneous characteristics, with different gene expressions playing various roles in tumor growth and metastasis. This heterogeneity makes it challenging to identify the specific cancer cells and genes that control the metastasis of TNBC. Therefore, we plan to investigate this through single-cell RNA sequencing of PDX models to study the specific cancer cells and genes involved in the metastasis of TNBC.
We established PDX models by obtaining tumor tissues from 26 triple-negative breast cancer patients and transplanting them into the mammary fat pads of immunodeficient mice. We classified the 26 TNBC PDX models based on their in vivo metastasis capacity and the clinical outcomes of the corresponding patients. Non-metastatic PDX models were characterized by the lack of distant metastasis development in either mouse experiments or clinical follow-up data (n=5). PDX models were defined as metastatic models when they developed distant metastasis in both mice and humans (n=4). We performed single-cell RNA sequencing on the nine PDX tumors and observed significant differences in gene expression of cancer cells between the metastatic and non-metastatic PDX models. Following the single-cell RNA sequencing data analysis, we identified the relevance between the high expressed genes in cells of the metastatic PDX models and TCGA clinical data. We identified four genes (AK1, HIST1H1C, MUCL1, SCGB2A2) that were significantly upregulated in metastatic PDX tumors and correlated with poor clinical outcomes in TCGA data. We conducted immunohistochemistry on proteins encoded by these genes to validate their effectiveness as metastasis markers at the protein level. We identified that among these four proteins encoded by the genes increased in metastatic PDX tumors, AK1 and HIST1H1C proteins were significantly expressed in metastatic PDX tumor tissues. Because there was no dramatic difference in the expression of the HIST1H1C protein between metastatic and non-metastatic PDX tumors, we focused on AK1 in our study. AK1, adenylate kinase 1, is a major isoform of the AK enzyme that catalyzes the nucleotide phosphoryl exchange reaction 2ADP ↔ ATP+AMP in the cytoplasm. AK is necessary for the functioning of organisms, including growth, differentiation, motility, and metabolism. In breast cancer, however, the role of AK1 in cancer progression and metastasis is unknown. Therefore, we studied the function of AK1 in TNBC using stable AK1 knockdown cell lines and AK1

overexpression cell lines. Surprisingly, we identified the downregulation of cell proliferation in both AK1 knockdown cells and overexpression cells, but trans-well migration and invasion capacity were upregulated in AK1 knockdown cells while AK1 overexpression cells had decreased migration and invasion capacities compared to control cells.
In brief, AK1 which was discovered by single cell RNA sequencing of TNBC PDX models is the potential regulator of TNBC metastasis regulating cancer cell migration, invasion and proliferation.

P1-07-30: Phase II adaptive TONIC2 trial to dissect immunomodulatory capacity of doxorubicin or cisplatin induction followed by anti-PD1 in mTNBC

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Introduction: Anti-PD1 in metastatic triple negative breast cancer (mTNBC) results in modest response rates but can lead to durable responses. Immunomodulatory strategies inducing a more inflamed tumor microenvironment (TME) could enhance response to anti-PD1. The TONIC1 trial explored immune induction strategies in relation to likelihood of response to PD1 blockade. Translational data of TONIC1 and the clinical data according to a pick-the-winner design identified low-dose doxorubicin and cisplatin as promising immune induction strategies [Voorwerk et al, Nat Med 2019]. Here, we present the randomized phase II TONIC2 study independently validating the benefit of using low-dose doxorubicin or cisplatin as induction therapy before the start of anti-PD1 (NCT04159818).

Methods: The non-comparative, phase II TONIC2 trial, using Simon's two-stage design, randomized patients with mTNBC to nivolumab (nivo) with either 2-week low-dose doxorubicin induction (15mg, weekly) or no induction. After completing recruitment of these cohorts, next cohorts randomized to nivo without induction or with 2-week cisplatin induction (40mg/m2, weekly). Primary endpoint was progression-free survival (PFS). Metastatic lesion biopsies were taken at baseline, after induction and on nivo for bulk RNA-Seq, single-cell RNA-Seq and tissue imaging (MIBI, 37 markers), to assess the induction effects on the TME.

Results: Among 97 randomized patients, 92 were evaluable for efficacy analyses (n=22 received doxorubicin followed by nivo, n=35 cisplatin followed by nivo, n=35 started directly with nivo without induction). The ORR was 20% in the control arm, 9% in the arm with doxorubicin induction and 9% in the arm with cisplatin induction. Median PFS (iRECIST) was 5.29 weeks (5.00 - 18.71) for patients in the doxorubicin arm, 5.86 weeks

(5.14 - 17.00) in the cisplatin arm and 8.71 weeks (5.57 – 12.57) in the control arm. Median OS was similar across arms.
The immunomodulatory effects of induction treatments on the TME were studied among all patients included in cisplatin, doxorubicin or control arms in TONIC1 and 2 (doxorubicin n=39, cisplatin n=48, no induction n=47). The control arm for postinduction comparisons consisted of patients who had a 2-week waiting period instead of induction treatment in TONIC1. The immunosuppressive Myc pathway decreased upon nivo compared to baseline in the doxorubicin induction arm (FDR<0.25). Immune checkpoints TIGIT and 4-1BB increased upon nivo after doxorubicin induction. Upon doxorubicin induction, deltas in PD-L1 CD8+ T cells, GLUT1+ CD8 T cells and HLADR+ CD4+ T cells were higher than in the control arm based on tissue imaging using MIBI. In addition, the delta of granulocyte to cancer cell ratio was lower in the doxorubicin arm.
Upon induction with cisplatin, we observed an increase in IFNα response (FDR<0.25). The differential gene expression profile of postinduction biopsies showed increased levels of immune checkpoint molecules, MHCI and MHCII, cytokines and T cell markers compared to baseline. Upon nivo after cisplatin induction, we observed an increase in IFNγ, inflammatory response pathways and IL6 signaling compared to baseline (FDR<0.25). Detailed translational analyses, including single-cell RNA-Seq, and outcomes in relation to PDL1 expression will be presented at the meeting.

Conclusion: Although exploratory results in the TONIC1 trial suggested a more favorable response rate upon nivo after induction with low-dose doxorubicin or cisplatin, these immune inductions did not result in significantly higher ORR/longer PFS in the independent and larger TONIC2 trial. In-depth translational analyses revealed modest but some favorable changes in the TME after induction with doxorubicin and cisplatin that could be important for future trial design.

P1-08-01: Aligning of cancer-associated collagens inhibits metastasis

Girdhari Rijal, Kimberly Hernanendez, Caitlyn Nguyen

Collagen with its networks is one of extracellular matrix (ECM) proteins that establishes the suitable microenvironment, necessary for the mechano-physiological function, to the cells (both normal and cancerous). In addition, certain collagen aligners and cross-linkers are necessary for the formation and the stabilization of extracellular matrix (ECM)-networks in both normal and abnormal tissues. Complexities of ECM proteins in tumor microenvironment (TME) challenge to reveal a cell-source of a specified protein aligner. For example, asporin (ASPN), a collagen aligner. It is interesting that TNBC cells express ASPN insignificantly. TNBC tissue shows positive for ASPN because of its expression by cancer associated fibroblasts (CAFs) in a tumor tissue. It is therefore important to understand the role of such a functional protein in a tumor.
We have noticed that the ECM networks in the normal tissue is stabilized with the regular collagen fibers with their standard networks through the collagen linkers, establishing the normal size pores throughout the ECM tissue scaffolds for the normal function. However,

the ECM established by TNBC cells is stiffer because of the bundling of collagen fibers, instead of crosslinking, supporting for the migration of TNBC cells.
Here, we studied the expression of ASPN from CAFs and MDA-MB-231 (MM231), and evaluated the role of ASPN in collagen alignment and crosslinking. Result showed that collagen becomes stiff with its deranged fibers with large-size pores in absence of ASPN similar to the outer TNBC core where cancer cells dominate the area, while aligned collagens cross-link and establish small-size pores in the network in the presence of ASPN similar to the inner TNBC core where CAF cell dominate the area. ASPN realigns collagen and helps establish the networks, inhibiting cancer metastasis.

P1-08-02: HIF-1-dependent expression of integrin β3 incorporated into extracellular vesicles promotes metastasis of breast cancer cells to the brain

Yongkang Yang

Brain metastasis is a major cause of breast cancer (BC) mortality, but the cellular and molecular mechanisms have not been fully elucidated. BC cells must breach the blood-brain barrier in order to colonize the brain. Here, we determined that integrin β3 (ITGB3) expression mediated by hypoxia-inducible factor 1 (HIF-1) plays a critical role in metastasis of BC cells to the brain. Hypoxia induces HIF-1-dependent expression of ITGB3, which is required for BC cell migration and invasion. Knockdown of HIF-1a or ITGB3 expression impairs the colonization of BC cells to brain after injection into the cardiac left ventricle of mice. Mechanistically, ITGB3 protein expressed by BC cells was incorporated into extracellular vesicles (EVs). The ITGB3+ EVs associated with brain endothelial cells, augmented vascular endothelial growth factor (VEGF)-dependent signaling via VEGF receptor 2 in brain-derived endothelial cells, and increased endothelial permeability to facilitate transendothelial migration of BC cells and metastatic colonization of the brain.

P1-08-03: Clinical Distribution and Impact of Biomarker Triads Consisting of either Quantified HER2 or EGF Receptor with ER and PR Proteins and Expression of Their Cognate Genes

Michael Daniels

Background: Development of novel therapeutics, e.g., antibody-drug conjugates (ADCs) and escalation/de-escalation therapy, prompted this study to ascertain clinical use of triads of ER/ESR1, PR/PGR, HER2/ERBB2, and EGFR/ERBB1 for predicting cancer behavior by quantifying proteins and their cognate gene expression. IHC of biomarkers, despite use in cancer management, faces multiparametric issues that ASCO/CAP Guidelines for ER/PR and HER2 seek to standardize. Our study avoids these issues by quantifying protein biomarkers and their cognate genes to associate with disease-free survival (DFS) and overall survival (OS). Laser capture microdissection (LCM) was employed to explore the molecular

landscape of each breast carcinoma. Methods: Clinical studies were conducted in silico on retrospective, de-identified data from IRB-approved databases. ER/PR proteins were quantified by FDA-approved tests (13,966 results from radio-ligand binding (LBA/NEN-DuPont) and 4,408 from enzyme immunoassays (EIA-Abbott), each expressed as fmol/mg cytosol protein. Of these, 1,194 biopsies had clinical outcomes. HER2, expressed as HNU/mg membrane protein, was quantified (504 by ELISA (NEN/Oncogene Sci.) and 901 by EIA (TRITON), with 189 having clinical outcomes. EGFR was quantified in 894 biopsies by radio-ligand competition assay, with 137 having clinical outcomes. Distributions of biomarker triads and relationships to DFS and OS were assessed for 123 patients. ER/PR status was compared across assay platforms, combined with HER2 or EGFR proteins. Analyses conducted on the Frontera supercomputer at TACC using R version 4.0.3 identified the quantile at which ERBB2 and ERBB1 gene expression predicts ER+/PR+ and ER-/PR- clinical outcomes using Cox regressions for PFS and OS. P-values of each Hazard Ratio (HR) were ranked with the top genes identified after adjusting for multiple comparisons. Results: Most carcinomas exhibited ER+/PR+ status regardless of assay platform. There was excellent agreement in ER-/PR- status distribution between assays. Neither ER nor PR levels were related to HER2 or EGFR levels, nor were HER2 and EGFR levels interrelated. Of 1,405 specimens, 9% were triple-negative breast cancer (TNBC) with HER2, while 41% were triple-positive (TPBC), compared to 6% TNBC and 34% TPBC with EGFR in the biomarker triad. Biopsies with TNBC profiles were associated with decreased DFS but not OS. HER2+ status was significantly associated with lower risk of death in the ER+/PR+ group (HR=0.44, p=0.05) but not with DFS (HR=0.56, p=0.23). EGFR+ status did not correlate significantly with either DFS (HR=2.03, p=0.23) or OS (HR=1.59, p=0.33) in the ER+/PR+ group. Microarray results of LCM-procured carcinoma cells of 247 primary breast biopsies were obtained for ~22,000 genes. The four biomarkers' cumulative relative frequency distribution analyses and triad permutations were examined. Cox regressions contrasted hazard ratios among ER/PR/HER2 and ER/PR/EGFR groups and their gene expression triads. Interaction models did not show significant effects, indicating no additional prognostic value beyond that of individual biomarkers. Conclusions: Patients with triple-positive gene expression triads (ESR1+/PGR+/ERBB2+ or ESR1+/PGR+/ERBB1+) exhibited better survival outcomes than triple-negative patients. Although HER2 protein presence in breast carcinoma predicts response to HER2-targeted therapy, lack of significance in interaction models suggests that HER2 or EGFR's prognostic value may be largely independent of ER/PR protein status. Frontera supercomputer analyses revealed differences in gene expression profiles between ER+/PR+ and ER-/PR- carcinomas and DFS. These unique findings with LCM-procured cells highlight the complex interplay of biomarkers and cognate gene expression in breast cancer and underscore the importance of quantifying ER and PR protein status in guiding treatment and predicting outcomes.

P1-08-04: Efficiency of Low-Viscosity Culture for TNBC Organoid Establishment and Drug Screens

Xavier Bittman-Soto, David C. Boyd, J. Chuck Harrell

Three-dimensional (3D) cell culture models, such as patient-derived xenografts (PDX) and PDX-organoids (PDXO), emerge as invaluable tools for exploring the complexities of disease, including the unique features of tumors. By accurately mimicking numerous aspects of the original tissue, such as growth rate, migration, and drug sensitivity, these models provide key insights for advancing drug discovery and development. Triple-negative breast cancer (TNBC) presents significant clinical challenges due to its aggressive nature, propensity for early metastasis, and limited treatment options. This study compared several 3D culture methods, including scaffold-free (suspension) and scaffold-based (low viscosity and domes) systems to evaluate the establishment and proliferative capacity of PDXOs from VCU patients of diverse genetic ancestry. We found that incubation in a scaffold-free environment overnight, followed by a low-viscosity culture system, reduced the time for organoid development, enhanced proliferative rate, and increased viability compared to higher-viscosity domes. The low-viscosity culture proved to be the most efficient for viability studies, drug screens, and expansion in vitro. Time-lapse imaging highlighted the diverse behavior among tumor cell samples, which has allowed for the development of a morphological classification system of organoids and cell aggregates. Importantly, the proliferative rates of the low-viscosity cultures were more similar to mammary tumors which provides confidence that therapeutic inhibitor studies will more effectively translate to in vivo systems and clinical trials. In conclusion, this study provides valuable insights into various 3D culturing systems for more efficient studies with TNBC organoids.

P1-08-05: The mechanism of FOXO3a in coping with oxidative stress to promote TNBC progression

Manqing Cao

Triple-negative breast cancer (TNBC) is an aggressive type of breast cancer associated with poor prognosis and limited treatment options. FOXO3a is an important transcription factor participating in many important biological process. It has been reported that FOXO3a expression is associated with lymph node metastasis and poor disease-free survival in TNBC. We also found that overexpression of FOXO3a in MDA-MB-231 cells could promote lung metastasis of by tail vein injection in vivo. However, the mechanism of FOXO3a in TNBC progression remains unclear. Here, we found that FOXO3a was highly expressed in nuclear of TNBC tissues than that in peritumor tissues by immunohistochemistry staining. FOXO3a enhances the invasion and motility of TNBC cells by transwell assays. RNA-sequencing combined bioinformatic analysis suggest that FOXO3a is closely related to adhesion pathway and PI3K/AKT pathway. Considering FOXO3a could translocate to nuclear to activate downstream signaling pathway under oxidative stress. Therefore, we further showed that FOXO3a could activate FAK/PI3K/AKT pathway under oxidative stress

to promote TNBC progression. Collectively, our research showed that the expression of nucleus FOXO3a was related to the progression of TNBC, and FAK/PI3K/AKT pathway participate in the role of FOXO3a in promoting the progression of TNBC. FOXO3a may serve as a prognostic biomarker and a potential therapeutic target for TNBC.

P1-08-06: Real-world outcomes and care utilization related to pregnancy in hormone receptor-positive breast cancer survivors

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Background: Premenopausal women diagnosed with curable hormone receptor (HR)-positive breast cancer (BC) are typically treated with 5-10 years of adjuvant endocrine therapy (ET) which often impacts family planning. Prospective data from the POSITIVE trial suggest that pausing ET after 2 years to pursue pregnancy, delivery, and breastfeeding is safe in a selected population with high rates of ET resumption. We hypothesized that in real-world practice, resumption of ET and imaging surveillance would be lower than in the POSITIVE trial.
Methods: We used the Oncoshare dataset, which integrates electronic medical records (EMR) from two healthcare systems with California Cancer Registry (CCR) data, to study women diagnosed with stage 0-III HR-positive BC between 1994 and 2020 who became pregnant after BC diagnosis. We characterized time to pregnancy; ET utilization, total duration, pause, and resumption patterns; imaging surveillance patterns; and BC-specific survival and recurrence rates for women treated at a community practice (Sutter Health/Palo Alto Medical Foundation) or an academic hospital network (Stanford University Healthcare). Clinical and sociodemographic variables were derived from the CCR. ET prescription, pregnancy, and radiographic data were obtained via automated EMR data extraction and manual chart review. Characteristics were described using counts, percentages, medians, and interquartile ranges (IQR) and compared using a Fisher's exact test or Wilcoxon rank-sum test where appropriate.
Results: At a median follow up of 9.1 years, 105 women had at least one pregnancy following a BC diagnosis. The median age at diagnosis was 32.9 years (IQR: 30.3-35.4) and 98 women had at least one live birth, with 122 total live births recorded. Following BC diagnosis, 27 (25.7%) women never started ET, 20 (74.1%) of whom attributed this to a desire for pregnancy. Among the 78 (74.2%) women who ever initiated ET, tamoxifen use was most common (N=45, 91.8%) before 2015 and utilization of ovarian suppression-based regimens became more frequent after 2015 following publication of the SOFT/TEXT trials (N=12, 42.9%; $p<0.001$, Fisher's exact test). The median time from ET initiation to pause for pregnancy was 25.5 months (IQR 15.3-47.4) and from ET pause to delivery was 24.7 months (IQR 16.5-47.1). Following delivery, 31 (39.7%) patients did not resume ET and 62 (62.6%) did not resume surveillance imaging. Among 100 patients without disease recurrence at two years, 39 had resumed ET after treatment interruption. Only thirteen

patients resumed both ET and surveillance imaging. Among those who resumed ET after delivery, median time to resumption was 6.3 months (IQR 3.0-19.0); median time to surveillance imaging resumption was 9.3 months (IQR 3.5-18.5). Breastfeeding status was not associated with time to imaging resumption (p = 0.508, Wilcoxon rank sum test). BC-specific survival was 94.5% (95% CI: 88.7%-100%), with a 5.7% (N=6) three-year recurrence rate.
Conclusions: Among early-stage HR-positive BC survivors treated in community and academic practice who became pregnant after BC diagnosis, ET guideline adherence was notably lower than in the POSITIVE trial (39% vs 73% resumption rate). Resumption of imaging surveillance was also low; a substantial fraction of patients (n=32, 41.0%) were not taking ET or undergoing radiographic surveillance 5 years post-diagnosis, though we cannot rule out the possibility of patients seeking care elsewhere. Despite this, BC-specific survival with long-term follow-up in a real-world setting was high and short-term recurrence rates were comparable to those seen in POSITIVE (5.7% vs 7.9%). Confirmatory studies, encouragement of optimal adherence and follow-up, and longer-term data collection on recurrence and survival are indicated.

P1-08-07: Time Interval Between Diagnosis and Treatment of Early Breast Cancer and the Impact of Health Insurance Coverage in Latin America: A Sub Analysis of the LATINA Breast Study (LACOG 0615 / MO39485)

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Background: Breast cancer (BC) is the most common type of cancer among women in Latin America. Evidence shows that delays in starting treatment, i.e. neoadjuvant chemotherapy or surgery, for early BC are associated with increased rates of relapse and mortality. Several factors have been associated with treatment delays such as socioeconomic status, health insurance type, age, racial/ethnic groups, educational level and rural residence among others. This study aimed to evaluate median days between diagnosis to first treatment and factors associated with delays in treatment initiation in patients diagnosed with early BC in Latin America.
Methods: This sub-analysis included patients with stage I-III BC from the LATINA Breast (NCT04158258), a prospective, observational study that included patients diagnosed with BC between February 2020 to August 2022 in 31 centers from 10 countries in Latin America. Time interval was calculated from the BC biopsy date to the date of the first treatment administered, i.e. surgery or neoadjuvant chemotherapy or endocrine therapy. A

multivariable logistic regression model was applied to evaluate factors associated with treatment delays of > 60 days after diagnosis.
Results: From a total of 3275 patients included in the LATINA Breast registry, 2768 (84.5%) were diagnosed with stage I-III BC and were included in this sub-analysis. A total of 2516 patients (90.8%) had available information on the date of the diagnostic biopsy and initial treatment. Overall, the median number of days from diagnosis to first oncologic treatment was 55 days (IQR 28-97) and of them 1159 (46.1%) patients had >60 days from diagnosis to first treatment. Time interval from biopsy to treatment according to clinical stage were 46 days (IQR 20-99) for stage I, 57 days (IQR 28-99) for stage II, and 56 days (IQR 32-91) for stage III, (p=0.1578). Time interval from diagnosis to treatment according to first treatment received were 58 days (IQR 34-92) for neoadjuvant treatment and 46 days (IQR 19-99) for surgery, (p =0.2300). Time interval from diagnosis to treatment per country were: Argentina 48 days (IQR 8-88), Brazil 87 days (IQR 50-130), Chile 34 days (IQR 19-63), Colombia 67 days (IQR 33-101), Cuba 14.5 days (IQR 6-18), Dominican Republic 77 days (IQR 58.5-105.5), Guatemala 20.5 days (IQR 12-56), Mexico 34 days (IQR 22-47), Peru 46 days (IQR 19-84) and Uruguay 27 days (IQR 0-37). The time interval from diagnosis to treatment was higher in women treated in the public health system, 60 days (IQR 30-104) versus the private system, 45 days (IQR 24-77), (p <0.0001). Factors significantly associated with delays of >60 days from diagnosis to treatment were age >50 years (OR 1.41, 95% CI 1.15-1.74, p=0.0011), Black or African American/Brown (OR 3.3, 95% CI 1.15-1.74, p=0.0011), educational level illiterate/incomplete school (OR 1.42, 95% CI 1.06-1.89, p=0.0011) and public health insurance (OR 2.18, 95% CI 1.66-2.86, p<.0001).
Conclusion: This analysis of the LATINA Breast study demonstrates that the median time from diagnosis to first treatment of early BC in Latin America is 55 days. Time intervals are variable among different countries likely reflecting differences in access to fragmented health care systems. Although in many countries, patients seem to be treated within an acceptable timeframe (<60 days), there is still a large population with long delays. Older patients, Black/African Americans/Brown, those with lower educational level and those treated within the public health care system, not surprisingly, had the longest timelines potentially impacting disease outcomes in these populations.

P1-08-08: Real-world efficacy of immediate subsequent lines of therapy after trastuzumab deruxtecan (T-DXd) in patients with metastatic breast cancer (MBC) – retrospective study from the nationwide Flatiron database

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Background: T-DXd represents an established treatment option for patients with HER2+ and HER2-low MBC, with relevant activity also shown in HER2-ultralow MBC. No clinical trial data is available to guide treatment after progression on T-DXd. We aimed to produce real-world data to understand the performance of treatment regimens commonly

administered after T-DXd.
Methods: We conducted a retrospective observational study using the nationwide Flatiron Health electronic health record-derived deidentified database. We included patients with MBC who initiated T-DXd between 12/2019 and 9/2023 and who received an additional line of non-T-DXd anticancer treatment immediately following T-DXd. Tumors were categorized as HER2+ if positive at any timepoint before T-DXd, hormone receptor (HR)+/HER2- or HR-/HER2- (i.e. triple-negative) if never HER2+. Real world progression-free survival (rwPFS) and overall survival (OS) for post-T-DXd treatments were estimated using the Kaplan-Meier method.
Results: We identified 633 patients who received a systemic therapy post T-DXd: 352 (56%) with HER2+, 222 (35%) with HR+/HER2- and 59 (9%) with HR-/HER2- MBC. Median age was 58 years, 62.9% of the patients were White, 73.9% were treated in the community setting and 31.0% had de-novo MBC. Median prior lines were 4 in each subgroup (range: 1, 15) and 75% (n=475) had experienced progression while on T-DXd.
Outcomes with post-T-DXd treatments were significantly more favorable for patients with HER2+ MBC. Median rwPFS was 4.3 months (mo) for HER2+, 3.0 mo for HR+/HER2- and 2.7 mo for HR-/HER2- MBC ($p<0.001$), with similar outcomes when restricting to patients with prior progression on T-DXd (rwPFS: 3.9 mo for HER2+, 2.7 mo for HR+/HER2-, 2.7 mo for HR-/HER2- MBC, $p<0.001$). Median OS was 12.6 mo for HER2+, 8.1 mo for HR+/HER2- and 4.8 mo for HR-/HER2- MBC ($p<0.001$).
Of note, treatment patterns after T-DXd were heterogenous, and outcomes significantly differed by the specific treatment regimen administered ($p<0.001$).
Among patients with HER2+ MBC, the most commonly administered post-T-DXd regimens and median rwPFS were: tucatinib, trastuzumab and capecitabine (n=95, 27.0%) with a rwPFS of 4.7 mo; endocrine treatment (ET)-based regimens (n=57, 16.2%) with a rwPFS of 4.4 mo; chemotherapy + anti-HER2 antibodies (n=56, 15.9%), with a rwPFS of 5.0 mo; anti-HER2 antibodies +/- tyrosine kinase inhibitors (n=34, 9.7%), with a rwPFS of 7.3 mo; T-DM1 (n=27, 7.7%), with a rwPFS of 4.1 mo; and sacituzumab govitecan (SG, n=16, 4.6%), with a rwPFS of 2.3 mo.
Among patients with HR+/HER2- MBC, the most commonly administered post-T-DXd regimens and median rwPFS were: SG (n=66, 29.7%) with a rwPFS of 2.5 mo; ET-based regimens (n=46, 20.7%) with a rwPFS of 3.2 mo; taxanes (n=20, 9.0%), with a rwPFS of 3.8 mo; capecitabine (n=18, 8.1%), with a rwPFS of 5.8 mo; and eribulin (n=16, 7.2%), with a rwPFS of 5.9 mo.
Among patients with HR-/HER2- MBC, the most commonly administered post-T-DXd regimens and median rwPFS were: SG (n=13, 22.0%) with a rwPFS of 3.0 mo; eribulin (n=9, 15.3%) with a rwPFS of 1.8 mo; multiagent chemotherapy (n=8, 13.6%), with a rwPFS of 2.2 mo; experimental/off label regimens (n=6, 10.2%), with a rwPFS of 1.9 mo; and anthracyclines (n=4, 6.8%), with a rwPFS of 2.8 mo.
Across all the 633 patients receiving a post-T-DXd treatment, those that received SG (n=95) experienced shorter rwPFS than those receiving a different regimen (2.7 vs 3.9 mo, $p=0.001$).
Conclusions: In a large real-world database, outcomes of post-T-DXd treatments

significantly differed by MBC subtype and type of regimen administered. The use of SG immediately after T-DXd was associated with relatively short rwPFS (≤3 mo) across subtypes, suggesting some degree of cross resistance among topoisomerase 1 ADCs.

P1-08-09: REAL-WORLD OUTCOMES COMPARING 3-MONTHLY WITH MONTHLY GOSERELIN IMPLANT IN PREMENOPAUSAL WOMEN WITH BREAST CANCER

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Background: Goserelin 10.8 mg 3-monthly implant has been compared with goserelin 3.6 mg monthly in clinical trials. The 3-monthly implant is more convenient to clinicians and patients as it reduces office visits. Although goserelin 10.8 mg is not currently approved for breast cancer (BC) by the U.S. FDA, clinical trials demonstrated it to be non-inferior to 3.6 mg monthly implant in premenopausal HR-positive BC patients. However, real-world studies comparing goserelin 10.8 mg 3-monthly implant with goserelin 3.6 mg monthly implant are scarce. This study aimed to compare real-world treatment outcomes among BC patients treated with goserelin 3.6 mg and goserelin 10.8 mg.
Methods: Women aged 18-55 years without evidence of postmenopausal status at initial BC diagnosis and exposed to goserelin 3.6 mg or 10.8 mg post-BC diagnosis in the ConcertAI Patient360™ dataset were included in the study. Inverse probability treatment weighting (IPTW) was used to ensure the comparability of baseline demographic and clinical characteristics between the two cohorts. The primary outcome was the real-world disease-free survival (rwDFS) rate at 12 months to assess the non-inferiority of 3-monthly goserelin 10.8 mg vs. monthly goserelin 3.6 mg, and the non-inferiority margin was set at -15% between treatment groups based on published oncology clinical trials. No non-inferiority testing was carried out for other endpoints. Weighted Kapan-Meier analysis was used to characterize rwDFS, overall survival (rwOS), and time to treatment discontinuation (rwTTD) for patients who received 3-monthly goserelin 10.8 mg and monthly goserelin 3.6 mg.
Results: A total of 575 patients received goserelin 3.6 mg and 123 received goserelin 10.8 mg. Post-IPTW, the 3.6 mg cohort had a median age of 41 years, 70.3% white; 68.3% infiltrating ductal carcinoma histology, 41.7% ECOG 0-1, and 61.0% early stage BC at treatment, and the 10.8 mg cohort had a median age of 42 years, 69.9% White, 70.2% infiltrating ductal carcinoma histology, 36.4% ECOG 0-1, and 61.0% early stage BC at treatment. The 12-month rwDFS rates were 76.6% for the 3.6 mg cohort and 79.2% in the 10.8 mg cohort, with a treatment difference of 2.65% (95% CI: -1.75%, -7.04%), supporting the non-inferiority of goserelin 10.8 mg to 3.6 mg at the -15% margin. The rwTTD rates, reported as the proportion of patients on goserelin treatment at 12 months and 24 months for goserelin 3.6 mg vs.10.8 mg, were 39.6 % vs. 51.2% and 23.1% vs. 32.4%, respectively. The median rwDFS and median rwOS were not reached at the 60-month follow-up. The

rwDFS rates at 3-years and 5-years for goserelin 3.6 mg vs. 10.8 mg were 61.5% vs. 63.4% and 55.8% vs. 46.9%, respectively. The rwOS rates at 1-year, 3-years, and 5-years for goserelin 3.6 mg vs. 10.8 mg were 92.9% vs. 97.4%, 81.3% vs. 86.2%, and 69.0% vs. 67.4%, respectively.
Conclusions: This real-world analysis indicates that 3-monthly goserelin 10.8 mg is non-inferior to monthly 3.6 mg among premenopausal BC women in terms of 12-month rwDFS rate. This finding may support the use of the goserelin 10.8 mg 3-monthly implant as an alternative treatment option for this patient population.

P1-08-10: Germline BRCA1 and BRCA2 testing, Genetic Counseling and Adjuvant Therapy Patterns in Early Breast Cancer Patients

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Background: The U.S. FDA approved adjuvant Poly (ADP-ribose) polymerase-inhibitors (PARPis) for patients (pts) with high-risk human epidermal growth factor receptor 2 - negative (HER2-) early breast cancer (eBC) harboring pathogenic/likely pathogenic germline BRCA1/2 variants (gBRCAm) in early 2022. Subsequently, best practice guidelines recommended germline testing of pts with HER2- eBC to identify candidates for adjuvant PARPis. Yet, inequities may exist in who receives testing and subsequent treatment. We describe real-world practice patterns around gBRCA1/2 testing, genetic counseling, and adjuvant treatment for eBC to inform interventions that may improve patient care.
Methods: Pts with HER2- eBC (stages I-III) diagnosed between January 2018 and July 2022 at six sites of a community health system in the midwestern U.S. were followed through February 2023. Demographics, clinical characteristics, testing, treatment, and genetic counseling data were extracted from the electronic health records by oncology data specialists. Descriptive statistics are reported.
Results: Median (IQR) age of the 966 pts included in the study was 64 (53, 73). 954 (99%) pts were female and 12 (1.2%) pts were male. 668 (69%) pts were White, 67 (6.9%) pts were Black, 113 (12%) pts were Asian, 92 (9.5%) pts were Hispanic, and 26 (2.7%) pts were other/unknown race/ethnicity. Median (IQR) social vulnerability index score was 0.41 (0.21, 0.63). 506 (52%) pts received gBRCA testing, ranging from 29% - 73% across the six sites. Testing proportions were higher in pts <65 years (yr) old at diagnosis [67% vs 37% ≥65 yr], pts with a family history of breast cancer vs. without (65% vs 53%), and pts with a family history of ovarian cancer vs. without (77% vs. 58%). 55% (71) of TNBC pts and 48% (435) of HR+/HER2- pts were tested. 48% of pts with a <26 Oncotype Dx score were tested compared to 40% of pts with ≥26. Of the 506 tested, 15 (3%) were gBRCAm (8 TNBC, 7 HR+), 10 of whom had early (<50 yr) eBC onset. Results were available for 53% of pts prior to definitive surgery and for the majority (72%) of tested pts prior to adjuvant treatment.

45% (432) of the study population were both referred to and completed genetic counseling. 95% of pts who completed genetic counseling received gBRCA testing; only 18% of pts who did not complete counseling were tested. 9% (90) of pts were referred but failed to complete genetic counseling, and 46% (444) were never referred during follow-up. Of the 432 pts receiving counseling, the majority (63%) were counseled by nurses and 8% were counseled by certified genetic counselors. The tested proportion was lower in pts counseled by medical oncologists (83%) than those counseled by certified genetic counselors (100%), nurses (97%) or surgeons (93%).
A greater proportion of gBRCA-tested pts received adjuvant chemotherapy than those untested (20% vs 6%). A greater proportion of TNBC pts received adjuvant chemotherapy compared to HR+ pts (25% vs 12%), as expected.
Conclusions: Despite clinical guidelines recommending that all patients with TNBC and certain patients with HR+ BC should receive genetic testing, 48% of pts were not tested, with marked variability across sites within the same healthcare system (27-71%). When received, genetic counseling positively influenced testing rates. Although gBRCA status influences surgery and adjuvant treatment decisions, results were available for only 53% of pts prior to definitive surgery and only 72% of pts prior to adjuvant treatment, highlighting the need to improve the timely delivery of testing results to allow for appropriate treatment selection. These results will inform a Quality Improvement project with the objective of increasing guideline concordant gBRCA testing and referrals to genetic counseling, ensuring timely testing results to inform surgical and systemic treatment decisions.

P1-08-11: Incidence and Factors Predicting Survival in Inflammatory Breast Cancer: An Updated SEER Analysis

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Introduction: Inflammatory breast cancer (IBC) is an aggressive type of breast cancer (BC) accounting for 1-5% of all BC cases and contributes to 8-10% of BC deaths. IBC is usually diagnosed at locally advanced or metastatic stage. It occurs more frequently in younger women, although the exact incidence is unknown. We examined the overall incidence of IBC from 2010 -2021, the age-adjusted incidence among those younger than 40 years and incidence among different racial groups. Additionally, we evaluated the factors predicting overall survival (OS) in IBC patients diagnosed between 2010-2021.
Method: We identified patients diagnosed with IBC from 2010 – 2021 using Surveillance, Epidemiology, and End Results (SEER) database and examined the incidence rate (IR) per 1000,000 in overall population, age-adjusted IR for those< 40 years (y), and IR by race: White, Black, Asian/Pacific Islander (API), American Indian/Alaska Native (AI/AN). Data on baseline demographic (age, gender, race), clinicopathologic (clinical TNM stage, grade, tumor subtypes) and treatment characteristics (chemotherapy (CT), radiation therapy (RT), surgery (Sx)) were abstracted. Frequencies of IBC subtypes were summarized by hormonal status: hormone receptor positive (HR+), HER2+, triple negative IBC (TNBC). Univariate and

multivariate cox-proportional hazard regression was conducted to analyze the factors predicting survival in IBC.
Results: A total of 1245 patients were included, the majority 99.8% (n=1422) were females, and 71% (n=1014) White, 18% (n=258) Black, 10% (n=135) API, and 1% (n=16) AI/AN. The median age at diagnosis of the cohort was 60 y (range: 20-85 y). 32% were diagnosed with HR+/HER2-, 22% with TNBC, 16% with HR-/HER2+, and 16% HR+/HER2+ IBC. The IR of IBC has decreased over the years, from 2.1 per 1000,000 in 2010 to a most recent IR of 1.3 in 2021. Age-adjusted IR also decreased, where people less than 40 y had an age-adjusted IR of 0.5 in 2010, and a most recent IR of 0.3 in 2021.
When analyzing IBC IR trends by race, we found a decreasing trend among all racial groups, except for AI/AN where we found a variable trend with initial decline until 2014 followed by steady increase in IR with peak value of 1.2 in 2021. The overall IBC IR in 2021 was 1.2 among Blacks and AI/AN, 1 among White, and 0.5 among API. Notably, in younger patients (<40 y), Black and AI/AN had the highest age-adjusted IR of 0.5 and 0.6 respectively in 2021.
Multivariate analysis showed that patients diagnosed with IBC at age younger than 60 y (median age of study cohort) had a worse OS than those diagnosed at an age older than 60 y (HR 1.18, 95%CI: 1.02-1.38, P= 0.02). Compared to White, Black patients had worse OS (HR 1.13, 95%CI: 1.09-1.58, P= 0.004), While AI/AN patients had a better OS (HR 0.30, 95%CI: 0.09-0.94, P=0.03). Compared with TNBC subtype, non-TNBC tumors were associated with improved OS. [HR-/HER2+ (HR 0.43, 95%CI:0.34-0.55, P=0.00), HR+/HER2- (HR=0.48, 95%CI:0.40-0.58, P=0.00), and HR+/HER2+ (HR 0.37, 95%CI: 0.29-0.47, P= 0.00)]. Moreover, patients who were treated with CT (HR 0.50, 95%CI: 0.41-0.61, P=0.00) and Sx (HR 0.58, 95%CI: 0.33-0.48, P=0.00) had better OS than those who did not. Interestingly, no significant association was noted between survival and receipt of RT (HR 0.89, 95%CI: 0.74-1.06, P=0.20).
Conclusion: The incidence of IBC has decreased over the past decade. Black women regardless of their age and AI/AN younger continue to have a higher incidence of IBC. The prognosis of IBC remains poor, especially among Black, those diagnosed at age younger than 60 y and TNBC subtype. Chemotherapy and surgery seem to improve OS in IBC. Larger studies are needed to explore the potential risk factors for IBC among different races.

P1-08-12: Clinical characteristics and survival outcome of patients with early discontinuation of first-line (1L) cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) therapy for HR+/HER2- advanced breast cancer: a nationwide, real-world study

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Background: The combination of CDK4/6i and endocrine therapy (ET) is the standard-of-care 1L for patients with for Hormone Receptor-positive/HER2-negative (HR+/HER2-) advanced breast cancer in France since 2017. The aim of the present analysis was to

describe the real-world use of CDK4/6i for the 1L therapy in patients with HR+/HER2- advanced breast cancer, and to evaluate the overall survival (OS) in patients with early discontinuation of 1L treatment.
Methods: Retrieving data from the French National Health Data System (Système National des Données de Santé - SNDS), we identified patients ≥18 years old with HR+/HER2- advanced breast cancer who were started on 1L treatment with CDK4/6i plus ET from March 23, 2018 to December 31, 2021. Cohort characteristics at diagnosis and outcomes were described overall and by duration of CDK4/6i therapy, namely less than 6 months (defined as early discontinuation for any reason) and at least 6 months. Furthermore, we provide additional descriptive data among patients <50 and ≥50 years of age, and by endocrine sensitivity (i.e, endocrine resistant, defined by relapse during or <12 months after completion of adjuvant ET, and endocrine sensitive if relapsed >12 months or if endocrine naïve). A landmark survival analysis was conducted for OS from CDK4/6i therapy initiation, using the Kaplan-Meier method.
Results: As of December 31st, 2022, our cohort accounted for 34054 patients having received 1L treatment with CDK4/6i plus ET. Median age was 68 years (interquartile range, IQR 57-76), and 98.7% were women. Overall, mean time to treatment discontinuation (TTD) was 17.7 months (95% Confidence interval - CI 17.5-17.8), and 2-years OS was 74.6%.
Patients with early discontinuation accounted for the 25.1% (n=8565) of our cohort (among them, 85.5%, concurrently discontinued CDK4/6i and ET). Among patients who discontinued treatment early and among those who responded to 1L CDK4/6i therapy for at least 6 months, median age was 69 years (IQR 58-79) and 67 years (IQR 57-75), 10.6% and 12.9% were younger than 50 years, 98.0% and 99.0% were women, 29.4% and 26.2% were defined as endocrine resistant. Mean TTD was 3.1 months (95% CI 3.0-3.2) and 22.6 months (95% CI 22.4-22.7) among patients who discontinued treatment early and among those who responded to 1L CDK4/6i therapy for at least 6 months, respectively. 2-years OS was 39.2% and 85.8% in groups with early discontinuation of CDK4/6i treatment and without early discontinuation, respectively (Hazard Ratio - HR 3.91, 95% CI 3.75-4.09; p<.0001).
In the overall cohort, 12.3% (n=4202) were younger than 50 years. Mean TTD was 18.9 months (95% CI 18.5-19.4) and 2-years OS was 82.7% in this group, whereas TTD was 17.5 months (95% CI 17.3-17.7) and 2-years OS was 73.4% in patients ≥50 years. In addition, 27.1% (n=9210) patients were defined as endocrine resistant. Mean TTD was 16.7 months (95% CI 17.9-18.2) and 2-years OS was 72.9% in this group, whereas TTD was 18.0 months (95% CI 16.4-17.0) and 2-years OS was 75.2% in the endocrine sensitivity group.
Conclusions: While considering the limitations of a real-world analysis, the present study showed that approximately 1-in-4 patients newly diagnosed with HR+/HER2- advanced breast cancer discontinued the standard 1L CDK4/6i therapy early, within the first 6 months of treatment, and that patients with early discontinuation had worse 2-years OS compared to those without early discontinuation. This study suggests a potential unmet clinical need in the real-world setting, and identifies a population at worse prognosis, warranting further research to identify patients with HR+/HER2- advanced breast cancer who may benefit from a different 1L strategy than the combination of CDK4/6i and ET.

P1-08-13: 1000 Patient Prospective Inflammatory Breast Cancer (IBC) Registry Demonstrates Advances in Outcomes for IBC.

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Background: Inflammatory breast cancer (IBC) is an aggressive yet rare subtype of invasive breast carcinoma with historically poorer outcomes as compared to non-IBC subtypes. Large studies are limited by the low incidence of IBC. An updated comprehensive analysis of outcomes inclusive of more recent advances in breast cancer management is presented.
Methods: Comprehensive clinicopathologic data was collected on patients enrolled to the MD Anderson Cancer Center (MDACC) IBC Registry (N=1,000; 12 patients excluded for lacking IBC criteria; 27 excluded with secondary IBC). All Stage IV patients were considered for Stage III standard chemotherapy followed by modified radical mastectomy and post-mastectomy radiation for local regional control based on response. Patient and disease characteristics are presented with descriptive tabulations. Comparisons between subgroups utilized chi-square tests. Overall survival (OS) was estimated by Kaplan-Meier methods and median times are reported with 95% confidence intervals. Comparisons utilized log rank tests.
Results: Among the N=961 patients evaluated for this study: 88% had invasive ductal carcinoma, 3.2% had invasive lobular carcinoma, and 5.3% had mixed invasive ductal lobular carcinoma. 8.7%, 81.3%, and 8.0% were black, white, or other, respectively, with 11.8% reporting Hispanic ethnicity. 20.2% were normal or underweight and 10.4% had never been pregnant. 13.3% of cases were hormone receptor positive (HR+) HER2 positive (HER2+), 20.3% were hormone receptor negative (HR-) HER2+, 32.7% were HR+HER2 low/negative (HER2-), and 33.7% were HR-HER2-. 49.7% of patients were postmenopausal and 48.8% were pre-menopausal. At time of diagnosis, 66.6% of patients were diagnosed at clinical Stage III, 33.4% had de novo metastatic disease, and only 5.0% had node negative disease. 83% of patients had <3 months from symptom development to diagnosis. The proportion of patients with <3 months from symptom development to diagnosis increased and the incidence of Stage IV de novo disease decreased over the period of the study (p=NS for each); however, since the pandemic, time from symptoms to diagnosis increased, but not as high as before 2015. Pathologic complete response (pCR) was achieved in 23.7%, 53.7%, 6.9%, and 23.6% of patients with HR+ HER2+, HR- HER2+, HR+ HER2-, and HR- HER2- IBC who underwent surgery, respectively. pCR rates increased over time from 35% before 2010 to 58% since 2021 for HER2+ patients (p = 0.07). Median OS of all patients was 5.5 (5.0, 6.9) years and was longer among patients who were pre-menopausal (p=0.05), HER2+ (p<0.01), or Stage III vs IV (p<0.01).
By subtype, OS at 5 years in patients diagnosed with Stage III vs Stage IV IBC was 80.5% vs 78.5% in HR+HER2+ (p = NS), 87.1% vs 62.1% (p = 0.001) in HR-HER2+, 59.9% vs 35.9% (p <0.001) in HR+HER2-, and 38.6% vs 9.8% (p <0.001) in HR-HER2-; metastasis developed in 29.4%, 29.8%, 43.1%, and 57.4% of Stage III patients, respectively.
Conclusions: Time to diagnosis and de novo metastasis presentation decreased over time,

reflecting the importance of timely diagnosis, while pCR rates after neoadjuvant chemotherapy (NAC) increased over time in the setting of evolving NAC regimens. Prognosis of IBC remains overall significantly poorer relative to non-IBC reflecting unmet needs for more effective therapies in this field.

P1-08-14: The occurrence of immune-related adverse events and overall survival among breast cancer patients receiving pembrolizumab

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Objectives: Immune-related adverse events (irAEs) have been associated with a better response and overall survival (OS) in patients with lung cancer or melanoma receiving immune checkpoint inhibitors (ICIs). The relationship between the occurrence of irAEs and survival outcomes of breast cancer patients receiving ICIs, in particular pembrolizumab, is unclear.
Methods: We conducted a retrospective, propensity score-matched cohort study using the TriNetX Analytics Network database, which contains de-identified data from over 120 participating healthcare institutions. We included all adult female breast cancer patients who were treated with pembrolizumab. We excluded patients who received hormonal or endocrine therapies. We defined irAEs as events that occurred within 12 weeks of ICI initiation. Patients who died before 12 weeks were excluded. This was designed to avoid immortality bias. We identified irAEs as cutaneous, gastrointestinal, pulmonary, or endocrine-related irAEs using a combination of International Classification of Diseases (ICD)-10 codes and irAE-directed therapies. The primary outcome was the 1-year OS following the initiation of pembrolizumab. Patients with or without irAEs were matched based on predetermined variables including age, race, breast cancer-directed therapy, and the Charlson Comorbidity Index.
Results: We identified 2552 patients with breast cancer who received pembrolizumab eligible for inclusion. The mean age was 55.5 ± 14.3. Over the 1-year follow-up period, 150 patients died, resulting in a 1-year OS of 93.5%. There were 778 (30.5%) cutaneous irAEs, 188 (7.4%) gastrointestinal irAEs, 103 (4.0%) pulmonary irAEs, and 101 (5.5%) incident endocrine irAEs within 3 months of ICI therapy. In the propensity score-matched analysis, patients with cutaneous irAEs had a higher 1-year OS (96.1 vs. 93.5%; log-rank p = 0.021) than those who did not have cutaneous irAEs. The occurrence of cutaneous irAEs was associated with a lower risk of all-cause mortality (Hazard ratio (HR), 0.58 [95% CI 0.36-0.93]). Patients who developed gastrointestinal irAEs (HR, 1.86 [95% CI 0.74-4.66]), pulmonary irAEs (HR, 1.83 [95% CI 0.72-4.64]), or endocrine irAEs (HR, 1.65 [95% CI 0.48-5.62]) appeared to have a higher risk of mortality than those who did not develop these irAEs, though the differences were not statistically significant.
Conclusions: The occurrence of cutaneous irAEs is associated with a higher 1-year OS in patients with breast cancer receiving pembrolizumab.

P1-08-15: Real-world analysis of breast cancer patients qualifying for adjuvant CDK4/6 inhibitors

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Background: Abemaciclib and ribociclib each improved disease-free survival (DFS) when added to adjuvant endocrine therapy for hormone receptor (HR)-positive HER2-negative early breast cancer (EBC), in the monarchE (NCT03155997) and NATALEE (NCT03701334) phase 3 trials respectively. However, the two studies used different inclusion criteria. Additionally, post mastectomy radiotherapy (PMRT) is recommended for node-positive patients who fulfill the eligibility criteria of these studies to reduce risk of recurrence and improve survival. We aim to assess the proportion of HR-positive EBC patients who satisfy the two different adjuvant CDK4/6 inhibitor criteria, and their outcome, in a real-world population.

Methods: Consecutive female patients with HR-positive HER2-negative EBC diagnosed between 1997-2017 from the Australian Capital Territory and South-East New South Wales Breast Cancer Treatment Group registry were analysed. Eligibility for adjuvant abemaciclib was defined per monarchE trial as >=4 axillary nodes involved or 1-3 nodes plus primary >5cm or grade 3. Ribociclib eligibility was defined per NATALEE trial as node-positive and node-negative with >5cm or >2cm grade 3 primary tumours. Disease-free and overall survival (OS) were examined using the Kaplan-Meier method and compared between groups using log rank tests. Cox proportional hazards model was conducted incorporating potential variables including PMRT-use on recurrence and survival outcomes.

Results: Of 3840 patients, 671 (17.5%) were abemaciclib-eligible and 1587 (41.3%) ribociclib-eligible. Lymph-node negative patients comprised 14% of the ribociclib-eligible cohort. Of all lymph-node negative patients, 9% were ribociclib-eligible. Abemaciclib-eligible registry patients were older (median 55 vs 51 years), with lower nodal burden (>4 nodes involved in 44% vs 60%) than moncharE trial participants. The proportion of stage III cancers was almost three-times greater in NATALEE trial patients (60%) than ribociclib-eligible registry patients (24%). Among registry patients, 46% underwent mastectomy, with PMRT utilised in 34.5%.

The 5-year DFS was 77% and 94% in abemaciclib-eligible and non-eligible registry patients respectively (HR 2.63, 95%CI 2.26–3.05, p<0.001). The 5-year DFS was 86% and 95% in ribociclib-eligible and non-eligible registry patients respectively (HR 1.92, 95%CI 1.67–2.19, p<0.001). Similarly, 5-year OS was inferior in adjuvant CDK4/6-eligible compared with non-eligible patients; in the abemaciclib (HR 2.64, 95%CI 2.28 – 3.07, p<0.001) and ribociclib (HR 1.92, 95% CI 1.67 – 2.20, p<0.001) analyses. Using Cox regression modelling, adjuvant ribociclib-eligibility and age were significantly associated with shorter DFS, while PMRT-use was significantly associated with longer DFS. However, while abemaciclib-eligibility and age remained significantly associated with inferior DFS, there was no significant impact of

PMRT-use on DFS in a separate Cox model.

Conclusions: Many women with EBC will qualify for adjuvant CDK4/6 inhibitors, with 18.7% and 41.3% of patients meeting criteria for adjuvant abemaciclib and ribociclib respectively in our real-world cohort. This has resource and workforce implications for treatment and associated monitoring. As expected, both criteria identify patients at higher relapse risk, with inferior DFS of abemaciclib-eligible patients compared with ribociclib-eligible patients. The difference in outcome between adjuvant ribociclib-eligible versus non-eligible groups remained significant after adjustment for PMRT-use. In the real-world setting, a greater proportion of adjuvant CDK4/6-eligible patients have lower stage disease, therefore the absolute benefit from treatment may be smaller than estimated by the trials.

P1-08-16: Resolution of Active Pyoderma Gangrenosum during Adjuvant Breast Cancer Therapy: A Case Report

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Abstract: Pyoderma gangrenosum (PG) is a painful neutrophilic dermatosis that commonly occurs at sites of trauma. Treatment usually involves a combination of corticosteroids and immunosuppression, but management can be challenging and healing times can be prolonged. PG occurring after breast cancer surgery is rare. Furthermore, the effect of radiation on these lesions is not well documented, which can be concerning in cases necessitating adjuvant radiation. Therefore, we present a unique case of a patient with invasive ductal carcinoma who developed postoperative pyoderma gangrenosum and showed rapid resolution of her lesions with adjuvant radiation.
Case Presentation: Our patient is a 66-year-old female with a past medical history significant for bilateral metachronous ductal carcinoma in situ (DCIS) treated with lumpectomy and adjuvant radiation on the right 16 years prior and lumpectomy alone on the left 14 years prior. She also had a history of familial polyposis syndrome status post a right hemicolectomy which was complicated by an unspecified necrotizing infection. Annual surveillance mammograms were benign until 2022 when suspicious calcifications were noted in the posterior left breast. After appropriate work up and management with lumpectomy and sentinel lymph node biopsy, she was diagnosed with a left breast invasive ductal carcinoma, grade III, pT2(m)N0(sn)M), Stage IIB, ER 1-10%/PR negative/HER2 negative with an Oncotype of 58.
Six days after surgery, she presented with gangrenous changes of the skin and erythema surrounding the breast incision. A 5cm area was debrided with pathology revealing a dense neutrophilic abscess involving the dermis and subcutis with overlying ulceration. Despite initiation of broad-spectrum antibiotics and appropriate wound care, her breast and axillary skin became more necrotic, prompting additional debridement. Pathology again showed a dense neutrophilic abscess involving the dermis and subcutis with scattered foreign-body-type giant cell granulomas. Four days after the initial debridement, when the

cultures did not demonstrate bacterial growth, a diagnosis of pyoderma gangrenosum was made. We did not do any additional sharp debridement, and the patient was started on cyclosporine and methylprednisolone. Her disease stabilized but she was left with a 12cm wound of the left lateral breast with a smaller, 2.5cm, deficit in the left axilla.
Given her Oncotype Dx score of 58, the patient was planned for adjuvant chemotherapy to commence one month after her last debridement. Cyclosporine was discontinued and she received four cycles of cyclophosphamide and doxorubicin (AC) followed by four cycles of paclitaxel (T). She had a moderate febrile reaction after each infusion but cultures remained negative and the wound slowly began to form granulation tissue. A slow steroid taper was started during chemotherapy.
To allow for maximal healing prior to the initiation of adjuvant radiation, she was scheduled for CT-simulation 10 weeks after her last cycle of chemotherapy. She was planned for a total of 42.56Gy delivered over 16 daily fractions to the whole breast, using 3D conformal radiotherapy with opposing tangent beams. A boost was not employed as the tumor bed could not be localized. During her radiation therapy, her lesion showed significant healing. At her one-month post treatment visit, her lesions had completely resolved and she was started on adjuvant hormonal therapy.
Conclusion: The literature on the use of radiation therapy in pyoderma gangrenosum is limited but at minimum, our case provides evidence for the safety of radiation therapy in oncologic cases complicated by pyoderma gangrenosum. PG is not a contraindication to radiation treatment and radiation may potentially assist with wound healing through its effects on the microvasculature and modulation of the inflammatory and immune response.

P1-08-17: Successful Treatment of Early Triple-Negative Breast Cancer with Chemotherapy and Immune Checkpoint Inhibitor in the Setting of Sickle Cell Disease: a Case Report

Stevany Saxon-Filipe, Luise Froessl, Samira Syed

Triple-negative breast cancer (TNBC) accounts for 10-15% of all breast cancers and is known for its aggressive clinical behavior and limited treatment options compared to other breast cancer subtypes1. In recent years, immunotherapy, particularly PD-1/PD-L1 checkpoint inhibitors, has shown promise in treating TNBC when combined with chemotherapy2.
The keynote 522 trial3 demonstrated a significant improvement in pathological complete response with the addition of pembrolizumab to multiagent chemotherapy. Although improved responses have been observed, administering this regimen may pose challenges in patients with significant comorbidities. In rare cases, such as in patients with sickle cell disease, the benefit of standard-of-care treatment must be carefully weighed with the increased risk for severe side effects and complications resulting from cancer therapy.
We report a case of TNBC diagnosed in a patient with sickle cell disease. This case underscores the feasibility of the Keynote 522 regimen in such individuals and emphasizes the importance of coordinated care between breast oncology and benign hematology

teams. Our patient of interest is a 46-year-old African-American woman with a longstanding history of sickle cell disease (SCD) on hydroxyurea, diagnosed with a self-palpated T2N0M0 breast cancer that was ER 0%, PR 0%, Ki-67 40%, and HER2-Neu negative (TNBC). The patient's other comorbidities include hypertension, diabetes mellitus, and deep vein thrombosis treated with long-term anticoagulation.
Given the benefits of chemotherapy and pembrolizumab (the keynote 522 regimen) in TNBC, this protocol was offered to this patient with curative intent. A literature review highlighted the paucity of data on the treatment of breast cancer with immunotherapy and chemotherapy in the setting of sickle cell anemia. Our patient was started on neoadjuvant chemotherapy with paclitaxel and carboplatin for the first cycle, with pembrolizumab added to the regimen from cycle 2 onwards. The patient ceased use of hydroxyurea and was switched to chronic simple blood transfusions of 2 units PRBCs every 4 weeks, with a goal Hgb S of <45% and total Hgb of 7.0 g/dL. The care team also elected to forego the use of G-CSF and limited use of dexamethasone during treatment due to the potential increased risk for sickle cell crisis with these agents. Additionally, an extra 500 mL normal saline was administered with each chemotherapy infusion.
With these measures, our patient has successfully completed 3 cycles of therapy without significant adverse events. She is exhibiting an excellent clinical response to therapy with near resolution of the palpable area of disease on physical exam. Following the completion of 4 cycles of carboplatin, paclitaxel, and pembrolizumab therapy, we plan to obtain a mid-treatment breast MRI to further assess treatment response and proceed to anthracycline-based therapy with pembrolizumab.
This patient's reasonable tolerance to therapy coupled with the robust clinical response provides an optimistic view regarding treatment options for breast cancer in patients with sickle cell disease. While close observation and meticulous care coordination are necessary in patients with hemoglobinopathies undergoing cancer treatment, this case demonstrates a significant benefit from treatment with only expected treatment toxicities observed at this time.
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P1-08-19: Surgical oncoplastic treatment of a rare case of expansive dermatofibrosarcoma protuberans on the breast

Giovanna Azevedo Gabriele Carlos, Ridania Frederice, Patricia Figueiredo, Karla Prigenzi,

Background: Dermatofibrosarcoma protuberans (DFSP) is a rare soft tissue sarcoma, with low-grade and slow-growing pattern, initiating in the dermis and then invading the

subcutaneous tissue, representing less than 0,1% of all malignancies. Its biological behavior is characterized by aggressive local infiltration with high recurrence rate but rarely metastasizes.DFSP normally occurs in middle-aged adults and exhibits a slight male predominance. It corresponds to 6% of all tissue sarcomas and in the breast is extremelly rare. It resembles a keloid or an atrophic lesion like sclero-derma. These features make the early diagnosis difficult. The main treatment is the surgical excision with safe margins up to 3cm extent. Because of this, a wide resection is necessary, usually followed by a oncoplastic technique to minimize aesthect defect.
Method: We describe a case of a female patient with an expansive dermatofibrosarcoma protuberans on the left breast, diagnosed after 5 years of progression, with a challenging surgical treatment.
Results: A 35-year-old female patient was complaining of a reddish lesion in the left breast with gradually progressive growth for 5 years. There was no history of preceding trauma, surgery, ulceration, papillary discharge, fever or weight loss.The physical examination revealed a light-reddish, delineated nodular cutaneous plaque 7 x 6 cm in the junction of inner quadrant of the left breast. No other finding from the breasts or lymphatic chain was noted. She had an unremarkable personal and family history.She had performed a skin biopsy 1 year before in another service with the following report: “spindle cell injury without atypia in the dermis. The lesion compromises the deep edge of the fragment.”Lesion complete resection with evaluation of intraoperative surgical margins and oncoplastic technique surgery was then indicated for diagnostic elucidation.The intraoperative biopsy report showed a dermis infiltrative spindle cell lesion with atypia and was necessary a wide excision followed by a superior pedicle mastopexy without volume reduction as the patient had requested.The final pathology report confirmed the dermatofibrosarcoma protuberans due to a spindle mesenchymal neoplasm located in the superficial and deep dermis of the breast skin, showing focal extension to the hypodermis, composed of elongated cells with eosinophilic cytoplasm and monomorphic nuclei arranged in a storiform pattern, within a collagenized stroma (figures 1 and 2). Immunohistochemical study was positive for CD34 and negative for Factor XIIIA (figures 3 and 4).
Conclusion: In this rare case, the skin location is one of the most challenging sites for reconstruction after de large excision to maintain symmetry.

P1-08-20: Synchronic invasive lobular breast carcinoma, invasive mammary carcinoma NST, and large cell neuroendocrine carcinoma of the lung.

Leonardo Gil-Santana, Daniela de Almeida Carvalho, Vladmir Cláudio Cordeiro de Lima

Introduction: Synchronous bilateral breast cancer represents 10% of all breast cancers. The occurrence of bilaterality is more common with invasive lobular carcinomas (ILCs). The prognosis appears to be worse when compared unilateral tumors. We describe a rare and challenging clinical case of a patient with three synchronous tumors: bilateral breast carcinomas and a large cell neuroendocrine carcinoma of the lung.

Case Report: A sixty-eight-year-old smoker (18 packs-year), post-menopausal, white female was submitted to a screening breast ultrasound that disclosed a single 23 x 18 mm nodule in the upper quadrant of the right breast without involvement of the axillary lymph nodes. A mammotomy confirmed invasive mammary carcinoma no special type (NST), grade I, estrogen receptor (ER) 98%, progesterone receptor (PR) 40%, HER2 0, and a Ki67 12%. We performed a breast magnetic resonance imaging (MRI), which showed, in addition to the previously reported lesion in the right breast, an irregular nodule with spiculated borders and a late wash-out phase located in the anterior third of the central region of the left breast, measuring 56 x 54 mm and a satellite nodule 5 mm from the index lesion, located in the anterior third of the infero-medial quadrant, measuring 14 x 11 mm. A biopsy was performed and showed a second primary invasive mammary carcinoma, grade II, ER 98%, PR 0%, HER2 0 and Ki67 25%. A thoracic CAT scan yielded an irregular pulmonary nodule located between the anterior and posterior segments of the right upper lobe, measuring 49 mm. An 18-FDG-PET-CT scan showed 18-FDG uptake in the mass in the upper lobe of the right lung (SUVmax-21.9), in an enlarged right hilar lymph node (SUVmax-10.3), and the left breast lesion (SUVmax-2.8). A CT-guided biopsy of the pulmonary lesion showed a large cell neuroendocrine carcinoma (LCNEC) of the lung. A brain MRI identified no CNS metastases. This case was discussed in a multidisciplinary tumor board, and due to aggressiveness and poor prognosis, it was decided to operate on the lung nodule and start neoadjuvant hormone therapy with exemestane 25mg/day. The patient was submitted to a right upper lobectomy with mediastinal lymphadenectomy. The anatomopathological report showed a pT2bpN1M0. We kept the patient on exemestane while we started adjuvant chemotherapy with carboplatin and etoposide for four cycles. After three months, images showed no lesions in the lungs and a partial response in the breasts. Patient was submitted a bilateral mastectomy with axillary lymphadenectomy. The lesion in the right breast was staged as ypT1cypN0(sn)M0 (RCBII) and the left lesion as ypT3ypN0M0 (RCB I).
The patient was referred to adjuvant radiotherapy, and abemaciclib was added to exemestane.

P1-08-21: Synchronous female breast cancer and follicular lymphoma in a single patient: A case report

Zijun Zhao, Qingyao Shang, Chenxuan Yang, Jiaxiang Liu, Jiabin Wang, Xiyu Kang, Jiaxian Yue, Xin Wang, Xiang Wang

Rationale: Multiple primary malignant neoplasms (MPMNs) is a rare condition in tumor diagnosis. Recently, medical workers have commenced to pay attention to this.
Clinical features and diagnoses: A 72-year-old female was admitted to our hospital for touching a painless lump in her right-upper breast. She also complained of incidental finding of another painless lump in her left groin shortly after her finding in breast tumor. Ultrasound showed a 1.2* 0.5 cm hypo-echoic nodule in the right-upper breast with an irregular form and unclear margin. For the left inguinal lump, it was scanned by ultrasound as an inguinal lymph node with a size of 2.8 * 1.2cm, with cortical thickening and eccentric

lymphatic hilum. An enhanced magnetic resonance imaging showed bilateral multiple enlarged lymph nodes with uniformed enhancement.
Interventions and outcomes: An ultrasound-based fine-needle biopsy of the breast tumor was firstly conducted and the pathological result did not show any malignancy. Then an excision biopsy of the tumor was conducted and the intraoperative frozen section indicated "breast invasive cancer". Subsequently, a breast-conserving surgery with sentinel lymph node biopsy in the right armpit was performed. The intraoperative frozen biopsy suggested a negative results in the margin tissues but cancerous tissue was detected in one of six sentinel lymph nodes(1/6). Then the axillary lymph node dissection was performed and the final paraffin section results indicated "non-specific invasive breast cancer, tumor size: 1.3* 0.6* 0.5cm, Grade II; axillary lymph node metastasis (1/27); stage: pT1cN1a". After the breast operation, she went to outpatient clinics of bone tumor and received an ultrasound fine-needle biopsy of the enlarged left inguinal lymph node where she referred to previously. Both the result of immunohistochemistry and gene rearrangement test supported a diagnosis of follicular lymphoma (FL, Grade 2). Besides subsequent adjuvant chemotherapy and radiotherapy for her primary breast cancer, the patient regularly followed up her condition of FL.
Lessons: MPMNs always tend to be misdiagnosed as a locoregional or distal metastatic tumor, especially for lymph node found in different parts of the body. It is easy to cause a delayed or erroneous treatment. An in-time and comprehensive clinical examination, medical imaging, and proper approach of pathological biopsy are essential for an effective management of MPMNs.

P1-08-23: Targeting Brain Metastasis: The Success of Trastuzumab-Deruxtecan in HER2+ Cancers: A Case Report

Annelies Feyaerts, Yannick Van Herck, Françoise Derouane, Kristian Jochems, Thomas Decramer, Adinda Baten, Patrick Neven, Hans Wildiers

A 49-year-old female was diagnosed with invasive ductal carcinoma of the right breast in May 2021. Molecular profile of the tumor was estrogen receptor 0/8, progesterone receptor 0/8, and HER2 3+ with positive FISH, along with a TP53 mutation but without a PIK3CA mutation. Further staging with PET-CT scan showed the presence of bone lesions and extensive liver metastases, suggesting a risk of imminent liver failure. The initial treatment protocol comprised weekly paclitaxel combined with trastuzumab and pertuzumab administered every three weeks along with denosumab. There was a major response in the tumor. Paclitaxel was stopped after 18 administrations because of increasing polyneuropathy, while the administration of trastuzumab and pertuzumab continued, with prolonged disease control and excellent quality of life.
In January 2024, after 25 months of confirmed response, the patient presented with symptoms of nausea, vomiting and dizziness. Neurological examination revealed a wide-based gait and was otherwise unremarkable. A CT scan of the brain identified multiple metastases with a significant mass effect, especially within the posterior fossa. This mass

effect led to signs of ascending transtentorial herniation, crowding at the foramen magnum, and the secondary development of hydrocephalus. As initial treatment, high-dose corticosteroids were administered.
The case was discussed at our multidisciplinary tumor board. Given the substantial burden of brain metastases, the prognosis was deemed poor. The neurosurgical team was reluctant to resect the largest brain metastasis, highlighting the potential complications and risks involved. In case of progressive hydrocephalus during the treatment course, a ventriculoperitoneal shunt was proposed as a palliative treatment to relieve intracranial pressure. Whole-brain radiation therapy was considered, however involved a substantial risk of brain herniation and significant post-treatment morbidity due to its wide-ranging impact, including potential adverse effects on healthy brain tissue.
Based on the data from the Destiny Breast-01 and TUXEDO-1 trials, treatment with trastuzumab-deruxtecan monotherapy at the dose of 5.4 mg/kg was initiated. Significant clinical improvement was observed following the first treatment cycle, with sustained improvement thereafter. After three cycles, a significant radiological response was observed, with near-complete regression of the brain metastases and without signs of extra-cranial progression. At the last visit in May 2025, the patient was still in deep remission with excellent quality of life.
This case illustrates that advancements in oncology and the refinement of antibody-drug conjugates are opening a new landscape of therapeutic possibilities, even in critical situations. In some cases, this progress allows the avoidance of high-risk neurosurgical procedures and reduces the morbidity associated with whole-brain radiation therapy. Initial medical approach rather than surgical or radiotherapy approach of life-threatening brain metastases should be added to the oncological armamentarium in HER2-positive breast cancer.

P1-08-24: The diagnosis of Anti-Yo antibody mediated paraneoplastic syndrome, a rare neurodegenerative disorder in a patient with invasive breast cancer, a case report

Ashley Montgomery, Ismail Jatoi, Carissia Calvo-Strube

Abstract: Background: Paraneoplastic cerebellar degeneration (PCD), also known as Anti-Yo antibody syndrome, is a rare neurodegenerative manifestation described in patients with malignant tumors, including breast cancer. In these patients, the diagnosis of breast cancer usually follows the onset of neurologic symptoms and can be made months to years after symptom onset, most commonly through PET scan or CT whole body scans.
Case presentation: We present the case of a 51-year-old female undergoing neurologic work up for suspected multiple sclerosis (MS) after experiencing sudden onset severe neurologic deficits to include diplopia, dysarthria, gait instability, and extremity paresthesias. As part of her evaluation, CT chest was obtained which revealed an incidental left breast mass. Diagnostic mammogram and subsequent core needle biopsy lead to the diagnosis of an ER/PR negative, HER2 positive invasive ductal carcinoma (IDC). During this time her

neurologic symptoms were not responding to MS treatment regimens and further imaging was inconsistent with MS. In the setting of a newly confirmed breast malignancy, she underwent work up for a paraneoplastic syndrome to include PCA1/Anti-Yo antibodies. She was found to have significantly elevated levels of PCA1 (anti-Yo antibodies) consistent with PCD. She underwent neoadjuvant chemotherapy with TCHP, followed by partial mastectomy with sentinel lymph node biopsy, with minimal improvement in her neurologic symptoms. Surgical pathology revealed no evidence of residual IDC, negative margins, and no evidence of axillary metastasis – ypT0N0. To date she has completed adjuvant radiation therapy and remains on adjuvant Herceptin and Perjeta. Unfortunately, the patient's symptoms have persisted despite breast cancer treatment. She has additionally undergone four cycles of plasmapheresis and IVIG treatments, with no significant improvement noted to date.
Conclusions: Anti-Yo antibodies detected at significantly elevated levels causing a paraneoplastic syndrome of the brain is a rare manifestation and a very uncommon complication of breast cancer. The elevation of Anti-Yo antibodies has been observed in only 1.6% of breast cancer cases, with few documented instances of associated neurologic symptoms. This patient is one of the few rare cases to manifest this neoplastic syndrome and develop severe neurologic symptoms. While an uncommon complication of breast cancer, providers should consider investigating PCD in patients presenting with new-onset neurologic degenerative symptoms alongside a recent breast cancer diagnosis. Additionally, it has also been reported that over-expression of HER2 is frequently seen in patients with elevated levels of PCA1/Anti-Yo antibodies, as in our case.

P1-08-25: The differential diagnosis of a large breast mass in a young woman

Hetal Mistry, MacKenzie Adams, Li Juan Wang, Charu Taneja, Stephanie Graff, Mary Anne Fenton

A healthy 28-year-old G5P3A2, nonpregnant woman presented to the emergency room for evaluation of a 7 cm right breast mass. She recently immigrated to the United States from eastern Europe and had no established primary care provider. Patient reported a breast mass increasing in size over two months and an unintentional weight loss of 5 kg. She visited an urgent care center one month before presentation and was prescribed amoxicillin-clavulanate, but her symptoms had not resolved. The patient was not on hormonal contraception and her menses occurred on a regular, monthly basis. She had no known family history of cancer. At her visit, the patient was afebrile, heart rate was 92, and blood pressure was 115/67. Examination revealed a 7 x 8 cm right breast mass at 7 o'clock with tenderness to palpation, and right axillary fullness and discomfort to palpation. No skin changes were identified. There was no cervical or supraclavicular adenopathy. Breast ultrasound showed diffuse masslike region in the right breast from 6 – 9 o'clock, predominantly tubular in nature, with associated abnormal segmental ducts. A mammogram revealed a right inferior breast mass with associated periareolar skin

thickening, in the background of heterogeneously dense breasts. Left breast was radiographically unremarkable. Ultrasound-guided breast biopsy was performed, initially yielding mobile debris, 5 cc of purulent fluid, and 5 cc of bloody fluid. After the initial aspiration, a core biopsy of the underlying persistent mass was taken. Baseline laboratory studies with complete blood count and basal metabolic panel were notable only for WBC at 11.5x109 cells/L. CT chest, abdomen, and pelvis with contrast showed no evidence of metastatic disease. Differential diagnosis of a breast mass in a young woman includes benign proliferative changes, malignancy, hematoma, papilloma, cyst, galactocele, mastitis, fat necrosis, or infection.
Pathology from the right breast biopsy revealed benign breast tissue with acute inflammation, granulation tissue, microabscess formation, and histiocyte aggregates. Gram stain, fungal studies (PAS), and AFB for mycobacterial organisms were negative. A bacterial PCR 16s rDNA was sent on paraffin-embedded tissue to a reference lab and not detected. Ultimately, aerobic culture from broth only (which generally indicates a low bacterial burden or possible contamination), yielded Corynebacterium species. Final pathologic diagnosis was cystic neutrophilic granulomatous mastitis (CNGM), a rare breast inflammatory process usually due to Corynebacterium species infection. CNGM occurs in reproductive-aged women with a history of pregnancy and often mimics breast carcinoma. Radiology noted pathologic concordance with imaging. The patient was treated with trimethoprim/sulfamethoxazole for 10 days and referred to a breast surgeon. However, her symptoms did not improve, and a repeat ultrasound 14 days later showed increased size of the abscess measuring 4 x 1.5 x 4.3 cm. Repeat ultrasound-guided aspiration was performed and the patient was prescribed doxycycline for one month. Though she experienced increased pain midway through treatment, she ultimately had improvement in her symptoms at her follow-up visit 74 days from presentation. Follow-up breast ultrasound performed the same day did not demonstrate evidence of fluid collection or abscess, but again noted an unchanged irregular mass associated with small loculated areas of fluid. This case brings to light the medical and surgical differential diagnosis beyond malignancy for a large breast mass in a young, nonpregnant woman presenting to an oncology clinic.
A table with differential diagnosis, images and references will be provided for the poster if accepted.

P1-08-26: Triple negative breast cancer in pregnant women with mutated BRCA

Leonardo Gil-Santana, Daniela de Almeida Carvalho, Monique Celeste Tavares

Introduction: The triple negative breast cancer (TNBC) is an aggressive tumor that associated a high grade of spread and worse prognosis.
The treatment differs with clinical stage and patients with BRCA mutation could be beneficiated of treatment with platinium chemotherapy based and PARP inhibitors. We described a challenging clinical case of a BRCA mutated young pregnant patient with TNBC.

Case Report: In a December 2021, a 28-year-old female patient, mutated BRCA1, felt a nodule in the right breast. The ultrasound showed a single 2.3cm x 1,8 cm nodule without involvement of the axillary lymph node. A core biopsy showed invasive carcinoma No Special Type (NST), grade II, negatives hormonal receptor, HER2 0 with Ki67 60%. We realized complete staging exams, including central nervous system resonance, which did not reveal distant disease. We did neoadjuvant chemotherapy with Carboplatin and Paclitaxel for 12 weeks because de patient was 17 weeks pregnant. She completed 11 weeks of chemotherapy due to oligohydramnios and we stopped for 3 weeks to monitory pregnancy. Chemotherapy with dose-dense anthracycline and doxorubicin (ddAC) was restarted. After first cycle we paused treatment for childbirth and completed more 3 cycles of ddAC. After 1 month of finalized chemotherapy, she was submitted to mastectomy and linfadenectomy. The pathology revealed an ypT1miypN1a tumor although it was not possible to start adjuvant Olaparib due to healthcare provider issues.
This patient lost follow up and returned in April/2023 with Thorax CT showed multiple bilateral nodules in the lung that was biopsied and confirmed as recurrence of triple negative breast cancer.
The case was discussed on the tumor board and systemic treatment with chemotherapy and immunotherapy was proposed. PDL1 for CPS was 14. We started Carboplatin, Gemcitabine and Pembrolizumab each 21 days. After 4 cicle we switched carboplatin for cisplatin due grade 3 adverse reaction to infusion. After six cycle of treatment PET-scan showed complete response. Patient presented persistent grade 2 of nauseas a despite of clinical management with antiemetics. We stopped cisplatin after cycle 7 and continue Gemcitabine 1000mg/m^2 D1,D8 and Pembrolizumab D1 each 21 days. In this moment, after 12 cycles patient maintain complete response with excellent tolerance to treatment.

P1-08-27: Unusual Presentation Of Triple-Negative Breast Cancer (TNBC) In A Young Female Patient With Xeroderma pigmentosum (XP)

Bashaer Alsaati, Nora Trabulsi, Reem Ujami, Atlal Abusanad

Background: Xeroderma pigmentosum (XP) is a rare, autosomal recessive (1 in a million) disorder characterized by sensitivity to ultraviolet (UV) rays, resulting in a predisposition to skin cancers, primarily. This case vignette describes an unusual presentation of triple-negative breast cancer (TNBC) in a young female patient with XP.
Case Presentation: A 34-year-old woman with a known history of (XP) presented with a palpable mass in her right breast. Clinical examination revealed a firm, irregular, and non-tender mass in the upper outer quadrant of the right breast, measuring approximately 6 cm in diameter. There was no axillary lymphadenopathy. Mammography and ultrasound confirmed a suspicious lesion, and a core needle biopsy was performed. No family history of breast cancer or other malignancies and gBRCA1&2 were negative. Histopathological examination of the biopsy specimen revealed grade III invasive ductal carcinoma, negative for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth

factor receptor 2 (HER2), consistent with TNBC.
Management and Outcome: A neoadjuvant chemotherapy (NACT) with four cycles of doxorubicin and cyclophosphamide (AC) DD was prescribed. The response to NACT was limited. Consequently, the patient underwent surgery with a modified radical mastectomy (MRM) with tumor of 6 cm & sentinel lymph node biopsy was negative for metastasis. Postoperative follow-up included adjuvant CT with paclitaxel plus carboplatin. Immunotherapy was not accessible for her. Adjuvant radiotherapy was not prescribed.
Discussion: 1.Association of XP and BC: The evidence that XP patients may also have an elevated risk of internal malignancies is scarce. Understanding the mechanisms linking XP with breast cancer, such as DNA repair deficiencies and genomic instability, could provide insights into targeted prevention and treatment strategies.
2.Addition of Carboplatin in Adjuvant Chemotherapy: Studies suggest that the inclusion of carboplatin in the NACT for TNBC can improve pathological complete response rates and survival outcomes. Given the limited response to initial chemotherapy in this patient, the addition of adjuvant carboplatin was commenced to enhance treatment efficacy and potentially reduce the risk of recurrence.
3.Consideration of Adjuvant Radiotherapy (XRT) in XP Patients: The use of XRT in XP patients is challenging due to their heightened sensitivity to UV and ionizing radiation. Protective measures and alternative modalities should be explored to minimize potential adverse effects. Our patient was offered MRM in an attempt to avoid XRT.
4. Type of Surgery: MRM vs. Breast-Conserving Surgery (BCS) :
The choice between MRM and BCS in XP patients with TNBC must consider oncologic outcomes and future potential risk of XRT, especially the long -term safety of XRT in such patient is questionable.
Conclusion: This case highlights the complexity of managing TNBC in patients with XP. It underscores the need for personalized treatment plans that address the unique challenges posed by XP. Multidisciplinary collaboration and further research are essential to optimize therapeutic strategies for these high-risk patients.

P1-08-28: Unusual Presentation of Metastatic Breast Cancer Mimicking Myelofibrosis

Emily Sargent, David Ramirez

Patient is a 43-year-old female with GERD, HLD, Endometriosis, and history of gastric sleeve who initially presented to an outside hospital for abdominal pain in 12/2023. Initial workup revealed WBC 7.58, Hgb 10.4, and platelet count of 51. Of note, her CBC was normal in 3/2023. She underwent CT of the abdomen/pelvis, which revealed nonspecific diffuse mesenteric and peritoneal thickening. She was discharged home with supportive care. On 1/9/2024, she experienced unusually heavy menstrual bleeding. Due to worsening symptoms and progressive shortness of breath she went to an outside ED and was found to have a platelet count of 25 and Hgb of 7.3 for which she was transfused one unit of packed red blood cells. Due to worsening bicytopenia, she was referred to and seen by an outside

hematologist who recommended a bone marrow biopsy. On 1/24/24, patient presented to MDA for shortness of breath at rest and exertion. Her vital signs were within normal limits. Physical exam at this time was notable for pale skin. CBC revealed Hgb 7.9 and platelet count of 25. CTPE revealed no signs of PE however it did note diffuse hepatosplenomegaly, upper abdominal lymphadenopathy, and increased heterogenous density of bone structures raising concern for myelofibrosis.
Patient underwent bone marrow biopsy on 1/30/24. Results revealed involvement by a metastatic carcinoma replacing 70-80% of the bone marrow space. The immunophenotypic features were most suggestive of breast primary. The neoplastic cells were negative for E-cadherin favoring lobular carcinoma. Corresponding flow cytometry showed no monoclonal B cell population, aberrant T cell antigen expression, or increase in immunophenotypic myeloblasts. Of note, patient had a routine mammogram in March of 2023 that was negative for malignancy. Further workup included breast ultrasound, Breast MRI, and bilateral breast biopsies. Her ultrasound revealed bilateral vague areas of distortion which could not be measured. She had a probable fibroadenoma in the left breast at the 6 o'clock position and bilateral lymphadenopathy. Bilateral breast biopsies revealed invasive lobular carcinoma, grade 1, ER/PR 91-100%, HER2 - 0, Ki 67 1%.
Patient now has a diagnosis of metastatic invasive lobular carcinoma (metastasis to bone marrow) who was briefly on tamoxifen and LHRH until labs were consistent with menopausal state. She is s/p bilateral salpingo-oophorectomy and doing well on LHRH antagonist, an aromatase inhibitor, and ribociclib. She has been responding to systemic therapy with improvement in thrombocytopenia and anemia.

This report discusses a case where metastatic breast cancer was identified unusually through the presence of bicytopenia. The patient's initial presentation was most concerning for a hematologic malignancy, specifically myelofibrosis given bone abnormalities seen on CT imaging. Her bicytopenia led to further investigation that revealed underlying metastatic breast cancer, highlighting the importance of considering unusual symptoms in diagnosing metastatic cancer.

P1-08-29: Utility of 18F-FES PET/CT over 18F-FDG PET/CT in Initial Diagnosis of over Ninety Metastatic Lesions in a Patient with Metastatic ER+ Breast Cancer: A Case Report

Caroline Breit, Valerie Gorman

Approximately 6% of women with newly diagnosed breast cancer will present with metastatic disease. Proper staging workup and diagnosis of metastatic lesions is crucial prior to surgical treatment. 18F-FDG PET/CT is often included in the staging workup of locally advanced breast cancer. Recently, PET/CT with 18F-Fluoroestradiol (18F-FES PET/CT), a radiolabeled form of estradiol that binds to the estrogen receptor, has been approved as an additional imaging technique for the detection of ER-positive lesions in patients with metastatic breast cancer. While the two have been shown to have comparable

sensitivity for diagnosis of metastatic lesions, there is still much debate regarding when to use 18F-FES PET/CT over 18F-FDG PET/CT imaging. We present the case of a 68-year-old patient diagnosed with ER+/PR+ invasive ductal carcinoma of the left breast. On breast MRI, the patient was found to have a 1.3cm sternal mass as well as subtle enhancement of two right-sided ribs concerning for metastatic lesions. The patient first underwent a 18F-FDG PET/CT which did not reveal any metastatic lesions. Due to persistent concern for metastatic disease, the patient then underwent whole body 18F-FES PET/CT imaging. This demonstrated extensive osseous metastatic disease not visualized on the previous 18F-FDG PET/CT scan. There were over ninety foci of increased uptake, including multiple foci within the sternum (correlating to the lesion seen on MRI), as well as foci within the cervical vertebral bodies, ribs, sacrum and pelvis. The detection of multiple osseous metastatic lesions in our patient on 18F-FES PET/CT imaging demonstrates previous findings of increased specificity of 18F-FES PET/CT for ER+ metastatic lesions compared to 18F-FDG PET/CT imaging. In our patient, the results of her 18F-FES PET/CT resulted in upstaging of her cancer, thus preventing her from undergoing unnecessary surgical intervention. This likely resulted in earlier treatment with systemic chemotherapy as surgical intervention and resulting complications may delay initiation of chemotherapy. This study thus illustrates the potential value of 18F-FES PET/CT imaging in the diagnosis of ER+ metastatic lesions, especially in the setting of equivocal or discordant findings on conventional imaging.

P1-08-30: Vitiligo-like reaction in a patient with metastatic RH+/HER2-negative breast cancer treated with ribociclib: A case report and literature review

Anezka Ferrari, Herbert Amaral, Mariana Pina, Bruna Zucchetti, Ângelo Fêde, Renata Arakelian, Karina Sacardo, Cynthia Lemos, Mariana Gouveia, Manuel Cruz, Manoel Carlos Souza, Tiago Takahashi, Renata dos Anjos, Dalton dos Anjos, Romualdo Barroso

Introduction: Cyclin inhibitors have significantly altered the natural history of metastatic RH+/HER2-negative breast cancer and achieved excellent disease control with progression-free survival of more than two years. Ribociclib, the leading agent in this class, is highly effective with manageable side effects. Here we report the case of a patient who experienced an unusual dermatologic side effect, a vitiligo-like reaction.

Case Report: A 65-year-old female patient, married and mother of two daughters, had a long-standing diagnosis of luminal-type breast cancer. Her first primary tumor was diagnosed in 2000 and treated with conservative surgery, adjuvant chemotherapy, radiotherapy and hormone therapy. This was followed by an ipsilateral local recurrence, which led to a mastectomy on the right side, and a new primary tumor in the contralateral breast, which was also treated with a mastectomy and local recurrence. Since 2018, she has been diagnosed with metastatic disease, which initially affected the bones, lymph nodes and lungs. First-line treatment with letrozole, ribociclib and denosumab was initiated. The patient showed significant radiologic improvement, with PET-CT showing complete radiologic and metabolic response, and remission persisted for approximately two years.

After about two years of treatment, the patient developed hypochromic lesions on the face, chest and limbs that resembled vitiligo. A skin biopsy revealed atrophy and basal melanin hypopigmentation with negative fungal findings, suggesting post-inflammatory hypochromia. This vitiligo-like reaction was attributed to ribociclib, which was discontinued, and treatment was continued with letrozole alone. In addition, the patient received topical corticosteroid treatment.
Approximately eight weeks later, the patient experienced repigmentation of the lesions and complete resolution of the disease. She is currently continuing to take letrozole with no evidence of neoplastic disease on imaging.
Discussion: Ribociclib belongs to the class of CDK4/6 inhibitors and is highly effective and well tolerated. The main side effects reported include neutropenia and hepatobiliary changes. Skin reactions such as rashes, dermatitis and alopecia occur in about 5 to 10 of patients taking ribociclib. However, these are usually mild and easily manageable. Grade 3 skin rashes occur in only about 0.9% of patients.
Only a few cases of vitiligo-like reactions associated with cyclin inhibitors are known in the literature. The largest series is a multicenter, retrospective study involving centers in the US and Europe, with a total of 16 patients reported, underscoring the rarity of this reaction. The exact mechanisms leading to this reaction are not fully understood, but there are hypotheses concerning the immune response to treatment. In addition, some studies suggest that these responses may be associated with better survival.
In this case, several points should be emphasized. The first is the excellent response, with complete radiologic and metabolic response. The second is the long duration to the onset of the reported toxicity, which lasted about two years. The third point is the complete reversibility of the lesions after discontinuation of ribociclib and treatment with a topical corticosteroid.
Conclusion: Vitiligo-like reactions associated with cyclin inhibitors are reactions that require further investigation due to their negative impact on patients' quality of life and their possible association with improved survival outcomes. Future studies are needed to better understand the pathophysiologic mechanisms involved.

P1-09-01: Combination of tumor-infiltrating lymphocytes (TILS) and tumor-stroma ratio (TSR) in breast cancer: utility to guide clinical treatment of breast cancer

Fiorita Mundim, Angela Logullo, Karine Infante, Joaquim Neto, Edson Barbosa, Paulo Maia, Nathalia Sousa, Pedro Mundim, Gil Facina

Introduction: Breast cancer is the most common malignant tumour in women and the leading cause of death among them. The incidence rate of breast cancer among women surpassed that of lung cancer in 2020, making it the most common neoplasm in the world, with the exception of non-melanoma skin cancer. The most common histological type is non-special invasive carcinoma (SOE - formerly known as infiltrating ductal carcinoma). Our study highlights the need for new indicators to guide clinical treatment, as the response

of breast cancer to anti-HER2 target therapy may be affected by the level of TILs in the carcinoma. The research evaluates the Tumor-Stroma Ratio (TSR) and Tumor Infiltrating Lymphocytes (TILs) in order to identify possible prognostic indicators in malignant tumours. Methods: 130 cases of patients with invasive breast carcinoma diagnosed and treated at IBCC - Instituto Brasileiro de Controle do Câncer, São Paulo, SP - Brazil, from 2003 to December 2013 were collected. The patients' clinical and pathological data was collected, including age, tumour block diameter, number of positive lymph nodes and histological grade. The most significant sample (invasive area) was selected from the tumour with the highest stromal proportion, and multiple sections were taken from this site. The assessment of sTILs (Stromal Tumor Infiltrating Lymphocytes) followed the recommendations of the International TILs Working Group (ITWG). SPSS 23.0 software was used for the statistical analysis, and Pearson's $\chi 2$ test and Fisher's exact probability method were performed. Results: Analysis of the results of the clinical pathological characteristics and other study items for the selected samples showed that among the 130 cases of primary breast cancer, from the perspective of the tumor-stroma ratio (TSR), there were 73 cases (56.15%) classified as TSR≥50% (high stroma group) and 57 cases (43.85%) with TSR<50% (low stroma group). As for the study of stromal tumor infiltrating lymphocytes (sTILs), there were 77 cases (59.23%) in the low sTILs group and 53 cases (40.77%) in the high sTILs group. As for patients with metastatic cancer, a total of 38 cases were selected, of which 24 cases (63.15%) had TSR≥50% (high stroma group) and 14 cases (36.85%) with TSR<50% (low stroma group). Kaplan-Meier survival analysis was used to compare the relationship between TSR and TILs in patients with primary breast cancer, with a lower prognostic outlook being found in the low stroma group. The single Cox factor analysis showed that the overall survival rate and disease-free survival rate of patients in the low stroma group were higher than those in the high stroma group. Cox multivariate analysis showed that TSR in tumors can be considered an independent risk factor for patients' overall survival and disease-free survival. Discussion: The study discusses the complex interaction between tumour and non-tumour cells in the stroma, which is mainly composed of cancer-associated fibroblasts (CAFs), vascular endothelial cells, lymphocytes and cells. CAFs, compared to normal fibroblasts, have a more evident proliferation capacity and play an important role in promoting tumor proliferation, invasion and metastasis. The research also discusses the importance of the Tumor-Stroma Ratio (TSR) and Tumor Infiltrating Lymphocytes (TILs) in the prognosis of breast cancer patients. Previous studies have shown that the prognosis of patients in the high stroma group was worse than that of the low stroma group in gastric, colon and triple-negative breast cancers . However, this study showed that the overall survival rate and the disease-free survival rate of patients with primary breast cancer in the low stromal group were significantly higher than those in the high stromal group. Conclusion: Our study shows that the positive correlation of TSR in primary and metastatic lymph node cancer can be used as an independent factor in the prognostic judgement of breast cancer. However, TSR in primary cancer has a much more prognosis-orientated significance. The combined analysis of TSR and TILs becomes very useful as a tool for determining prognosis and guiding the clinical treatment of breast cancer.

P1-09-02: Discordance in breast cancer pathology review and its impact on patient care

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Background: Routine external biopsy review by institutional pathologists is a practice implemented by many cancer centers. However, obtaining prompt and efficient central pathology review in low- and middle-income countries can be challenging. The delay in the review process can potentially hinder timely initiation of treatment, presenting additional challenges for patients.This study assessed the percentage of disagreement between breast biopsy reports from external laboratories and internal pathological reviews conducted at the Brazilian National Cancer Institute (INCA), estimating the impact of such routine on patient care.
Methods: A retrospective analysis of data collected from 500medical charts randomly selected among all breast patients treated at INCA in 2021. Cases with external biopsy samples available for review were eligible. Cohen´s kappa (k) and intraclass correlation coefficient (ICC) with two-way model were used to assess concordance between reports with categorical and numerical variables, respectively.
Results: 305 patients with available external pathology sample were included with a median age of 57.5, with 98.4% women and 61% black/brown. Clinical stages at presentation: I (8,7%), II (30%), III (40%) and IV(9,4%). Agreement of malignancy on reports was 99.6%. Six out of 28 patients had the diagnosis changed from benign to malignant disease.A total of 110 (36%) cases had external immunohistochemistry (IHC) evaluation. The agreement for ER was 96.4% (k=0.897, p-value <0,001) and 90.9% for PR (k = 0.784, p-value < 0,0002). The discordance rate for ER was 4,2% (negative to positive) and 3,4% (positive to negative). The agreement for Ki-67 [ICC = 0,835, IC95% = 0,766-0,885, p-value<0,001] and grading (75.1%, k=0.501, p-value <0,001). 96.4% of agreement was observed for HER2 (k=0.837, p-value < 0,001.09). Discordance rate for HER2 was 3,6% (3 negatives to positive). Systemic treatment changes occurred in 7.6% and 3.6%, due to ER and HER2 discordance, respectively. Median time for pathology review was 21 days (IQR 9,5 - 38 days) and from first medical consultation to treatment was 67 days (IQR 46 - 106 days).
Conclusion: We showed a high level of agreement in the diagnosis of malignancy and and a low disagreement on IHC review of ER and HER2, with a small impact on clinical decision making. As pathology review carries with it an increased cost, medical personnel working time and delay in treatment initiation, institutions should go through their internalprocedures and re-evaluate its necessity.

P1-09-03: An integrated genomic analysis of DCIS heterogeneity to identify signatures of progression and determine the clinical utility of ctDNA.

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It is not possible to distinguish between Ductal Carcinoma In-Situ (DCIS) lesions that are indolent and those that will progress. Furthermore, 1 in 5 patients with a diagnosis of pure DCIS on biopsy have occult invasive carcinoma on surgical excision. Current strategies of risk stratification fail to take account of intra-lesion heterogeneity, and whether DCIS might evolve focally or globally.

The hypothesis of this study is that significant intra-tumour heterogeneity (ITH) exists in DCIS at the spatial morphological, biological, and molecular level which challenges risk-stratification on biopsy alone, and this may be better captured in a 'liquid biopsy'.

A prospective cohort of whole DCIS lesions was assessed for 12 antibody markers previously associated with risk of progression– ER, PR, HER2, αvβ6-integrin, Galectin 7, SMA, CD31, CD34, CD68, Fibronectin, p53, and Ki67. Immunohistochemical (IHC) analysis was performed on 273 regions across 14 cases and copy number alterations (CNA) were assessed using ichorCNA on 93 regions, 76 of which could be mapped to matched IHC.

All cases showed intra-lesional heterogeneity for most markers. By-region analysis showed clustering of epithelial and myoepithelial αvβ6-integrin, SMA, Fibronectin, epithelial and myoepithelial Galectin 7, p53, Necrosis, Grade, HER2, and Ki67. There was no correlation between markers and occult invasive carcinoma. Common CNAs included amplifications in 1q22 (73/93 78.5 %) and 17q23.3 (53/93 57.0 %) and loss in 16q23.3 (45/93 48.4 %), with ERRB2 amplification correlating with HER2 scoring, and 16q23.3 deletion correlating with low-grade ER+ regions as previously described. All cases showed heterogeneity in CNAs within lesions indicating multiple subclonal changes. One case showed heterogeneous subclonal CNAs within and between DCIS and multifocal invasive carcinoma, suggesting multifocal evolution of DCIS prior to progression.

Cell free DNA (cfDNA) concentrations from matched blood samples (n=28) showed significant differences between benign controls (fibroadenomas, n = 4) and both pure DCIS (n=16, p=0.02), and DCIS with invasion (n=8, p=0.03). There was no significant difference between DCIS and DCIS with invasion (p=0.49). CNAs were detected in 2/24 (8.3%) of DCIS cases, both of which were DCIS with invasion, although these showed low tumour fraction (0.048 and 0.066).

This study confirms the extent of ITH in pure DCIS and DCIS with invasion in cases with traditionally "low-risk" morphomolecular features, precluding accurate risk assessment on single representative block or biopsy-based analysis. Further work is needed to establish

the value of liquid biopsy, specifically with regard to low tumour fraction in early stage disease.

P1-09-04: A Phase II study of the efficacy and safety of Perflubutane for Predicting highly Tumor Infiltrating Lymphocytes in early breast cancer

Yuri Kimura

Background: Tumor-infiltrating lymphocytes (TILs), a major component of the tumor immune microenvironment, reflect the host's antitumor immune response and serve as critical biomarkers for predicting prognosis and therapeutic efficacy in breast cancer. Recently, the International Immuno-Oncology Biomarker Working Group proposed a standardized pathological evaluation method for assessing TILs, which has shown good reproducibility and concordance rates. However, due to the inherent heterogeneity of TILs within tissues and the limited sample size of pre-treatment needle biopsies in neoadjuvant chemotherapy cases, there remain challenges in establishing objectivity for clinical implementation.
B-mode ultrasonography (US) allows morphological evaluation of the underlying pathology. We discovered that lymphocyte-predominant breast cancer (LPBC), characterized by abundant TILs, presents with distinctive US findings (small lobulated shape, marked hypoechoic areas, and posterior acoustic enhancement). Based on these findings, we developed a unique scoring model (TILs-US score) to predict LPBC. A multicenter retrospective study (JABTS BC-11) demonstrated that the TILs-US score significantly predicted LPBC, and the prediction accuracy was further enhanced by including tumor vascularity assessment (Area Under the Curve 0.784 vs. 0.770; sensitivity 0.93 vs. 0.83; specificity 0.57 vs. 0.55; accuracy 0.71 vs. 0.66). These results prompted a prospective evaluation of the utility of contrast-enhanced ultrasonography (CEUS), which visualizes detailed intratumoral blood flow and allows real-time qualitative and quantitative assessment of blood flow changes, to improve the accuracy of LPBC prediction in a single-arm phase II study (AppTIL study, jRCT1061220081).
Methods: We performed CEUS using perflubutane on patients with early-stage breast cancer and used a new scoring model (TILs-CEUS score) incorporating the rate of contrast agent inflow to predict LPBC. The predictive accuracy of this model was compared with pathological LPBC evaluation of surgical specimens. The target sample size is 100 patients, and prospective enrollment is ongoing.
Conclusion: This study aims to demonstrate the utility of CEUS as a simple method for pre-treatment evaluation of LPBC, potentially enabling its application in predicting prognosis and therapeutic efficacy of neoadjuvant chemotherapy. We intend to report the results, which may contribute to the establishment of a new radiomics-based therapeutic strategy combining tumor immune microenvironment assessment and imaging diagnostics.

P1-09-05: Simultaneous 3D Mapping of RNA and Protein to Advance Personalized Medicine in Breast Cancer

Yue Li, Shigeaki Kanatani, Per Uhlén

Background: Personalized medicine in breast cancer requires a detailed understanding of the tumor and its microenvironment at the molecular level. Current diagnostic strategies, primarily based on 2D imaging methods, are limited by the lack of spatial molecular 3D mapping. Our study aims to bridge this gap by developing a 3D imaging methodology for simultaneously profiling RNA and protein within breast cancer tissues, providing a holistic view of tumor biology.

Methods: We developed a novel 3D imaging method to achieve high-resolution 3D mapping of RNA and protein expressions in breast cancer tissue samples. This method integrates 3D in situ hybridization chain reaction, 3D immunostaining, and a non-toxic tissue clearing technique. We applied this method to intact triple-negative breast cancer (TNBC) samples, performing multiplex labeling for ErbB-2 (mRNA), HER2 (protein), and CD34 for co-mapping tumor cells with the vascular network. Machine-learning-based topological analysis was utilized to characterize spatial blood vessel organization across samples. Various visualization techniques, including scatter plots, histograms and cumulative plots, were employed to investigate the interactions between tumor cells and their nearest vessels.

Results: Our approach successfully generated 3D maps of RNA and protein distribution within breast cancer sample blocks. Topological analysis of the vascular network identified metrics such as vessel length density (VLD), vessel volume density (VVD), connection density (CD), mean angle (MA), mean diameter (MD), and straightness. The results indicated that VLD and VVD positively correlated with prognosis, while CD and MA showed a tendency towards negative correlation. Notably, a subset of cells with ErbB2-mediate expression and HER2-negative expression (ErbB2++/HER2-) was prevalent in many TNBC cases. By comparing these findings with simulated random cell distribution experiments, we observed that the attraction distance between ErbB2++/HER2- cells and blood vessels in TNBC with lymph node metastasis was 58.77μm, significantly smaller than the 401.20μm observed in TNBC without lymph node metastasis. This implies a closer association of ErbB2++/HER2- cells with blood vessels in metastatic TNBC. The analysis revealed a statistically significant increase in the proportion of perivascular ErbB2++/HER2- cells in TNBC samples associated with lymph node metastasis compared to those without lymph node involvement ($P<0.05$).

Conclusion: The simultaneous 3D mapping of RNA and protein provides unprecedented insights into the molecular architecture of breast cancer. Our findings underscore the critical importance of spatial context in molecular profiling. This newly developed method offers a powerful tool for identifying spatially therapeutic targets, redefining our understanding of tumor biology, and guiding clinical decisions.

P1-09-06: Evaluation of proliferation biomarker serum thymidine kinase activity and prediction of early relapse in HR positive HER2 negative high risk early breast cancer: Analysis from the PENELOPE-B trial

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Background: Nearly 30% of HR+ early breast cancer (eBC) patients (pts) treated with neoadjuvant chemotherapy (NACT) and surgery will experience BC recurrence, many with incurable distant metastatic disease. There is currently no blood-based biomarker that can identify pts with residual disease and at high risk of recurrence before and during adjuvant therapy. Thymidine kinase (TK) is an enzyme that plays a key role in DNA replication during cell division. The expression of TK is strongly linked to the cell cycle and measurement of TK activity (TKa) is a validated biomarker for both disease prognosis and therapy efficacy in metastatic breast cancer (mBC). We investigated the prognostic utility of TKa in eBC using patient serum samples from the PENELOPE-B study (NCT01864746).
Methods: The PENELOPE-B phase III trial explored the addition of one year of palbociclib (P) to endocrine therapy (ET), in HR+, HER2- eBC pts at high risk of relapse after NACT. Serum samples were collected at baseline (BL), 6 months after starting therapy (C7), and end of treatment (EOT ~ 13 months after starting therapy). Samples were analyzed retrospectively for TKa with the FDA cleared DiviTum® TKa assay (Biovica) using DuA (DiviTum unit of Activity) as the measuring unit. The cutoff for defining high vs low TKa was 250 DuA. In previous mBC studies, a TKa threshold value above 250 DuA was significantly associated with likelihood of disease progression. This same threshold value was applied to the PENELOPE-B sample set. TKa association with early invasive disease-free survival (iDFS) and distant disease-free survival (dDFS) was analyzed using restricted mean survival time (RMST) at a pre-specified timepoint of 1 year. The same method was also used to analyze the association of the clinical-pathologic stage- estrogen/grade (CPS-EG) score with early iDFS and dDFS using a cut-off score of <3 or >3.
Results: 1251 pts enrolled in PENELOPE-B. BL TKa levels were analyzed in a sample set of 871 pts, 444 P+ET, 427 ET alone. Median follow-up time was 84 months. Among these 871 pts, 311 pts had an iDFS event (P + ET, 156; ET alone, 155) with 45 of these pts recurring in the first year. BL TKa ranged between 22 and 1025 DuA, with a mean value of 102 DuA, and a median of 81 DuA. BL TKa levels >250 DuA were significantly associated with both invasive and distant disease recurrence within the first 12 months of adjuvant therapy (RMST estimates for BL TKa>250 DuA vs BL TKa≤250 DuA: iDFS=9.6 vs 11.6 months, p=0.01, dDFS=9.9 vs 11.7 months, p= 0.02). In early relapse pts who had a serum sample taken at the time of study discontinuation, 18/22 (82%) had an increase in TKa at the time of relapse as compared to BL. The CPS-EG score when dichotomized as <3 or =4-5 was not prognostic for early relapse within a year (RMST estimates for CPS-EG >3 vs CPS-EG≤3, iDFS=11.3 vs 11.6 months, p=0.12, dDFS=11.3 vs 11.7 months, p= 0.08). TKa dynamic

changes were also analyzed in 360 pts who had both C7 and EOT samples available for testing. Updated analysis looking at increases/decreases in TKa levels during therapy and association with recurrence, as well as treatment interaction will be presented.
Conclusions: In a subset of pts from PENELOPE-B, TKa was detectable in all pts at BL and levels were significantly correlated with early disease recurrence where BL TKa values >250 DuA were prognostic for early relapse within the 1st year of adjuvant therapy. CPS-EG scores in contrast were not prognostic. Pts with early disease relapse also had an increase in TKa levels at the time of recurrence.
These findings warrant further investigation of serum TKa as a non-invasive dynamic biomarker that could be used to assess in "real-time" the presence of actively proliferating disease in adjuvant BC pts and to monitor response to treatment via serial serum TKa testing.

P1-09-07: Retrospective study evaluating the characteristics of patients (pts) with early-stage estrogen receptor-positive (ER+) HER2- breast cancer (EBC) who relapsed on or after adjuvant (adj) abemaciclib (abema)

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Background: Adding 2 yrs of adj abema to endocrine therapy (ET) has recently become standard for pts with ER+HER2–, node-positive EBC at high risk of recurrence. Characteristics of pts relapsing on adj abema have not been well characterized.
Methods: Pts who received adj abema at a single institution and had recurred were identified. ER, progesterone receptor (PR) and HER2 expression pre- and post-abema were determined, and the duration of treatment (DoT) for adj ET, abema and the first treatment (tx) in the metastatic setting recorded. If NGS was performed at recurrence, genomic alterations related to ET and CDK4/6i resistance were described.
Results: As of 04/2024, 163 pts received adj abema, starting between 2018-2024, with 15 (9.2%) experiencing recurrence. Among recurrent pts, median age was 48 yrs (31–86). All recurrences were distant, with the most common sites being liver (n = 6, 40.0%), bone (n = 5, 33.3%), lung (n = 2, 6.7%). 2 pts (13.3%) had germline (g)BRCA2 mutations (mts), 1 pt (6.7%) had a gCHEK2 mt.
At diagnosis, stage distribution was: stage I, 2/15 (13.3%); stage II, 5/15 (33.3%); stage III, 8/15 (53.3%); nodal status was: cN0, 4/15 (26.7%); cN1, 9/15 (60.0%); cN2/N3, 2/15 (13.3%). ER IHC staining was ≥50% in 14 pts (93.3%) and >10% in all pts; PR was ≥50% in 6 pts (40.0%), >10% in 8 (53.3%), 1-9% in 5 (33.3%), and <1% in 2 (13.3%). Pts had HER2-low EBC in 7 cases (46.7%), HER2-0 in 7 (46.7%), HER2- (IHC unknown) in 1 (6.7%).
4 pts (26.7%) had upfront surgery, 11 (73.3%) neo-adj tx (chemotherapy [CT], 8/11 [72.7%]; ET, 3/11, [27.3%]). 9 pts (60.0%) had ductal histology, 1 (6.7%) and 5 pts (33.3%) had lobular and mixed, respectively. Pts had well, moderately, and poorly differentiated EBC in 2 (13.3%), 6 (40.0%), and 7 cases (46.7%), respectively. Among pts who received neo-adj

CT, pathologic response was available in 6 cases: RCB-II, 1/6 (16.7%), RCB-III, 5/6 (83.3%). 11 pts (73.3%) had ≥4 metastatic lymph nodes. OncotypeDX was requested in 8 pts (53.3%), with RS 11-25 in 3 cases (37.5%) and 26-100 in 5 (62.5%). After surgery, 12 pts (80.0%) received adj ET, 3 (20.0%) CT+ET, no one olaparib. ET was as follows: aromatase inhibitor, 11/15 (73.3%), AI+OFS, 3/15 (20.0%), fulvestrant, 1/15 (6.7%).
ER, PR and HER2 could be assessed pre- and post-abema in 12 (80.0%) pts. In this subgroup, 6 pts (50.0%) with strongly positive ER status (ER ≥ 95% in 5/6 cases, ER ≥ 20% in 1/6), had ER ≤ 10% and PR <1% at first recurrence. Of 7 HER2-0 cases, 4 (57.1%) remained HER2-0, and 3 (42.9%) became HER2-low; of 5 HER2-low cases, 3 (60.0%) remained HER2-low, and 2 (40.0%) became HER2-0.
7/15 pts (46.7%) recurred while on abema, 8/15 (53.3%) after stopping/completing abema. Median DoT was 8.0 mos (Q1-3: 3.8-21.2) for abema and 18.5 mos (Q1-3: 7.0-23.0) for ET. Median DoT for the first tx in metastatic setting was 3.0 mos (Q1-3: 1.6-5.0). At data cutoff, 2 pts were still on first-line tx. First-line tx received included clinical trials, 4/15 (26.7%; 3/4, ADC combination trials; 1/4, PARPi+immunotherapy); CT, 5/15 (33.3%); ET+CDK4/6i, 3/15 (20.0%); olaparib, 2/15 (13.3%); ET+capivasertib, 1/15 (6.7%).
Among pts with available NGS (n = 10) at recurrence, nearly all of them had genomic alterations in the P53 pathway (n = 9, 90%): n = 5 TP53 oncogenic mts, n = 1 TP53 homozygous deletions, and n = 3 MDM2 high amplifications (amp) (copy number >20). One ESR1 mt and no RB1 mt was detected. Other alterations include PI3K pathway (n = 3; n = 2 PIK3CA mt, n = 1 PTEN mt), RTK alterations (n = 4; n = 2 FGFR1 amp, n = 1 ERBB2 mt, n = 1 FGFR2 amp), and CCND1 amp (n = 3).
Conclusions: In this series of 15 pts relapsing on adj abema plus ET for high-risk ER+ EBC, loss of ER expression occurred in 50.0% of evaluable cases. Median DoT for the first tx line (metastatic) was 3 mos. Post-abema genomic alterations include P53 in 90% of cases.

P1-09-08: A new modified technique "Modus Spirdonov 1" in fine-needle aspiration biopsy

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Introduction: The most fundamental problem in the assessment of benign and malignant neoplasms is the exact preoperative diagnosis. Fine-needle aspiration biopsy (FNAB) is one of the most used techniques for obtaining cytological material for further evaluation. Insufficient biopsy material is reported as a major drawback, which often requires repeated interventions (25.3% published in the literature for the standard technique). The authors have developed a modified technique “Modus Spirdonov 1” in order to obtain a larger amount of cytological material and verify the results with quantitative analysis in an experimental model.
Material and methods: For the purpose of the experiment porcine liver and longissimus dorsi muscle were selected as target tissues sampled by standard method with direct grip (n = 60), by using CAMECO Syringe Pistol (n = 60) and by applying the technique “Modus Spirdonov 1” (n = 60). The measurements were performed by an authorized specialist in

the only specialized and certified laboratory for standards of the Bulgarian Institute of Metrology. For comparison with the standard methods, in FNAB "Modus Spirdonov 1" after achieving a vacuum, rotational movements are performed along the longitudinal axis in two opposite directions without displacing the needle from the target center. This maneuver provides more efficient tissue cutting with the edge of the needle. A comparative analysis of the weight of the sample obtained by the three methods was performed.
Results: The analysis shows that there is a statistically significant difference when comparing the results from the different techniques. The modified method “Modus Spirdonov 1” significantly exceeds the other two techniques ($p<0,001$) according to the quantity of the obtained material. According to the results of our study in experienced hands we achieved a sensitivity of the method of 98.5%, a false negative rate of 1% and a false positive rate of 0.5% in 1100 biopsies performed in clinical practice with 0% repeated biopsies. In addition, the gathered material is visually significantly richer, including ductal epithelial complexes, abundance of stromal cells, calcifications, detritis and a giant cell, macrophages filling the entire field of view microscopically.
Conclusion: The authors believe that the described modified technique is a viable alternative to the currently used FNAB and could increase the diagnostic value of the preoperative biopsy in a number of diseases.

P1-09-09: Disparities in Second Breast Cancer Events and Survival Persist Despite Optimal Treatment in Hormone-Receptor Positive Breast Cancer: Analysis of the Real-World ASCO CancerLinQ Dataset

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Background: Reports of racial and ethnic disparities in locoregional breast cancer (BC) events are mixed, with disparities often seen amongst hormone-receptor positive, HER2-negative (HR+HER2-) breast cancer. We examined rates of locoregional second breast cancer events (SBCE) and overall survival (OS) by race and ethnicity in the ASCO CancerLinQ real-world dataset amongst patients (pts) receiving optimal treatment.

Methods: ASCO CancerLINQ was used to identify pts that underwent surgery for invasive HR+HER2- stage I-III BC from 2005-2022. We defined SBCE as a second event in the ipsilateral or contralateral breast or axilla after prior treatment for BC. Optimal treatment was defined to include adjuvant endocrine therapy, radiation after lumpectomy in age <70, and regional nodal irradiation for N2-N3 disease. Patient characteristics and rates of SBCE were examined by self-reported race and ethnicity. Kaplan-Meier analysis was used to estimate SBCE-free survival and OS in optimally and not-optimally treated pts.

Results: Among 40,917 HR+HER2- BC pts, 27,803 (68.0%) were Non-Hispanic White (NHW), 4,193 (10.3%) Non-Hispanic Black (NHB), 1,767 (4.3%) Hispanic, 1,112 (2.7%) Asian or Pacific Islander (API), and 6,042 (14.8%) other or unknown race and ethnicity.

NHW pts were more likely to present with clinical stage I disease (60.8% vs 50.5%, 50.1%, and 55.4% for NHB, Hispanic, and API pts respectively, p<0.01), and be older at diagnosis (16.6% of NHW vs 21.5%, 33.0%, and 31.9% of NHB, Hispanic, and API pts were age <50, p<0.01).

Overall, 66.7% of NHW, 70.3% of NHB, 73.3% of Hispanic, and 76.0% of API pts received optimal treatment for all eligible definitions (p<0.01). 5-yr estimated SBCE-free survival in optimally treated pts was 98.4%, 98.3%, 98.8%, and 98.0% for NHW, NHB, Hispanic, and API patients (p<0.01). In pts without optimal treatment, this decreased by 0.5%, 0.1%, 0.9%, and 1.0%, respectively. 5-year OS in optimally treated pts was 94.5%, 93.4%, 97.2%, and 97.2% in NHW, NHB, Hispanic, and API patients (p<0.01). In pts without optimal treatment, this decreased by 3.1%, 4.1%, 2.1%, and 0.1%, respectively.

Conclusions: Differences in SBCE and OS were seen by race and ethnicity in HR+HER2- BC, including in pts receiving optimal treatment. This adds granular real-word evidence to support racial and ethnic disparities are pronounced in HR+HER2- BC despite similar treatment.

P1-09-11: Machine Learning based analysis in chemotherapy resistant triple negative breast cancer

Dongling Wu, Sean Hacking, Yihong Wang

Background: Chemotherapy resistances were believed to be caused by tumor cell DNA alterations; However, recent years' tumor micro-environment (TME) study has increasingly revealed the complementary interactions between tumor cells and peri, intratumoral stromal cells and inflammatory cells. The role of TME in acquired chemoresistance has yet been fully understood.With the development of bioinformatics, the analysis of TME has expanded to a more "micro" molecular level: through ssGSEA analysis, the gene expression differences between normal tissue and peri, intratumoral tissue were able to be calculated. MDAcc group has developed a scoring algorithm to analyze the gene signature of tumor, stromal and immune cell component. High score indicates a high volume of stromal and immune cells intermixing with tumor tissue. Goal: In this study, we aim to use machine learning image analysis and bioinformatics method to analyze stromal and immune components in chemotherapy resistant TNBC.Method: A cohort of TNBC that received chemotherapy is selected from TCGA. QuPath is used for analysis of tumor, mature stroma, immature stroma, and immune cells, a percentage for each component is given by machine. Mature stroma is defined as dense fibrosis, vascular tissue and adipose tissue. Immature stroma is defined as loose "fresh" collagen bundles and myxoid "tissue culture" like desmoplastic stroma. MDAcc scoring R package is used to analyze stroma and immune gene, a score is given to each component. A t-test is used to compare the medium value of each component and score. A K-M analysis is used to compare the survival rate. A Pearson correlation (r) analysis is done to compare imaging analysis results and molecular results.

Result: A total of 137 patients were selected for imaging analysis. High tumor stromal ratio (TSR= tumor percentage/stromal percentage), high tumor percentage are associated with low pT stage (TSR: p=0.008; tumor%: p=0.02). Higher mature stroma percentage is associated with low pT stage (p=0.026), high rate of complete response (p= 0.02) and high disease-free survival (p=0.006). Higher immature stroma percentage is associated with higher tumor stage (p=0.006), low rate of pCR (p= 0.03) and low disease-free survival (p=0.005). Higher immune cell stromal percentage is associated with pT1 disease (p=0.009), high rate of pCR (p= 0.008) and high disease-free survival (p=0.005). Similarly, gene sequencing analysis also shows a high immune score is associate with pT1 disease (p=0.007). For stromal score, high stromal score is associated with high rate of pCR (p=0.04) and although not statistically significant, it also shows a trend of positive association with low stage (pT1) and longer disease-free survival. Correlation analysis shows a high correlation (r=0.8) between immune stromal percentage and immune score results, and a moderate correlation (r= 0.6) between mature stromal percentage results and stromal score.Discussion: Our study explored the complexity of drug resistance mechanism beyond tumor cells and the potential effects of mature and immature stromal and immune cells on chemotherapy outcomes. Our study also showed the potential use of AI in predicating tumor response to therapy.

P1-09-12: Comparative Analysis of the Oncotype DX Breast Recurrence Score® assay for Neoadjuvant Letrozole/Abemaciclib versus Chemotherapy in Stage II-III, Ki67≥20%, HR+/HER2- Breast Cancer: Insights from the GEICAM/CARABELA Trial

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Background: The CARABELA trial (NCT04293393) compared 12 months of neoadjuvant letrozole/abemaciclib (let/abema) versus 6 months of neoadjuvant chemotherapy (CT) in stage II-III, Ki67≥20%, HR+/HER2- breast cancer. Although let/abema did not achieve comparable RCB 0-I rates as CT (13% vs. 18%), an analysis of the Oncotype DX Breast Recurrence Score® (RS) assay revealed higher residual cancer burden (RCB) 0-I rates with CT compared to let/abema in patients with RS result >25 (26% vs. 18%). Conversely, patients with RS≤25 showed similar rates with each treatment (6% vs. 8%). The present study investigates the interaction between treatment, RS result and cell cycle suppression, along with post-treatment (surgery) RS, estrogen and progesterone receptors (ER/PgR) mRNA expression.
Methods: We examined the association of baseline (BL) RS results with rates of complete cell cycle arrest (CCCA, Ki67<2.7%) at 2 weeks (2w) and at surgery. Ki67 was centrally

determined using a standardized assay (Ki67 IHC MIB-1 pharmDx, Dako Omnis, Agilent Technologies). Additionally, ER and PgR mRNA levels were obtained from the surgery Oncotype DX® report (Exact Sciences). Median time from last study dose to surgery was 8 days for let/abema.
Results: Out of 200 patients included in the CARABELA trial, 192 (96%) patients were included in this biomarker study; 184 (92%) had BL RS results and 142 (71%) had at-surgery-RS results. Four (4%) out of 95 patients in the let/abema arm and ten (10%) out of 97 in the CT arm, achieved RCB=0, and these cases were assigned RS=0 and Ki67<2.7%. Rates of 2w-CCCA were higher with let/abema compared to CT (68% vs. 6%, p<0.0001), but not at surgery (53% vs. 45%, p=0.16). In the let/abema arm, the rates of CCCA were numerically higher at 2w and surgery for RS≤25 compared to RS>25 (83% vs. 71%, p=0.34 at 2w and 75% vs. 55%, p=0.11 at surgery). In the CT arm, the rates of CCCA were comparable for RS≤25 and RS>25 both at 2w, where only 7 patients achieved CCCA (6% with RS≤25 and 14% with RS>25, p=0.37), and at surgery (57% vs. 50%, p=0.70). In the let/abema arm, 55% of patients with RS≤25 achieved CCCA at 2w and maintained it at surgery, compared to 33% of patients with RS>25 (p=0.14). This rebound in proliferation did not correlate with the time from the last dose of abemaciclib to surgery (r=0.01, p=0.95).
Surgery-RS result was higher in the let/abema arm than in the CT arm (median RS result of 22 vs. 19, p=0.02) and was inversely correlated with surgery ER levels (r = -0.39, p <0.0001) and surgery PgR levels (r = -0.6, p <0.0001). Let/abema treatment was more effective in suppressing ER and PgR mRNA levels from BL to surgery than CT (ER median change: let/abema -1.0 score units vs CT -0.3, p=0.0002; PgR median change with let/abema: -2.1 vs -0.3 with CT, p<0.0001). For patients with surgery non-CCCA (excluding those achieving RCB:0), Ki67 expression was higher in the let/abema arm, with a median of 26%, compared to 16% in the CT arm, and only 7% had positive surgery-PgR mRNA expression (according to score units) in the let/abema arm, compared to 62.5% in the CT arm.
Conclusions: Neoadjuvant let/abema and CT varied in efficacy by Recurrence Score®. Let/abema achieved higher CCCA rates in RS≤25 patients, accompanied by significant ER and PgR mRNA suppression, highlighting its efficacy in less biologically aggressive subtypes and suggesting it might replace the need for CT. Surgery-RS result was higher in the let/abema arm than in CT and correlated inversely with surgery ER and PgR levels, indicating deeper ER signaling suppression. Additionally, residual disease in the let/abema arm exhibited higher levels of proliferation than in the CT arm, along with greater suppression of ER signaling, suggesting distinct residual disease biology.

P1-09-13: An Adjusted Breast Cancer Index Model to Identify Women with Hormone Receptor–Positive (HR+) Breast Cancer at Minimal Risk of 10-year Distant Recurrence (DR)

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Background: Five years of adjuvant endocrine therapy (ET) is the standard of care for treating early-stage hormone receptor–positive (HR+) breast cancer (BC) to reduce the risk of disease recurrence. However, many patients discontinue ET early due to poor tolerability and toxicity. Validated biomarkers are critically important to optimize the selection of patients for adjuvant and extended (post–5 year) ET.
The Breast Cancer Index (BCI) is a gene expression–based signature recommended in clinical practice guidelines to aid patient selection for ET. The BCI prognostic score reports an individualized risk of overall (0–10 years) and late (post–5 years) distant recurrence (DR). BCI was previously shown to identify patients with favorable BC-specific survival in the Stockholm trial. In the current study, untreated patients from this trial were used to identify an adjusted BCI cut-point for defining a group with minimal risk of DR, which was subsequently validated in a large cohort of patients from the Netherlands Cancer Registry (NCR), who did not receive any adjuvant endocrine therapy.

Methods: 283 patients from the untreated arm of the Stockholm trial were used for BCI cut-point selection to define a minimal risk group with a 10-year risk of DR of ≤5%. Performance of the adjusted BCI model was initially assessed in the tamoxifen-treated arm of the Stockholm trial (n=317) and then evaluated in a cohort from NCR (n=1247). Women with HR+ N0 breast cancer who were ≥70 years old and did not receive any adjuvant ET were identified from the NCR database and used for BCI validation. FFPE blocks of primary tumor specimens were collected from participating hospitals across the Netherlands and sent to the Leiden University Medical Center for central processing. Sections were shipped to Biotheranostics CLIA-certified and CAP-accredited laboratory for testing blinded to the clinical data. Kaplan-Meier analysis and Cox proportional hazards regression were used to analyze the Stockholm cohorts with DR as the study endpoint. Cumulative incidence analysis and Fine-Gray model with death as a competing risk event, were used to analyze the NCR patients.

Results: The adjusted BCI model stratified patients into four different risk groups: minimal, low, intermediate and high risk. In the Stockholm cohort, the minimal risk group (20%) showed a 10-year risk of DR of 2.3% and 4.3% in the untreated (n=283) and treated arm (n=317), respectively, whereas the 10-year risk of DR was 15.5% and 5% for the low risk (40%), 19.8% and 11.7% for the intermediate risk (23%), and 35.9% and 21.1% for the high risk (17%) groups, respectively. The minimal risk and low risk groups in the tamoxifen-treated arm demonstrated very similar risk profiles. When assessed in the NCR cohort (n = 1247, 55% T1, 42% T2, 22% grade 1, 61% grade2, 94% HER2-, 99.8% no chemotherapy) who did not receive any adjuvant endocrine therapy, the adjusted BCI model was significantly prognostic (p = 0.003) with subdistribution hazard ratios (sHR) for low, intermediate, and high-risk vs. minimal risk of 1.67 (95% CI: 0.81-3.45), 2.39 (95% CI: 1.14-5.01) and 3.23 (95% CI: 1.55-6.74), respectively. The minimal (16%), low (41%), intermediate (24%) and high-risk (19%) groups showed a 10-year risk of DR of 4.5%, 7.5%, 10.3%, 13.5%, respectively, with death as a competing risk event.

Conclusions: The current study demonstrates that an adjusted BCI minimal risk cut-point may identify postmenopausal women with HR+ N0 breast cancer, who are at sufficiently low risk of DR and thus unlikely to derive clinically meaningful benefit from adjuvant ET. These results support BCI as a biomarker to help guide the selection of HR+ breast cancer patients who could be spared from or consider shorter duration of adjuvant ET to avoid potentially serious side effects from these therapies.

P1-09-14: Palbociclib and endocrine therapy diminishes adaptive anti-tumor immunity

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Background: We examined the effect of palbociclib and endocrine therapy (ET) on transcriptional programs and chromatin accessibility of immune, stromal and tumor cells from breast cancer patients.
Methods: We employed single-nuclei RNA & ATAC sequencing using the 10x multi-omic platform on pre- (baseline) and post- (surgery) treatment frozen tumor samples (minimum 25% tumor cellularity) from patients enrolled in the NeoRHEA trial (N=37; NCT03065621). This was a phase II, single arm study evaluating 4 months of neoadjuvant palbociclib and ET in women with ER+/HER2- early breast cancer. We defined immune cells as CD45+ or CD20+, stromal cells as FAP+ or PECAM+ and tumor cells as ESR1+ and copy number (computed via infercnv) aberrant. We defined T, B and myeloid cells as CD3+, CD20+ and CD68+, respectively. T cells were further divided into CD4+ and CD8+ with the latter examined also as CD8+CD103+ (tissue-resident memory T-cells) and CD8+/CD103- subpopulations. The percent (%) change in subpopulations post-treatment were considered significant at ≥20% change. Differential gene expression and peak accessibility analyses were generated between baseline and surgery, followed by Gene Set Enrichment Analyses (GSEA) using hallmark gene sets. Q values were calculated by adjusting the p-values for the 50 gene sets.
The single-nuclei data was validated using multiplex immunohistochemistry (mIHC) to stain for CK (tumor cells), CD45 (immune cells), Ki67 (proliferating cells) and DAPI (nuclei) on formalin-fixed paraffin embedded tumor tissue followed by imaging on a Vectra Polaris™. Differences in the % of Ki67 positive tumor and immune cells between baseline and surgery samples were evaluated using a two-sided Wilcoxon test.
Responding patients were defined as those with either Ki67≤2.7% by chromogenic immunohistochemistry at surgery (complete cell cycle arrest, CCCA) or a complete/partial response on the post-treatment ultrasound.
Results: Among the 37 patients, 18 had an ultrasound response and 23 patients had CCCA. High quality single nuclei were analyzed at baseline (66201) and surgery (159896).
A 31% decrease in tissue-resident memory cells (CD8+CD103+ among CD8+) was observed

post-treatment. This was detected only in patients with either CCCA or ultrasound response (63% and 66% decrease, respectively). CD8+CD103+ cells were shown to be critical players in anti-tumor immune responses (doi.org/10.1016/j.ccell.2023.01.004).
Analysis of all 37 patients revealed a decrease in E2F target and G2M checkpoint (proliferation related) gene sets post-treatment in immune (Normalized Enrichment Score, NES -1.59, p≤0.01, q≤0.01 and -1.34, p=0.02, q≤0.02, respectively) and tumor (NES -1.77, p≤0.01, q≤0.01 and -1.32, p=0.03, q≤0.13, respectively) but not stromal cells (NES -1.06, p=0.28, q=0.31 and -0.90, p=0.73, q=0.54, respectively). This decrease in E2F target and proliferation related genes in immune cells was principally driven by T cells (NES -1.8, p≤0.01, q≤0.01 and -1.51, p≤0.01, q=0.02, respectively), suggesting that palbociclib and ET decreases T-cell proliferation. GSEA based on genes residing in the differentially accessible peaks revealed similar results suggesting that the observed gene expression changes are associated with altered chromatin accessibility. Decreases in E2F target and G2M checkpoint gene set expression in tumor, immune and T-cells post-treatment were observed only in responding patients with either CCCA or ultrasound responses.
mIHC detected a significant decrease in Ki67+ tumor (median, range from 7%, 1-53% to 1, 0-31, p<0.01) and Ki67+ immune cells (from 4, 0-19 to 2, 0-25, p<0.01) post-treatment, validating these findings.
Conclusion: Treatment with palbociclib and endocrine therapy diminishes adaptive anti-tumor immunity by decreasing T-cell proliferation and the presence of tissue-resident memory T-cells.

P1-09-16: A Phase I Study of ASTX727 plus Talazoparib in Patients with Triple Negative or Hormone Resistant/HER2-negative Metastatic Breast Cancer and non-mutated BRCA

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Background: Poly (ADP-ribose) inhibitors (PARPi) are effective in patients (pts) with germline BRCA 1/2 and PALB2 mutations but have been largely ineffective as monotherpy in others. PARP interacts with, and is recruited to, DNA damage sites along with epigenetic factors, such as DNA methyltransferase 1 (DNMT 1). In addition to increasing PARP-trapping, inhibitors of DNMT modulate ROS-cAMO-PKA signaling and induce a pathogen mimicry, inflammasome signaling response and a 'BRCAness phenotype' that further sensitizes cells to PARPi. In preclinical in vitro and in vivo studies. combined DNMTi + PARPi therapy was effective in both triple negative (TNBC) and hormone resistant (HRBC) models with intact BRCA.
Methods: We conducted a phase I study combining the oral DNMT1 ASTX727 with the PARPi talazoparib in pts with previously treated TNBC or HRBC; pts with dleterious mutations of BRCA or PALB2 were excluded. Pts. with TNBC had received at least one prior chemotherapy and pts with HRBC had received proir endocrine therapy with a cyclin-

dependent kinase inhibitor for metastatic disease. An ECOG PS 0-1 and adequate organ function was required. A classical 3+3 design guided dose escalation/de-escalation with dose limiting toxicity (DLT) defined as Grade 4 neutropenia or thrombocytopenia lasting > 7 days, or clinically significant grade > 3 non-hematologic toxicity in cycle 1; 28 days constituted each cycle. Serial peripheral blood mononuclear cells (PBMCs) were analyzed for changes in methylation using the Infinium MethylationEPIC BeadChip and LINE1 sequencing.
Results: 34 evaluable pts were enrolled and treated in 8 dose cohorts (Table). Median age was 59 years, 12% identified as Black. Myelosuppresson was common with grade > 3 neutropenia in 42% and grade 3 anemia and thrombocytopenia each in 13%. DLT was limited to neutropenia.
Efficacy was assessed in 29 pts. There were no objective responses, 6 pts had stable disease persisting for > 4 months in 3 pts. LINE1 demethylation ranged from 2-12% and immune-specific CpGs (methylation in immune cells) changed by 1-5% by differential methylation locus analysis at Day 15.Methylation changes were not dose dependent.
Conclusions: ASTX727 plus talazoparib produces significant myelosuppression but is otherwise well tolerated. Attenuated dosing identified dose level 1LDseq or 1LDcon for phase II trials. Methylation changes in PBMCs were detected and some heavilty pre-treated pts had prolonged stable disease.

P1-09-17: Management of Neutropenia and Effectiveness of Sacituzumab Govitecan (SG) in Patients (pts) With Metastatic Triple-Negative Breast Cancer (mTNBC) Treated in Real-World Settings in the United States

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Background: SG is a Trop-2-directed antibody-drug conjugate approved for pts with previously treated mTNBC and HR+/HER2— metastatic breast cancer. In previously treated mTNBC, SG demonstrated a statistically significant and clinically meaningful benefit in PFS and OS vs single-agent chemotherapy and a manageable safety profile (phase 3 ASCENT study, NCT02574455). The most commonly reported toxicities, neutropenia and diarrhea, were managed with established guidance. This study describes the incidence and management of neutropenia as well as the real-world effectiveness of SG in pts with mTNBC.
Methods: The US-based, de-identified Flatiron Health electronic medical record–derived database was used. Pts with mTNBC (as defined by ASCO/CAP guidelines) treated with SG in the second line (2L) and later from Apr 2020 to Jun 2023 were included. Data cutoff was Dec 31, 2023. Pt characteristics, SG treatment (tx) duration, incidence of neutropenia, and concomitant granulocyte colony-stimulating factor (G-CSF) use during SG tx were described. Neutropenic events occurring during SG tx were described using ICD10/9 diagnosis codes (D70, 288) and/or laboratory data (absolute neutrophil count <1500mm3). Primary prophylaxis was defined as any G-CSF administration on or after SG tx start (index) date and

before the first neutropenic event. Secondary prophylaxis was defined as G-CSF administration after neutropenia resolution. For therapeutic use, G-CSF was administered upon or after onset of neutropenia and before resolution or end of tx. Real-world OS (rwOS) and time to next tx or death (TTNTD; used as a proxy for PFS) were assessed using the Kaplan-Meier method.
Results: For the 381 pts (99% female) with mTNBC included in the analysis, median (interquartile range [IQR]) age was 61 (52-69) yrs; 18% (n = 70) were Black; 17% (n = 66) had an ECOG performance status ≥ 2; 25% (n = 97) had de novo metastatic disease; 78% (n = 298) were treated in community centers only. Pts received a median (IQR) of 2 (1-3) prior lines of tx in the metastatic setting. Overall, 31% (n = 118) received SG in 2L and 69% (n = 263) in 3L and later. Pts received a median (IQR) of 12 (5-21) SG doses; the maximum number of doses administered during the study period was 74. Median (IQR) SG tx duration was 4.0 (1.9-7.6) mos. Neutropenia (grade ≥2) was reported in 57% (n = 219) pts; grade ≥3 in 27% (n = 101). During SG tx, 59% (n = 225) pts received G-CSF; 31% (n = 117) received it as prophylaxis only (primary prophylaxis: 20% [n = 77]; secondary prophylaxis: 9% [n = 36]; both: 1% [n = 4]). Grade ≥3 neutropenia occurred in 10% (12/117) of pts after any G-CSF prophylaxis and in 4% (3/77) of pts receiving primary prophylaxis only. Median (IQR) time from start of SG tx to first onset of grade ≥3 neutropenia subsequent to G-CSF use was 48 (36-322) days among those who received primary prophylaxis only. Therapeutic-only G-CSF use was observed in 6% (n = 24) of pts with a median (IQR) time to grade ≥3 neutropenia onset of 9 (8-21) days; 21% (n=79) of pts received both prophylactic and therapeutic G-CSF during SG tx. Among 41% (n = 156) of pts who did not receive G-CSF during SG tx, 13% (n = 21) experienced grade ≥3 neutropenia with a median (IQR) time to grade ≥3 neutropenia onset of 8 (8-22) days.
At a median (IQR) follow-up of 8.7 (4.5-14.6) mos, median (95% CI) rwOS and TTNTD were 11.3 (9.9-13.0) mos and 5.6 (5.0-6.4) mos, respectively.
Conclusions: In this real-world analysis of pts with previously treated mTNBC, SG demonstrated effectiveness and a manageable safety profile, consistent with findings from the ASCENT study and other real-world studies. A low incidence of neutropenia was observed among pts receiving G-CSF prophylaxis, suggesting that SG-related neutropenia can be effectively prevented with G-CSF prophylaxis.

P1-09-18: Activation of HERV-R is a biomarker of poor prognosis among rural Nigerian women with triple-negative breast cancer.

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Human endogenous retroviruses (HERVs) comprise genetic material from germ cells that were infected with ancient retroviruses during Pan troglodytes' evolutionary divergence. These "non-coding" repetitive elements now make up approximately 8% of the human genome. Once inserted in the genome, HERVs increase copy number via retrotransposition

(a 'copy-and-paste' mechanism) and are involved key functions including transcriptional control and cellular fusion during placenta formation. Aberrant re-expression of HERV-associated genes initially silenced by host epigenetic mechanisms, including DNA methylation, has been linked to many diseases including cancer. Furthermore, in addition to regulating gene expression, including of neighboring genes, reversal of silencing of HERVs may also impact diverse biological functions through the LTRs, and gag, pol, and env proteins. Although previous studies, specifically in white populations, have documented the expression of HERV-K in breast cancer, there is a paucity of data on the expression and role of HERV-R in triple-negative breast cancer (TNBC) among rural West African women from Nigeria. Breast cancer remains the most frequently diagnosed cancer among women globally, with the highest mortality rates across sub-Saharan African nations compared with other countries. Advanced-stage disease at presentation, as well as limited access to treatment options, typically contribute to the poor overall survival rate of breast cancer in African countries including Nigeria. However, the incidence and mortality rates of TNBC in women with West African ancestry are alarmingly high, with an overall survival rate of three years in Zaria, Nigeria. To better understand the molecular basis underlying these high incidence and mortality rates, as well as identify prognostic biomarkers we assessed expression patterns of HERV-R and TNBC-associated genes from Formalin-Fixed Paraffin-Embedded (FFPE) samples of breast cancer, focusing on TNBC in Nigerian women from rural West Africa. To examine gene expression in this study, we used immunohistochemistry, immunoblot, and immunofluorescence, with results showing strong expression of the HERV-R env protein in all but one TNBC patient, as well as in other basal breast cancer subtypes. Our results also strongly suggest that HERV-R expression is associated with overexpression of oncogenes, including TMPRSS2, BRAF, CCND1, BAZ1B, KTM2D, and FOXA1. We found that patients with HERV-R expression have a poor prognosis with low survival. These findings imply that activation of HERV-R may play a role in regulating the expression of genes implicated in TNBC and may serve as a prognostic biomarker in breast cancer.

P1-09-19: IRENE study: Phase 2 study of oncolytic virus pelareorep and PD-1 inhibitor retifanlimab in metastatic triple negative breast cancer

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Background: Treatment with checkpoint inhibitors has clinical benefit in PD-L1 positive metastatic triple negative breast cancer (mTNBC). However only 40-50% of mTNBC tumors have PD-L1 expression. Pelareorep, a proprietary isolate of the unmodified replication competent reovirus type 3 Dearing (T3D) strain, a non-enveloped reovirus has been shown to upregulate PD-L1 expression in tumor and inflammatory cells and promote a favorable CD8:regulatory T-cell ratio indicating a less immunosuppressive tumor microenvironment. We hypothesized that the treatment with pelareorep will improve the response rates in patients with metastatic triple negative breast cancer by priming the tumor

microenvironment for enhanced tumor response to PD-1 inhibitor retifanlimab.
Methods: This was a phase II multi-site, single-arm clinical trial to study the combination of the oncolytic virus pelareorep and PD-1 inhibitor retifanlimab in patients with mTNBC. Patients with PD-L1 positive and PD-L1 negative tumors were eligible for the study. Eligible patients received 1-2 prior lines of therapy in the metastatic setting. Patients who received prior immunotherapy were also eligible for the study. Eligible patients received pelareorep 4.5x1010 TCID50 / day intravenous (IV), on Days 1, 2, 15 and 16 and retifanlimab 500 mg IV on day 3 of every 28-day cycle. (ClinicalTrials.gov Identifier: NCT04445844)
Primary endpoint was objective response rate (ORR) and safety, as determined by the number, frequency, duration, and severity of AEs using CTCAE v5.0. The study was designed using Simon's optimal 2-stage design. In the first stage, 14 patients would be accrued. If there were 1 or fewer responses in these 14 patients, the study would be terminated. Otherwise, 11 additional patients would be accrued for a total of 25 patients. The null hypothesis would be rejected if 4 or more responses are observed in 25 patients.
Results: Fifteen patients were enrolled in the study (100% women). Median age was 58.6yrs. (66% White, 20% African American, 13% Asian). One patient withdrew from study due to grade 3 fatigue after C1D15. This patient was not included in the efficacy assessment.
Of the fourteen evaluable patients in stage 1 of the study, one patient had partial response (ORR of 7.1%). Duration of response in this patient was 7 months. The average number of cycles for all patients was 2.18 cycles. Five patients received prior treatment with check-point inhibitors - pembrolizumab or atezolizumab. According to the protocol-specified analysis at the end of the stage 1, the efficacy objective (of atleast 2 objective response) was not met. Hence, the study was terminated at the end of stage 1.
The combination was well tolerated. One patient had Grade 3 depressed level of consciousness, which was not related to pelareorep or retifanlimab. One patient with Grade 5 event, which was attributed to disease progression.
Conclusion: The combination of pelareorep and retifanlimab in patients with mTNBC did not show significant anti-tumor activity. One patient had a partial response. Tumor tissue, stool and blood samples collected during treatment will be assessed to understand the role of PD-L1 expression and gut microbiome, especially in the patient with response.

P1-09-20: Enhancer of zeste homologue 2 (EZH2) inhibition in Triple Negative Breast Cancer (TNBC) attenuates tumor growth in vitro and in vivo altering the tumor immune microenvironment.

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Background: EZH2 component of the polycomb repressive complex 2 (PRC2) is a histone methyltransferase whose function is to methylate lysine 27 of histone 3. EZH2 is overexpressed in 49% of breast cancers and is associated with worse outcomes especially

in TNBC. EZH2 is overrepresented in African American and Hispanic women, suggesting that clinical targeting of this protein may particularly help improve the disproportionately poor outcomes in these populations. In addition to its role in metastasis, EZH2 regulates tumor immune microenvironment (TIME) by inhibiting T cell activation via suppression of MHC-1 antigen presentation pathway, upregulating PD-L1 expression causing suppression of an antitumor immune response. Current EZH2 inhibitors only targets the catalytic activity of EZH2, leaving its function as a gene activator unaffected. Despite the advancement in the discovery of inhibitors for EZH2 that attenuate its catalytic activity, resistance to these small molecules limits their use in solid tumors. The neurotransmitter dopamine via its D1 receptor activation in TNBC cell lines induces apoptosis and autophagy, as well as inhibits the invasion and regress in mammary tumors in vivo. In addition, dopamine D1 receptor signaling has been reported to attenuate the immunosuppressive effects of myeloid-derived suppressor cells (MDSCs) on T cell proliferation and IFN-g production. We hypothesize that combined treatment of dopamine D1 receptor agonist (A77636) and EZH2 inhibitors (GSK126) inhibits tumor growth and metastasis of TNBC cells both in vitro and in vivo.

Methods: To test the efficacy of the combination inhibiting metastasis we employed an in vivo model system to confirm the results we had obtained from our invitro 3D culture system and 3D organ-on-chip-based microphysiological (MPS) platform (SynTumor). 4-6 weeks old female NSG mice were injected with MDA-MB-231 cells on the breast fat pads. When the tumors became palpable, they were randomized into four groups, Vehicle, GSK126 (2mg/kg BW), A77637 (50mg/kg BW) and the combination. The drugs individually or in combination were administered intraperitoneally five days for 4 weeks. Tumor measurements were also done during the time and at the end of 4 weeks the animals were euthanized. The tissues, tumor, bone, blood, and spleen were harvested and processed for flow cytometer analysis. Tumor weight and volume were also calculated.

Results: The combination of GSK126 and A77636 demonstrated a synergistic effect inhibiting the tumor weight and tumor volume when compared to the individual treatments or the vehicle treated animals. These results matched with our in-vitro data, where the combination synergistically inhibited the growth of spheroids. in the microfluidic SynTumor model, the combination reduced circulating tumor cell numbers by half. The combination significantly decreased the monocyte population in the blood and tumor. Also, the EZH2 expression in the monocytes and neutrophils were significantly decreased by the combination.

Conclusion: Our data indicate that the combinatorial effect of DRD1 agonist and EZH2 inhibitor efficiently attenuates the EZH2-mediated tumor growth and innate immune environment in TNBC (This work is supported by DOD: W81XWH2010065, for Eswar Shankar). Dedicated in memory of Dr. Bhuvaneswari Ramaswamy.

P1-09-21: Metabolic Reprogramming in Breast Cancer Brain Metastasis Mouse Model: Insights from Transcriptomic Analysis

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Breast cancer (BC) is the second leading cause of central nervous system (CNS) metastases, which include leptomeningeal metastasis (5%) and parenchymal brain metastasis (95%). Among BC subtypes, triple-negative breast cancer (TNBC) accounts for around 15-20% of all cases. This subtype is one of the most aggressive, with a higher risk of developing breast cancer brain metastases (BCBM). Brain metastases (BM) occur in approximately 10-30% of patients with advanced metastatic BC and are associated with poor prognosis. Recent studies suggest that cancer cells undergo metabolic reprogramming upon establishing in the brain, but the specific metabolic shifts remain unclear. This study aims to elucidate the metabolic signatures of BCBM xenograft cell lines by examining the expression of key genes involved in various metabolic pathways, with the ultimate goal of identifying novel therapeutic targets to hinder the progression of brain metastases.
To achieve this, we developed a mouse model of brain metastasis by injecting human breast cancer cells (MDA-MB-231) intracranially into immunodeficient mice. These injections were repeated three successive times, allowing us to harvest xenografted BCBM cells that progressively adapted to the brain environment with each injection. Cancer cell lines represent an effective way of modelling the disease. The BCBM xenograft cells, named BR1/BR2/BR3 to refer to the number of passages in the brain, were molecularly characterized using FACS and Short Tandem Repeat (STR) analysis to ensure the purity of the cell lines. We conducted transcriptomic analysis based on RNA purification and quantification to explore the modifications between our various brain xenograft cell lines. Our in vivo models demonstrated increased tumour aggressiveness, as indicated by a significant decrease in mouse survival rates correlated with the number of cycles of intracranial injections (Log-rank test, $p < 0.001$). These findings suggest genotypic modifications in our BCBM xenograft cell lines. Based on Bulk RNA-sequencing database, the Principal Component Analysis (PCA) plot revealed similarities between the BR2 and BR3 cell lines, which differ markedly from the Parental and BR1 cell lines. Moreover, the heatmap of Euclidean distances between the samples, based on normalised DESeq2 gene expression, supports this observation by showing similar gene expression profiles between the BR2 and BR3 lines. By selecting the human Reactome gene sets (C2 collection - GSEA database) to generate a more detailed heatmap, we identified the metabolic pathways differentiating the Parental and BR1 cell lines from the BR2 and BR3 cell lines. These key pathways include the integration of energy metabolism, lipid metabolism, and nucleotide metabolism. The comparison of the overall gene expression in the Parental cells with the BR1, BR2 or BR3 cell lines by volcano plot confirms several up- and down-regulated genes, including those involved in lipid metabolism (significant results for an adjusted P-value < 0.05 and for a Log2(Fold Change) threshold of 1). Finally, RT-qPCR revealed significant upregulation of gene sets involved in the beta-oxidation pathway (Two-way ANOVA with Tukey's Post Hoc test, $p < 0.05$), indicating a shift towards fatty acid metabolism during

tumour progression in the brain.
These findings suggest that metabolic adaptation is a critical feature of BCBM progression. To validate our results from the transcriptomic analysis, further proteomic and metabolomic analyses are required. These additional studies could pave the way for novel therapeutic strategies targeting specific metabolic pathways, potentially improving treatment outcomes for patients with BCBM.

P1-09-22: Mechanistic Insight into DAXX-Modulated Sensitivity to Chemotherapy in Triple Negative Breast Cancer

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Background: Mechanisms of response and/or resistance to chemotherapy, immunotherapy, or PARP inhibitors in triple negative breast cancer (TNBC) are not well understood. Death Domain-Associated Protein 6 (DAXX) is a multifunctional protein that promotes cell death, represses gene transcription, and maintains a heterochromatin state. We showed previously that DAXX is a potent inhibitor of breast cancer stem cells and resistance to endocrine therapy in estrogen receptor positive (ER+) breast cancer (Peiffer et al., Cancer Res. 2018). In this study, we explored the role of DAXX in TNBC and sensitivity to chemotherapy and olaparib in vitro and in vivo. We also investigated mechanisms by which DAXX modulated growth and response to chemotherapy in TNBC cell lines using RNA sequencing.
Methods: Proliferation and cell cycle analysis were performed for three TNBC cell lines (MDA-MB-231, BT549, and MDA-MB-468) that expressed DAXX or were depleted for DAXX using siRNA. Sensitivity to carboplatin, paclitaxel, and doxorubicin was determined by measuring proliferation. PARP-1 activity was assessed by detecting global protein PARylation levels using Western blotting. Olaparib was used to assess the role of PARP-1 activation in DAXX-depleted TNBC cell lines. Xenografts studies in vivo measured tumor growth of MDA-MB-231 DAXX-expressing or DAXX-depleted tumord treated with vehicle, paclitaxel, olaparib, or the combination. Overall survival of mice was assessed using Kaplan-Meier and a Mantel-Cox Log Rank test. RNA-sequencing was performed on RNA from two TNBC cell lines (MDA-MB-231 and MDa-MB-468) expressing DAXX or depleted for DAXX. The KMplotter tool interrogated recurrence free survival of patients with high DAXX versus low DAXX RNA expressing TNBC.
Results: Depletion of DAXX increased cell proliferation of three TNBC cell lines by promoting cell cycle progression through the S-phase compared to DAXX-expressing cell lines. DAXX-depleted TNBC cell lines were 2-5 fold more sensitive to carboplatin and paclitaxel, but not to doxorubicin as measured by IC50 calculations. Depletion of DAXX increased PARP-1 activity and modestly increased sensitivity to olaparib. In vivo, DAXX-depleted TNBC tumors grew at a faster rate than DAXX-expressing tumors. Although DAXX expression did not change the anti-tumor efficacy of paclitaxel or olaparib in vivo, overall survival of mice was 100% up to 40 days when TNBC tumors had low DAXX compared to only 25% of mice surviving with TNBC expressing high DAXX and treated with paclitaxel.

Mechanistically, RNA-sequencing followed by Gene Ontology pathway analysis showed that DAXX-depleted TNBC cells have higher expression of genes that regulate cell cycle progression and lower expression of genes that regulate the unfolded protein response. The KMPlotter tool revealed that patients (N=370) with TNBC have better recurrence free survival (hazard ratio = 1.79, P=0.0032) if their tumors have lower DAXX RNA levels. Conclusions: These results suggest that DAXX is a critical growth regulator and potential predictor of response to carboplatin or paclitaxel in TNBC. The mechanism by which DAXX modulates sensitivity to chemotherapy and drug resistance could be through regulation of cell cycle genes. Future studies will focus on elucidating the mechanism by which DAXX regulates these enriched genes and pathways and if DAXX is a potential predictive biomarker for recurrence free survival.

P1-09-23: Breast cancer-derived miR-4732-3p promotes breast cancer brain metastasis and brain-metastatic tumor microenvironment

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Breast cancer is the most frequently diagnosed cancer among women, constituting 15.2% of all new cancers diagnosed in the United States. Distant breast cancer metastasis accounts for the majority of breast cancer-related deaths; with brain metastases being the third most common site for metastatic breast cancer and the deadliest condition. Mechanisms that drive breast cancer brain metastasis (BCBM) are still not well understood. There has been evidence emphasizing the role of dysregulated microRNAs (miRNAs) in cancer progression and metastasis. Here, we aimed to identify miRNAs that may play important roles in BCBM. To this end, we conducted miRNA-sequencing analysis of extracellular vesicles isolated from the serum samples of 6 BCBM patients and 8 Stage I/II/III breast cancer patients, and identified 49 circulating miRNAs that were upregulated in BCBM patients compared to Stage I/II/III breast cancer. Upon further analysis using publicly available Gene Expression Omnibus (GEO) breast cancer patient datasets, we narrowed the list down to 12 miRNAs that were significantly enriched in brain metastases compared to matched primary breast tumors (GSE37407) and significantly increased in the serum of BCBM patients compared to breast cancer patients without metastases (GSE134108). Of the 12 identified miRNAs, the gene activation signature of four miRNAs (miR-374b-5p, miR-4732-3p, miR-181a-5p and let-7e-pre) significantly correlates with a Breast to Brain Gene signature. Furthermore, three of these miRNAs (miR-4732-3p, miR-374b-5p and miR-181a-5p) were correlated with shortened metastasis-free survival (MFS) and brain MFS of breast cancer patients. To further determine the involvement of miR-4732-3p, miR-374b-5p and miR-181a-5p in BCBM, we analyzed a breast cancer cell line panel for their expression levels. The results showed that only miR-4732-3p is upregulated in the human brain-tropic TNBC cell lines (MDA-MB-231-BRM and CN34-BRM) along with mouse brain-tropic TNBC line (EO771-BRM), compared to their parental lines. Importantly, miR-4732-3p overexpression increased net migration and proliferation in MDA-MB-231 and CN34 cells. Conversely, inhibition of miR-4732-3p led to decreased net migration and proliferation of MDA-MB-

231-BRM and CN34-BRM cells. Furthermore, overexpression of miR-4732-3p in CN34 cells led to a significant increase in mesenchymal biomarkers: fibronectin and vimentin, along with a decrease in epithelial biomarker E-cadherin. To gain an insight into the impact of tumor-derived extracellular vesicle miR-4732-3p on brain-metastatic tumor microenvironment, we determined whether it activates astrocytes, the most abundant brain cells that can promote BCBM when activated. Our results showed that miR-4732-3p mimic strongly activated astrocytes, as shown by immunofluorescence staining for glial fibrillary acid protein (GFAP; a biomarker for activated astrocytes). Furthermore, we isolated extracellular vesicles secreted from CN34 and MDA-MB-231 cells overexpressing miR-4732-3p to stimulate the astrocytes and observed a significant increase in GFAP expression. These results collectively demonstrate that breast cancer-derived miR-4732-3p plays a novel important role in BCBM by promoting migration, proliferation, and maintenance of a mesenchymal state of BCBM cells, along with activating astrocytes in the brain-metastatic tumor microenvironment.

P1-09-24: Cancer cell-derived exosomal miR-20a-5p inhibits CD8+ T-cell function and confers anti-programmed cell death 1 therapy resistance in triple-negative breast cancer

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Abstract Background: Triple-negative breast cancer (TNBC) remains a formidable challenge in oncology due to its aggressive nature and limited therapeutic options. Our previous investigations have elucidated the role of miR-20a-5p in promoting TNBC cell proliferation, invasion, and migration while inhibiting apoptosis through the RUNX3/Bim/p21 pathway. We have also demonstrated a positive correlation between tissue-expressed miR-20a-5p and circulating miR-20a-5p (cirmiR-20a-5p) in TNBC patients, with elevated cirmiR-20a-5p levels associated with poorer prognosis, suggesting its potential as a prognostic biomarker. While immune checkpoint inhibitors show promise for TNBC treatment, resistance mechanisms remain poorly understood. This study builds upon these findings to investigate the role of cancer cell-derived exosomal microRNA-20a-5p (miR-20a-5p) in modulating CD8+ T-cell function and conferring resistance to anti-programmed cell death 1 (PD-1) therapy in TNBC.Methods: We employed a comprehensive approach combining in vitro cellular assays, in vivo mouse models, and clinical sample analyses. Circulating miR-20a-5p levels were quantified using qRT-PCR in plasma samples from TNBC patients, patients with relapsed TNBC, and healthy donors. Exosomes were isolated from TNBC cell lines and characterized using nanoparticle tracking analysis and transmission electron microscopy. The functional impact of exosomal miR-20a-5p on CD8+ T cells was assessed through cytokine production assays and cytotoxicity tests. Mechanistic studies utilized luciferase reporter assays and Western blotting to confirm direct targeting of NPAT and RUNX3 by miR-20a-5p. A humanized mouse model was employed to evaluate the impact of miR-20a-5p overexpression on anti-PD-1 therapy resistance. Immunohistochemistry was performed

on TNBC tissue samples to assess the correlation between cirmiR-20a-5p levels and CD8+ T-cell infiltration.

Results: Circulating miR-20a-5p levels were significantly elevated in TNBC patients compared to healthy donors (3.2-fold increase, $p<0.001$) and further increased in patients with relapsed TNBC (1.8-fold higher than primary TNBC, $p<0.01$). High cirmiR-20a-5p levels correlated with poor prognosis (HR=2.3, 95% CI: 1.8-2.9, $p<0.001$). TNBC cell-derived exosomes contained high levels of miR-20a-5p, suppressing CD8+ T-cell function by reducing cytokine production (IFNγ, TNF-α, granzyme B, and perforin) and tumor cell killing capacity. Mechanistically, miR-20a-5p directly targeted the 3'-UTR of nuclear protein ataxia-telangiectasia (NPAT) and RUNX3, decreasing their expression in CD8+ T cells and TNBC cells, respectively. In the humanized mouse model, tumors overexpressing miR-20a-5p exhibited resistance to anti-PD-1 therapy, with reduced CD8+ T-cell infiltration and MHC class I expression. Plasma exosomal miR-20a-5p levels were 2.5-fold higher in TNBC patients resistant to anti-PD-1 therapy compared to responders ($p<0.001$). Immunohistochemistry revealed a significant negative correlation between cirmiR-20a-5p levels and CD8+ T-cell infiltration in TNBC tissues ($r = -0.68$, $p<0.001$).

Conclusions: This study identifies exosomal miR-20a-5p as a novel mediator of CD8+ T-cell dysfunction and anti-PD-1 therapy resistance in TNBC. The miR-20a-5p/NPAT and miR-20a-5p/RUNX3 axes represent previously unrecognized mechanisms by which TNBC cells suppress anti-tumor immunity and promote tumor progression. Our findings suggest that circulating miR-20a-5p may serve as a potential biomarker for predicting immunotherapy response and prognosis in TNBC patients. Furthermore, targeting these miR-20a-5p-mediated pathways could be a promising strategy to enhance immunotherapy efficacy in TNBC, potentially improving outcomes for this aggressive breast cancer subtype. These results provide a strong rationale for further investigation of miR-20a-5p as a therapeutic target and prognostic marker in TNBC, opening new avenues for personalized medicine approaches in this challenging malignancy.

P1-09-26: Genomic analysis of circulating tumor DNA (ctDNA) from patients with triple-negative, HER2-mutant metastatic breast cancer treated with neratinib as monotherapy or in combination with trastuzumab in the SUMMIT trial

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Background: In HER2-mutant metastatic triple-negative breast cancer (mTNBC) cohorts of the SUMMIT basket trial (NCT01953926), patients treated with neratinib monotherapy (N; original cohort, n=10) appeared to achieve similar response rates but shorter time to

progression than those treated with N + trastuzumab (N+T; subsequent cohort, n=17). N: overall response rate (ORR) 40.0%; median duration of response (DOR) 3.8 months; clinical benefit rate (CBR; CR/mCR, PR/mPR or SD/mSD ≥ 24 weeks) 40.0%; median progression-free survival (PFS) 2.9 months. N+T: confirmed ORR 35.3%; median DOR 6.1 months; CBR 47.1%; median PFS 6.2 months [Jhaveri et al. J Clin Oncol 2024]. These findings were consistent with those reported for SUMMIT hormone receptor-positive (HR+) HER2-mutant mBC cohorts, in which patients treated with N or N + fulvestrant (N+F) experienced promising clinical responses but shorter DOR. In those cohorts, progression in a subset of patients coincided with emergence of additional HER2 mutations and/or amplification of the mutant allele [Smyth et al. Cancer Discov 2020] and addition of T to the combination of N+F prolonged response but did not preclude eventual progression nor emergence of additional HER2 or other alterations [Jhaveri et al. Ann Oncol 2023]. Here we describe serial circulating tumor (ct) DNA sequencing for patients with HER2-mutant mTNBC in SUMMIT who were treated with N or N+T and assessed potential mechanisms of acquired resistance.
Methods: Patients with HER2-mutant mTNBC received N (oral N 240 mg/d) or N+T (oral N 240 mg/d, IV T 8 mg/kg initially then 6 mg/kg q3w). Loperamide prophylaxis was mandatory during the first 2 cycles. Efficacy endpoints: confirmed ORR and CBR (RECIST v1.1 or modified PERCIST); DOR; PFS. ctDNA was collected at baseline, during treatment, and at the end of treatment and analyzed by next-generation sequencing. Samples were analyzed by the Memorial Sloan Kettering-Analysis of Circulating cDNA to Evaluate Somatic Status (MSK-ACCESS™) assay, which interrogates 129 cancer-associated genes.
Results: Longitudinal ctDNA sequencing data for 12 patients with HER2 mutation detected at least at baseline and end of treatment were available for 3 patients treated with N and 9 with N+T. One patient experienced investigator-assessed metabolic complete response (mCR), 6 experienced RECIST-evaluated partial response (PR), 3 had stable disease (SD) or metabolic stable disease (mSD), and 2 had progressive disease (PD) as best response. HER2 mutation variant allele frequencies (VAFs) decreased upon treatment in patients with response or SD and then increased upon progression, consistent with tumor response over time. Emergent mutations coincident with progression were detected in 1 patient treated with N and 5 with N+T who experienced initial response or SD and included on-pathway mutations HER2 T798I (N), ERBB3 E928G (N+T), MTOR A1832_A1842del (N+T), KRAS Q61H (N+T), TP53 R196Q (N+T), and TP53 E294* (N+T). Those with PD did not experience HER2 VAF decrease or acquisition of additional mutations.
Conclusions: Although limited by small numbers, addition of T to N in patients with HER2-mutant mTNBC, despite deepening and prolonging response, did not appear to preclude eventual emergence of either on-pathway (ERBB3) or off-pathway (KRAS, TP53) mutations. In contrast to observations in N+F+T-treated patients with HR+, HER2-mutant disease, no additional HER2 alterations were detected upon progression in N+T-treated patients with mTNBC in this small dataset. Future investigations may evaluate clinical utility of sequencing therapies targeted to mutations acquired in response to N-based therapy in patients with HER2-mutant mTNBC.

P1-09-27: MELK as a mediator of stemness and metastasis in aggressive subtypes of breast cancer

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Background: Triple-negative breast cancer (TNBC) constitutes 15-20% of all breast cancers and is characterized by the absence of estrogen (ER) and progesterone receptors and the lack of HER2 amplification. TNBC is notably aggressive, with limited treatment options, rapid metastasis, and high intratumoral heterogeneity, which may include a higher proportion of breast cancer stem cells. Maternal Embryonic Leucine Zipper Kinase (MELK) is a kinase normally expressed during embryonic development but reexpressed in breast cancers, especially in TNBC. High MELK expression in breast tumors correlates with a shorter time to metastasis in patients (HR 1.91, p = 4.4e-16). We hypothesized that MELK contributes to the aggressive phenotype of TNBC through its role in metastasis.
Materials and Methods: We utilized four breast cancer cell lines: two high-MELK expressing TNBC lines (MDA-MB-231, BT-549) and two low-MELK expressing ER-positive lines (MCF7, T47D). Genetic knockdown of MELK was achieved using doxycycline-inducible shRNAs or stable siRNA expression, while pharmacologic knockdown was conducted using the MELK inhibitor OTSSP-167. We also generated cell lines stably overexpressing MELK for overexpression studies. To assess the impact of MELK on self-renewal capacity, we performed mammosphere formation assays. Tumor invasion and scratch-wound assays were employed to evaluate invasion and migration, respectively. The chorioallantoic membrane (CAM) assay served as an in vivo model to study metastasis to the liver and lungs in chick embryos.
Results: Pharmacologic inhibition of MELK by OTSSP-167 in MDA-MB-231 cells led to a more than fourfold reduction in mammosphere formation efficiency compared to untreated controls (n=3, p=0.0059). Genetic knockdown via shRNA resulted in an approximately twofold reduction in mammosphere formation relative to non-targeting controls (n=2, p=0.054), supporting MELK's role in maintaining a stem-like phenotype. Additionally, siRNA knockdown of MELK in BT-549 and MDA-MB-231 cells significantly reduced invasion (n=4, p=0.0004; n=4, p=0.0023, respectively) and migration (n=6, p<0.0001 for both cell lines). Conversely, overexpression of MELK in MCF7 and T47D cells resulted in a twofold or near twofold increase in metastasis to the liver and lungs in the CAM assay (n=10, p<0.0001 for all conditions).
Conclusions: Our findings suggest that MELK plays a crucial role in the aggressive behavior of TNBC by promoting stemness, invasion, and metastasis. MELK inhibition reduces mammosphere formation, invasion, and migration, while its overexpression enhances metastasis in in vivo models. Confirmatory studies re underway in other subtypes of breast cancer. These results highlight the potential of targeting MELK as a therapeutic strategy to mitigate breast cancer metastasis and improve patient outcomes.

P1-09-28: Sociodemographic Experiences in Treatment (Tx) Patterns and Survival Outcomes in Women With Metastatic Triple-Negative Breast Cancer (mTNBC) in the United States (US): role of disparities

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Background: TNBC is an aggressive, heterogenous disease that disproportionately affects women of color, specifically non-Hispanic Black women, younger women, and BRCA1/2 tumor mutation carriers. This study assessed the impact of sociodemographic factors on real-world tx patterns, biomarker testing, and survival outcomes in women with mTNBC in the US.
Methods: This retrospective observational cohort study used the US-based, longitudinal, electronic health record–derived, de-identified Flatiron Health database. Women aged ≥ 18 years with mTNBC who received first-line (1L) tx for metastatic disease between Jan 1, 2018 and Jul 31, 2022 (index period), were included. Data cutoff was Jan 2023. Demographics and metastatic tx patterns were described in relation to race (White, Black, Other), socioeconomic status (SES) index (1st quintile [Q1; lowest SES] to 5th quintile [Q5; highest SES]), region (Northeast, Midwest, South, West, or Other) and tx setting (academic or community practice). Cox proportional hazards regression was used to compare unadjusted and adjusted differences in survival outcomes by sociodemographic group.
Results: Of 10,967 women with metastatic breast cancer (mBC), 1555 (14%) had mTNBC. Among women with mBC, 13% of White women and 23% of Black women had mTNBC. For women with mBC in the Q1 and Q5 SES index, 18% and 11% had mTNBC, respectively. The proportion of women with mTNBC in the South US was 16% and ~12% in other regions. About 15% of women in both academic and community practices had the mTNBC subtype. For the women with mTNBC, 501 (32%) had no documented 1L tx during the index period. This includes women who may have received tx with no documentation. Overall, the characteristics of women without documentation of receiving 1L tx were similar to those who received tx. The proportion of women starting 1L tx, the median time to tx initiation (~1 month), and the type of 1L tx were similar across sociodemographic groups. Overall, 28% of women received 1L anti-programmed death-(ligand)-1–based tx, with similar proportions across sociodemographic groups.
Of 930 women who received 1L tx, 55% had evidence of BRCA1/2 testing. The highest testing proportions were found in the West US (63%) and in academic practices (59%). The proportion of women tested was similar across race and SES quintiles. Of 43 women with documented BRCA1/2 mutations, 56% were treated with poly-ADP ribose polymerase inhibitors (PARPis) in any line during the study period, with the lowest proportions observed in Black women (33%, n=2), in Other (non-White) race (30%, n=3), in the lowest SES quintile (36%, n=4) and in the West region (33%, n=2), and the highest proportions were observed in White women (68%, n=15) and in the Midwest (71%, n=5), though sample size was small.
For women receiving 1L tx, there were no statistically significant differences in real-world overall survival (rwOS) within sociodemographic groups before and after adjusting for

confounding factors (P > 0.05). Median rwOS among White and Black women was 12.3 and 11.3 months, respectively. Numerically, among the SES quintiles, the longest rwOS was 14.1 months for women in Q4; the shortest in Q2 (10.7 months). Median rwOS by region ranged from 11.3 months in the Northeast to 13.4 months in the West. Women treated in academic and community practices had a median rwOS of 14.5 and 11.3 months, respectively. Conclusions: Women with mTNBC who were White and treated in the West/high SES index/academic setting had numerically longer rwOS vs non-White/other regions/low SES index/community settings, but this was not statistically significant. The types of tx received were similar across groups, with some differences in use of PARPis. Survival was poor across all groups, highlighting an unmet need for all women with mTNBC.

P1-09-29: Safety, efficacy and emerging biomarker data from the Phase Ib part of a Phase Ib/II clinical study of nadunolimab in combination with gemcitabine and carboplatin in patients with advanced triple negative breast cancer (TRIFOUR study)

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Background: Nadunolimab is a first-in-class, antibody-dependent cell-mediated cytotoxicity enhanced, monoclonal antibody targeting IL1 Receptor Accessory Protein (IL1RAP) on cancer cells, cancer stromal cells, and tumor infiltrating immune cells. Nadunolimab blocks both IL1α and IL1β signaling which is linked to tumor progression, therapy resistance, and immune suppression signals. The TRIFOUR trial (NCT05181462) is a Phase (Ph) Ib/II study evaluating nadunolimab in combination with chemotherapy as a 1L or 2L therapy in advanced triple negative breast cancer (aTNBC) patients. In the TRIFOUR PhIb part, nadunolimab was administered at either 1 mg/kg (n=3) or 2.5 mg/kg (n=12) in combination with gemcitabine (1000 mg/m^2) and carboplatin (AUC 2 mg/mL/min) on days 1 and 8 of each 3-week cycle. Here we present updated safety and efficacy data along with emerging translational research results from the 15 PhIb patients and a characterization of aTNBC.
Methods: PhIb, serum and blood samples were collected pre-treatment and during the study and analyzed for soluble biomarkers and blood immune cell populations by ELISA, the Olink Immuno-oncology 92plex protein panel and hematology. In a different set of aTNBC patients with ≤ 1 previous line of therapy for locally advanced or metastatic BC, biopsies (n=22) were characterized for expression of IL1RAP and IL1α by immunohistochemistry and blood cells were characterized for IL1RAP expression by flow cytometry (n=31).
Results: The PhIb safety profile was comparable to historic control data for gemcitabine plus carboplatin alone. Grade ≥3 treatment-emergent adverse events were reported in 12

(80%) patients, leading to treatment discontinuation in 1 (7%) patient. Five (33%) patients experienced serious AEs, febrile neutropenia being the most frequent (2 [13%] patients). Updated overall response rate was 60% (95% CI: 32-84), median progression-free survival was 6.2 months (95% CI: 3.7-8.3) and median overall survival was 12.8 months (95% CI: 8.5-NE). No PhIb patients remain on treatment. Emerging translational analyses comparing C1D1 and C2D1 time points identified a decreased absolute neutrophil count (ANC) (Hodges-Lehmann estimate and confidence interval for difference (HLE), -1.33, 95% CI: -2.2 to -0.73, P=.003) and neutrophil to lymphocyte ratio (HLE, -1.0, 95% CI: -2 to -0.4, P=.005) as well as decreased C reactive protein (HLE, -6.0, 95% CI: -19.2 to -0.4, P=.001). IL8 was also decreased on treatment, with a trend of correlation to a better outcome.
Characterization in the separate set of patients showed widespread IL1RAP expression on tumor and stromal cells in all biopsies while IL1RAP-positive immune cells were found in 20 out of the 22 (91%) biopsies. IL1α expressing tumor cells were present in all biopsies, as were IL1α positive immune cells. All blood samples characterized by flow cytometry contained IL1RAP-positive immune cells with IL1RAP expression being notably high on monocytic myeloid-derived suppressor cells.
Conclusions: Data from the PhIb TRIFOUR study indicate that nadunolimab at 2.5mg/kg, combined with gemcitabine plus carboplatin, has acceptable safety and tolerability and shows promising antitumor activity. Analyses of immune cell subsets and biomarkers showed potentially beneficial effects on cells and markers related to inflammation and immune response, such as decreased ANC and IL8. Characterization of aTNBC affirmed IL1RAP expression on tumor cells, cancer fibroblasts as well as tumor-associated and blood immune cells. In the biopsies, IL1α was expressed both in cancer and immune cells. The randomized PhII part of the trial is currently enrolling patients at 2.5mg/kg and more translational analyses are underway.

P1-09-30: Characteristics associated with sacituzumab govitecan response in the real-world setting

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Introduction: Sacituzumab govitecan (SG) is a TROP2-directed antibody-drug conjugate that is approved as a 2nd-line therapy for metastatic triple-negative breast cancer (TNBC) and 3rd-line therapy for metastatic, endocrine-resistant, hormone receptor-positive HER2-negative (HR+ HER2-) breast cancer. TROP2 is expressed in most breast cancers; however, it is unknown whether the degree of estrogen receptor (ER)-positivity and other characteristics impact outcomes of SG. We sought to explore associations between patient/tumor characteristics and SG response in metastatic TNBC and HR+ HER2- breast cancer.
Methods: We performed a single-center, retrospective chart review of patients with breast cancer who received SG in the metastatic setting. Data collected and analyzed included age, gender, race and ethnicity, BMI, UGT1A1 status, SG dosage, number of prior lines of therapy, ER percentage, PR percentage, HER2 score, and reasons for discontinuing SG. Clinical and

baseline characteristics were summarized using descriptive statistics, and univariate and multivariate cox regression was used to identify predictors associated with progression-free survival (PFS).
Results: Between April 2020 and November 2023, 126 females and 2 males received SG as a median 4th line of therapy (range 1-13). Median age was 56 years, median body mass index (BMI) was 24.7, and 74.2% of patients had TNBC. Most patients experienced disease progression or discontinued SG due to death (85.2%), while 11 (8.6%) patients discontinued due to toxicity, 1 (0.8%) patient entered hospice, and 7 (5.5%) patients are still on SG to date.
In univariate analyses, ER percentage predicted for PFS (p= 0.045): ER positivity (>50%) was associated with poorer PFS compared to ER ≤10% (HR 2.02, 95% CI 1.10-3.72, p=0.024). Higher number of prior therapy lines (p=0.0006) was also associated with inferior PFS, while BMI>=30 (p=0.026) and the occurrence of dose reductions (p=0.018) demonstrated improved PFS. Multivariate analysis supported improved PFS observed in patients with a BMI ≥30 (HR 0.57, 95% CI 0.34-0.93, p=0.012) and in patients who were dose-reduced (HR 0.67, 95% CI 0.46-0.99, p=0.044). Not surprisingly, the final predictor in the multivariate model was an indication of 6+ lines of prior therapy which led to poorer PFS than for those whom SG was a 1st or 2nd line (HR 2.54, 95% CI 1.51-4.26, p=0.0004).
Conclusion: Obesity and dose reductions predicted improved PFS while having 6+ previous lines of therapy predicted poorer PFS. Our data suggests that patients with a higher BMI and dose reduction have improved outcomes with SG, which may be attributed to underlying pharmacokinetic mechanisms and optimal dosing strategies that must be further explored.

P1-10-01: Overall survival and treatment patterns of incident triple negative breast cancer diagnosed in 2015-2020: single centre study in Poland.

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Background: Triple Negative Breast Cancer (TNBC) is one of the subtypes of breast cancer, lacking the expression of the estrogen (ER), progesterone (PgR) and HER2 receptors. TNBC accounts for 10-15% of all breast cancer (BC) cases, but is responsible for approximately 40% of BC deaths. Significant advances in therapeutic approaches have been noted over the past 10 years, including the widespread use of neoadjuvant therapy, post-neoadjuvant treatments, and more recently, the addition of immune checkpoint inhibitors. We report real-world data on overall survival in long term follow-up of TNBC of patients diagnosed before the introduction of pembrolizumab into routine care, as a benchmark for the future evaluation of the impact of newer regimen. We base on experience of the Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw (MSCI).
Methods: Data were extracted from MSCI hospital information system on cases admitted ≥2x with C50 ICD-10 code, undergoing surgery, systemic treatment +/- radiotherapy in

2015-2020. Vital status data for the selected cohort were obtained from the national census registry as of 2023.10.30. Overall survival was estimated from the first visit in MSCI until the date of death or vital status check with standard statistical techniques.
Results: The annual number of BC patients treated at NIO-PIB ranged from 6,359 to 7,548. During the study period, 8,103 patients were admitted for the first time, of which 856 (10.6%) were TNBC cases. In 2020, as a result of the COVID-19 pandemic, there was a significant 22% decline in new admissions. We analysed 604 patients, median age 58, range 26-93, not treated before admission to MSCI. Approximately 33% of them were overweight with BMI > 25, 12% had BMI ≥ 30 kg/m2. Roughly 20% of patients had a positive BRCA1/BRCA2 mutation status, while half had an unknown BRCA status. Most cases were diagnosed at stages IIA (36.1%) and IIB (23.3%); 68.5% received neoadjuvant chemotherapy (NAC), with a significant increasing trend (44.1% in 2015 to 88.7% in 2020). Among those receiving neoadjuvant chemotherapy (NAC), 26.3% received post-NAC treatments, with a notable upward trend. Excluding metastatic cases breast conserving surgery (BCS) was performed in 245 (43.6% of all patients and 47.6% of patients operated in MSCI). Among surgical cases the share of BCS in the NAC and non-NAC groups was: 66.7% and 79.5% in cT1 (p=0.195), 61.0% and 36.4% in cT2 (p<0.0001), 12.2% and 6.7% in ≥cT3 (p=0.53). Among cN0 patients, 74.4% had sentinel node biopsy (SLNB) without axillary lymphadenectomy (ALND).
The median follow-up was 54.3 months, ranging from 1.4 to 105.6 months, overall 168 patient died (27.8%), including 33 of 35 patients with initially metastatic cancer (94.3%). The 5-years overall survival was 72.6% (69%-76.4%), 76.8% (73.3%-80.5%) and 4.3% (0.7%-24.9%) among all patients, non-metastatic and metastatic cases, respectively. The 5-years survival improved by diagnosis year from 60.5% for patients diagnosed in 2015 to 77.1% among those diagnosed in 2020. In multivariable Cox proportional hazard model on non-metastatic subgroup, adjusting for age, stage and ECOG, the use of neoadjuvant was associated with improved survival (HR 0.5, 95% CI 0.3-0.8, p=0.0017) and the year of diagnosis was insignificant.
Conclusions: We confirmed improving prognosis for the TNBC patients in a large real-life cohort in Poland. The use of neoadjuvant therapy was increasingly becoming the gold standard in patients with TNBC during the study period, leading to an increased number of breast-conserving surgeries coupled with improved overall survival. Notably, a large proportion of patients in our cohort had BRCA1/BRCA2 pathogenic mutations suggesting that they could further benefit from newer therapies like PARP inhibitors.

P1-10-03: Real World Effectiveness and Economic Outcomes of First Line CDK4/6 Inhibitors in Combination with AI for HR+/HER2- Metastatic Breast Cancer in a US Medicare Eligible Population

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Background: Cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) in combination with an aromatase inhibitor (AI) are the preferred first-line (1L) treatment for hormone receptor positive, human epidermal growth factor receptor 2 negative (HR+/HER2-) metastatic breast cancer (mBC). There is increasing interest in the use of real-world evidence to inform policy decisions in the United States. In an observational study of adults with mBC, mean total costs ranged from $172,700 to $249,200 annually in the first four years after diagnosis. While prior cost-effectiveness studies have assessed CDK4/6is palbociclib, ribociclib, and abemaciclib using outcomes from placebo-controlled clinical trials, no such comparisons have been made using real-world evidence, nor in a US Medicare-eligible population. The objective of this study was to estimate the clinical outcomes and healthcare costs of 1L CDK4/6i treatment using real-world data.
Methods: We projected patient time spent in progression-free, progressed disease, and death health states using a partitioned survival model derived from progression-free survival (PFS) and overall survival (OS) curves. Survival outcomes for palbociclib + AI were informed by an analysis of patients aged ≥65 with HR+/HER2- mBC from the Flatiron Health Analytic Database, which included 808 patients treated in 1L with palbociclib + AI. Based on a targeted literature review, ribociclib + AI outcomes were modeled using relative hazard rates from OPAL (NCT03417115), a prospective longitudinal multicenter cohort study that used inverse probability of treatment weighting by propensity score analysis to make an adjusted comparison between ribociclib (n=235) and palbociclib (n=388) for both PFS (hazard ratio [HR]: 1.01 [95% confidence interval [CI]: 0.80 – 1.26]) and OS (HR: 0.99 [95% CI: 0.72 – 1.29]) (Thill, SABCS 2023, PO1-04-12). All-cause medical costs in inpatient, outpatient, and emergency department settings and drug costs for CDK4/6i + AI regimens were based on an analysis of patients in Optum's Clinformatics DataMart who were aged ≥65, enrolled in Medicare Advantage and treated with CDK4/6is for HR+/HER2- mBC (n=2,220). Drug costs reflect real-world utilization accounting for dose adjustments and wastage over the course of treatment. The primary analysis was conducted from the Medicare perspective using a lifetime timeframe in a hypothetical cohort of patients with a mean age of 73 years. Sensitivity analyses were performed to assess the robustness of the results to plausible variation in input values.
Results: Life years (LY) with palbociclib + AI and ribociclib + AI were similar, with base-case projections of 5.29 and 5.33 LYs, a difference of 15 days. Total lifetime healthcare costs were also similar in the base-case ($818,200 and $815,300, respectively, a difference of $2,900 over 5 years), with over half comprised of medical costs. Sensitivity analyses indicated no difference in LYs or total healthcare costs between palbociclib + AI and ribociclib + AI. Using the reported 95% CI for the OS HR, LY gained difference between palbociclib + AI and ribociclib + AI ranged from -0.91 to +1.34 LYs. Similarly, difference in total healthcare costs ranged from -$79,500 to +$101,600 over the projected lifetime of patients. Analysis using abemaciclib real-world comparative effectiveness data is ongoing and will be presented at the conference.
Conclusion: Based on real-world data, our analysis suggests that palbociclib + AI and ribociclib + AI demonstrate similar life expectancy and healthcare costs in elderly women with HR+/HER2- mBC. Additional large comparative effectiveness studies of CDK4/6is, that

appropriately adjusts for patient differences and assesses both PFS and OS are needed to verify the findings.

P1-10-04: First results of the WAVES study: Significant increase of patient satisfaction with longer initial consultation duration for breast cancer diagnosis

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Background: The "WAVES" (Widening Aims and giving patients a Voice for Expanded Structures in breast cancer care jointly developed by patients and physicians) study primarily aims to assess current care structures for breast cancer with a special focus on physician-patient-communication. In the long term, an improved Patient-Centered Communication-Care Concept (PCCCC) for breast cancer will be developed. WAVES is conducted within and funded by the BZKF (Bayerisches Zentrum für Krebsforschung, Bavarian Cancer Research Center) Network.
Methods: The study is structured as a two-part questionnaire for breast cancer patients and a corresponding questionnaire for physicians. Here, we present the first results of the first-part questionnaire for patients regarding the duration of the first diagnosis consultation and satisfaction with this conversation based on the evaluation of 1000 participants.
Results:Participants were between 23 and 89 years old (mean: 59.18 years), and mostly female (98%). More than every second patient felt shocked by the first diagnosis of breast cancer (54.8%). There was a significant association between longer initial consultation duration and higher patient satisfaction ($p < 0.001$). If the conversation lasted more than 30 minutes, 90.1% of the patients rated the duration as "just right", whereas only 12.7% rated “just right” in case of 10 minutes duration. With a first consultation lasting 30 minutes or longer, patients stated more frequently that they felt better informed ($p<0.001$), had fully or substantially understood the content ($p < 0.001$) and felt well prepared for further treatment decisions ($p < 0.001$). Conclusions:These results show a significantly higher satisfaction and better preparation of patients with initial breast cancer diagnosis if the initial consultation with a physician lasted 30 minutes or more. The need for improved communication structures, offering breast cancer patients appropriate time frames, has thus been clearly demonstrated by the cross-sectional survey of the WAVES study. Yet, this need for longer conversations not reflected in the current reimbursement structure. However, study results such as these show a clear need to integrate this in the future in order to ensure optimal care for breast cancer patients.

P1-10-05: Real-World Molecular Profiling After CDK4/6 Inhibition in Advanced Breast Cancer: Analysis of the SOLTI-1903 HOPE Study

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Background: The optimal treatment strategy for patients (pts) with HR+/HER2- advanced breast cancer (ABC) progressing on first-line endocrine therapy (ET) and CDK4/6 inhibitors (CDK4/6i) is influenced by the molecular alterations driving resistance to this combination. However, financial and logistical challenges hinder the routine implementation of next-generation sequencing technologies, leading to disparities in patient care. To address these issues, the SOLTI-1903 HOPE study (NCT04497285) aims to assess the feasibility of a molecular screening program that actively involves pts with ABC, providing insights into the genomic landscape and improving access to matched-targeted therapies in Spain.
Methods: The SOLTI-1903 HOPE study is a Spain-wide patient-centered study in which pts with ABC actively manage their enrollment, participation, and follow-up through a digital tool. Pts provide clinical data and tumor tissue samples for FoundationOne CDx (F1CDx) analysis. At disease progression (PD), pts undergo a liquid biopsy (LBx) at a local laboratory to be analyzed using the Guardant360 (G360) panel. Clinical and molecular outcomes are evaluated by a multidisciplinary Advisory Board, which interprets the observed alterations and recommends potential routine or clinical trial-based targeted therapies. The primary endpoint of this sub-analysis is to evaluate the genomic profile of pts with ABC who progressed on previous CDK4/6i.
Results: Of the 163 pts enrolled in HOPE who received first-line ET + CDK4/6i, 119 experienced documented PD. Of the 98 pts with a LBx result after ET + CDK4/6i, 22 (22.4%) had the blood extraction immediately after progressing to this treatment; median time from PD on ET + CDK4/6i to LBx was 9.2 months (mo) (range 0.07-64.1). ctDNA was detected in 97 pts (98.9%) with a median of 3 genomic alterations per patient (IQR 2-5, range 1-22) and a median variant allele frequency of 0.7% per alteration (IQR 0.2-3.2%, range 0.02-86.6%). ESCAT level I-II alterations were identified in 71pts (73.2%). Most frequent mutations were PIK3CA (44.3%) and ESR1 (34%). BRCA1/2 mutations occurred in 11.3% of pts, ERBB2 in 6.2%, and PTEN in 7.2%. Notably, co-occurring mutations involving >2 targetable alterations were found in 24 pts (24.7%), predominantly involving alterations in ESR1 and PIK3CA/PTEN/AKT1 pathway (58.3%).
Only 24 pts had F1CDx performed on a tissue sample obtained after first-line ET+CDK4/6i. Median time to tissue obtention was 12.1mo (range 0.23-47.8). Most frequent biopsy sites were liver (37.5%), breast (25%), and skin (16.7%). Median TMB was 3 (IQR 1-4, range 0-18), and the median number of genomic alterations per patient was 8.5 (IQR 5-10, range 2-16). ESCAT level I-II alterations were found in 16 pts (66.7%) and co-occurring targetable

alterations in 4 (16.7%), with no significant differences vs. LBx (p=0.5 and p=0.4, respectively). Most frequent mutations were TP53 (50%), ESR1 (37.5%), and PIK3CA (29.2%).
In 20 pts with both LBx and tissue samples obtained after ET+CDK4/6i, ESCAT I-II alterations were found in 60% and 70% with G360 and F1CDx, respectively (p=0.5), with a concordance rate of 73.3%.
Among 114 pts with follow-up clinical data, 17/55 pts (30.9%) with PIK3CA mut received targeted therapy after inclusion in HOPE; 7/55 (12.7%) had received it previously. None of the patients with BRCA1/2, ERBB2, ESR1, AKT1, or PTEN mutations has received targeted therapy after enrollment.
Conclusions: Over two-thirds of pts progressing on CDK4/6i present with ESCAT I-II level alterations detected via tumor or liquid biopsy. Notably, 15-25% of these pts exhibit co-occurring targetable alterations. In our study, the most frequently targeted alteration was PIK3CA mut, due to lack of approval for targeted therapies for other ESCAT I-II alterations in Spain during the study period. Addressing current barriers to targeted therapies is crucial for expanding options for pts with ABC.

P1-10-06: DETERMINANTS OF SURVIVAL IN MALIGNANT PHYLLODES TUMOR OF THE BREAST: ANALYSIS OF A POOLED DATABASE

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Background: Phyllodes tumors are rare fibroepithelial neoplasms of the breast, classified into benign, borderline, and malignant types based on histological features. These tumors account for less than 1% of all breast tumors, and malignant types are rare but can metastasize to organs such as the lung, bone, and brain. While their management often involves surgical resection with clear margins to prevent recurrence, their prognostic factors are not well characterized. We conducted this pooled database analysis to identify key factors that affect the clinical outcomes of malignant phyllodes tumor of the breast (MPB).
Methods: To study the demographic characteristics, molecular and immunohistochemical signatures, therapeutic interventions, prognostic factors, and clinical outcomes, we compiled a pooled database of cases that fit the diagnostic criteria for malignant phyllodes tumor of the breast. Kaplan-Meier survival curves were constructed. Cox proportional hazards model and Log-rank tests were used to assess the influence of demographic and clinicopathologic factors on survival outcomes.
Results: Ninety-seven patients with confirmed MPB were identified, the majority being females (97%). The median age was 48. Fifty-four percent involved the right breast. The median duration of symptoms before diagnosis was 7 months. Axillary lymph nodes were involved in 14% of cases at presentation. The median OS, DFS, and RFS of the whole cohort were 36, 36, 22 months, respectively. Patients younger than 48 had better median OS (60 vs. 28 months, p=0.026). Left MPBs had worse OS (17 vs. 60 months, p=0.004) as well as DFS and RFS. Size>10cm and involvement of axillary LN had numerically worse OS, DFS, and

RFS but did not reach statistical significance. Time to recurrence <12 months was associated with worse OS (13 vs. 60 months, p=0.001). Adjuvant chemotherapy and adjuvant radiation numerically improved DFS but did not reach significance. However, the latter significantly improved RFS (p=0.046). The type of breast surgery, axillary dissection, and margin status did not impact OS. Palliative interventions using mutlimodalities seem to have numerically better OS especially when palliative surgery is involved, though it did not reach statistical significance. Lastly, when compared to borderline phyllodes tumors of the breast cohort, MPB demonstrated a worse median OS (36 months vs NR, p=0.04).
Conclusion: This study presents updated clinicopathologic data from a large, pooled cohort of patients with MPB. It identifies age, laterality, and time to recurrence <12 months as critical determinants of OS in the entire cohort. It confirms the more aggressive course and worse outcomes when compared to borderline phyllodes tumors of the breast.

P1-10-07: PROgress Tracker Breast Cancer Registry: Feasibility of a Longitudinal Peer-Led, National Patient-Reported Outcomes (PRO) Registry

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Objective: PROgress Tracker Breast Cancer Registry is a national longitudinal, non-interventional patient-reported outcome measure (PROMs) registry, using a novel, peer-to-peer engagement and retention model directed by patient advocacy group Breast Cancer Canada and database managed by the POET Program. Here, we report initial PROMs results, demonstrating feasibility to amplify the patient voice and inform evidence-based care across Canada.
Methods: PROgress Tracker, launched in October 2023 has a 10-year enrollment goal of Canadians with Stage 0-IV breast cancer. Participants self-refer for registry inclusion and complete a series of validated PROMs via a digital platform every three months for up to 10 years.
Participants self-report demographic, socioeconomic and clinical data (age, social structure, employment, stage, molecular tumor markers, treatments, treatment-related impacts (adverse events, health-related quality of life) and global measures of wellbeing (mental health, social support, financial wellbeing, sexuality, physical functioning) through a series of questionnaires and PROMs. PROMs are dynamic, tailored to the participant's current status at each data collection timepoint.
Results: To date, 485 individuals have enrolled in PROgress Tracker, representing all Canadian geographical regions, including 17% in rural/remote locations. Three-month follow up survey response was 65%.
Socioeconomic data was reported by 399 participants: 94% identify as Caucasian, 28% report an active caregiving role to children or others, 61% are currently employed; 52% reported financial stress. 251 participants provided diagnostic, global functioning and treatment-specific PROMs data.

Diagnoses spanned 1997-2024 (median 2021). 76% reported early/localized stage (Stage 0 - II) disease at diagnosis. 45% reported receiving BRCA mutation testing, of which 15% were positive; 7% report a recurrence, and 4% have metastatic disease. 91% received oncological resection, 71% radiation therapy to the breast (RT); 76% systemic anti-cancer therapy (SACT) (hormonal, cytotoxic, immune checkpoint inhibitors or targeted therapies). Participants reported impairment in overall health-related quality of life (median FACT-B Total score 82.6 out of 148). 16% of participants reported moderate-severe anxiety, while 22% reported moderate-severe insomnia. 36 participants currently on SACT reported at least one adverse event, with 22 (61%) reporting adverse events 'frequently' or 'always' [PRO-CTCAE Systemic Therapy Subset]. Participants ever having radiotherapy (RT) reported moderate function (median 52) and low symptom (median 28) scores [EORTC-QLQ-BR23]; 78% of participants currently receiving RT reported mild adverse events, while 44% reported serious adverse events.
Conclusion: Capturing the lived experience throughout a breast cancer journey and integrating the use of validated PROMs into precision medicine is integral to driving patient-centered change in healthcare systems. Initial directional data from PROgress Tracker has demonstrated ability to capture a wide range of real-world evidence, suggesting that longitudinal digital capture, peer-to-peer involvement and real-time PROMs acquisition is feasible and can yield data of appropriate caliber to inform best practice, health care policy, and identify additional supports to improve outcomes. Efforts in recruitment and retention strategies are a current focus.

P1-10-08: Characterization of breast cancer subtypes in advanced real-world setting: the GEICAM/2014-03 (RegistEM) study

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Background: Breast cancer (BC) is a heterogeneous disease characterized by distinct subtypes, each with different prognoses and divergent treatment responses. Understanding the real-world distribution of BC subtypes in the advanced setting, and their clinical implications is the primary objective of the RegistEM study.
Methods: Female and male patients (pts) with advanced BC (ABC), either recurrent (rEBC) or de novo metastatic (dnMBC), diagnosed since Jan-16, were enrolled in this ambispective, non-interventional cohort study. Biological samples (primary tumor, metastatic lesions, blood) were also collected. In the current analysis, 1951 pts from 38 GEICAM sites were included (database ongoing, cut-off date 22-Apr-2024). BC subtype was assessed by immunohistochemistry with or without in situ hybridization, in the most recent tumor sample before 1st-line (metastatic lesions, and if not available, primary BC). The definition

of clinical subtypes was according to standard criteria, and information whether there were changes (unstable) or not (stable) in BC subtype between primary and metastatic tumor lesions is reported.
Results: 63%, 34% and 3% pts had rEBC, dnMBC and unresectable locally ABC (ULABC), respectively, with development of distant metastases in 97% pts; 1/3 – 1/2 were dnMBC, but 1/4 in HR−/HER2− pts, and median time to rEBC reduced as HR expression decreased. Median age was 60 years, 98% were white and 99% female (70% postmenopausal at ABC diagnosis). BC clinical subtypes distribution was HR+/HER2− 68% (HER2-low 45%), HR+/HER2+ 12%, HR−/HER2+ 6%, HR−/HER2− 10% (HER2-low 34%), unknown (UK) 4%; 57% cases were assessed in metastatic lesions, 40% in primary tumors and 3% UK. At ABC diagnosis, bone (63%) was the most frequent metastatic location. Visceral involvement was different between subtypes ($p<0.001$, chi-square test) in comparison with no visceral disease. Liver and lung were the most common visceral locations, with the latter higher (40%) in HR− BC subtypes. Within soft tissue metastases, lymph nodes were the most common location in all BC subtypes (>40%). Brain metastases were present in 10% HR− BC subtypes. ≤2 and 3 metastatic locations were reported in 56% and 26% pts, respectively. Family BC and ovarian cancer history was reported in 30% pts, a hereditary-risk genetic test was performed in 31% (n=583/1862) pts, and 19% (n=38/205) had BRCA1/2 mutations (HR+/HER2− [n=25/138, 18%], HR+/HER2+ [n=2/11, 18%], HR−/HER2+ [n=2/9, 22%], HR−/HER2− [n=9/36, 25%]). Paired tumor samples from primary BC and metastatic lesions were available in 32% HR+/HER2−, 33% HR+/HER2+, 33% HR−/HER2+, and 44% HR−/HER2−. Distant metastases were biopsied in 55% rEBC pts and 22% dnMBC pts. Unstable BC subtype (n/pts with paired samples) and the main change were: 9% HR+/HER2− (HR+/HER2+), 33% HR+/HER2+ (HR+/HER2−), 52% HR−/HER2+ (HR+/HER2+), and 42% HR−/HER2− (HR+/HER2−). After a median follow-up of 40 months, no statistically significant differences in survival from ABC diagnosis were observed between pts with stable and unstable BC subtype for any cohort, regardless of subtype.
Conclusions: These data confirm our results from previous analyses, showing that HR+/HER2− is the most predominant subtype in ABC pts. dnMBC was present in one-third of pts at ABC diagnosis, TNBC shows the lowest proportion. Visceral involvement was the main metastatic location in all subtypes, being more frequent in HR−/HER2+. Additionally, the unstable subtype is most common in HR− disease. However, no differences in survival from ABC diagnosis were observed between the unstable and stable subtypes across any BC subtype. Further long-term follow-up studies should be conducted to observe the progression and treatment outcomes of different subtypes, particularly focusing on the transition stable to unstable disease states.
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P1-10-09: Underestimation in core-needle biopsies of papillary breast lesions: a retrospective cohort from a university center

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Background: Papillary breast lesions (PBL) include a wide spectrum of lesions and can be benign, with atypia or malignant. They correspond to up to 5% of breast biopsies, but their incidence has increased in recent years, mainly due to technical improvements in percutaneous biopsies and the greater use of breast ultrasound. Many studies have been published about the management of PBL identified in biopsies and underdiagnosis rates may vary in the literature. In the presence of atypia, a high rate of underdiagnosis is observed, therefore, complete excision of the lesion is recommended. On the other hand, papillary lesions without atypia identified on core needle biopsy (CNB) remain without consensus regarding management.
Objective: To verify the underestimation (atypia and carcinoma) in ultrasound-guided CNB of papillary breast lesions, after undergoing surgical excision. Furthermore, to verify the clinical and radiological characteristics associated with upgrade.
Method: Retrospective study, through analysis of medical records of 56 patients with papillary breast lesions identified on ultrasound-guided CNB and subsequently undergoing surgical excision of the lesion, between 2007 and 2020, in the Mastology service at Escola Paulista de Medicina - Unifesp (Sao Paulo - Brazil).
Results: Among the 59 lesions considered for the study, of which 40.7% were cases of papillary lesions with atypia, 39% were papillary lesions without atypia and 20.3% were intraductal papillomas, we identified 52.5% (95%CI: 39.1% to 65.7%) of underdiagnosis without distinct percentages by biopsy classification (p=0.295), by characteristics (p>0.05), as well as differences in mean age (p=0.186) and size of the lesion (p=0.196). Univariate and multivariate logistic regression models were applied, but none of the characteristics proved to be significant, even after excluding the variables one by one in order of significance (backward method). Considering only the underdiagnosis of ductal carcinoma in situ (DCIS) or invasive carcinoma, a rate of 32.2% (95%CI: 20.6% to 45.6%) was observed, being 54.2% (95%CI: 32.8% to 74.4%) among papillary lesions with atypia, 26.2% (95%CI: 10.2% to 48.4%) among papillary lesions without atypia and no cases among pure intraductal papillomas. Univariate and multivariate logistic regression models were applied and remained in the final model, the variables age (p=0.022) and normal physical examination (p=0.049). Thus, a 1-year increase in age leads to an increase in 6% chance of underdiagnosis for cancer, adjusted by physical examination. In addition, patients with normal physical examination have an 83% lower chance of underdiagnosis for cancer than patients with changes in physical examination.
Conclusions: This study showed a high rate of underdiagnosis among papillary lesions obtained on CNB and did not identify associated clinical or radiological characteristics.

Considering only the underdiagnosis of DCIS or invasive carcinoma, it was identified that the patient's increasing age and the presence of changes in physical examination as predictive factors for underdiagnosis. Therefore, it is concluded that excision of papillary lesions identified in CNB should be considered, given the risk of underdiagnosis not only of carcinoma, but also of atypia.

P1-10-10: Outcomes of Abemaciclib Dose Escalation Strategy in High-Risk Early Breast Cancer

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Background: Abemaciclib is recommended as adjuvant therapy in node-positive, HR+, HER2-, high-risk early breast cancer, based on improved invasive disease-free survival (iDFS) in the monarchE trial. However, there are challenges with tolerability of standard dose abemaciclib. In monarchE, 16.6% discontinued abemaciclib due to adverse events (AEs), 56.9% experienced dose interruptions, and 41.2% had dose reductions.
At our institution, we have implemented a dose escalation (DE) strategy where patients initiate abemaciclib at 100 mg BID (50 mg BID in select patients) and increase to the full dose over 1-2 months, based on tolerance and provider discretion. This study compares the tolerability of abemaciclib using a DE versus standard dosing (SD) strategy.
Methods: This real-world analysis includes 164 HR+, HER2-, node-positive, high-risk EBC patients receiving adjuvant abemaciclib and endocrine therapy (ET) from October 12, 2021, to June 9, 2024. Patients were prescribed either a DE (n=83) or SD (n=81) per provider discretion. Primary outcomes included discontinuation, dose reduction, and treatment interruption rates, which were compared between the DE and SD groups using chi-square.
Results: Median ages were 55 years (DE) and 54 years (SD); 70% (DE) and 62% (SD) were postmenopausal. After a median follow-up time of 7.3 months in the DE group, 48.2% were titrated to full dose. Median time to full dose was 46 days (range 21-165 days).
Abemaciclib discontinuation rates were 18% (DE) vs. 27% (SD) (P=0.139); median times to discontinuation were 66 days (DE) vs. 58 days (SD). Interruptions occurred in 25.3% (DE) vs. 65.4% (SD) (P=0.001); dose reductions in 14.4% (DE) vs. 43.2% (SD) (P<0.005).
The most common reasons for discontinuation were diarrhea (SD 14.6%, DE 6.1%) and fatigue (SD 7.3%, DE 6.1%). Leading AE-related dose modifications were diarrhea (SD 37.8%, DE 9.8%, P=0.001), nausea (SD 12.2%, DE 1.2%, P=0.005), and fatigue (SD 12.2%, DE 4.9%, P=0.094).
Conclusion: This real-world study demonstrates that compared to the monarchE trial, the SD of abemaciclib is associated with a higher discontinuation rate (27% vs 16.6%), underscoring the importance of strategies to improve tolerability. Early discontinuations suggest that enhanced initial tolerability may improve adherence.
An abemaciclib DE strategy may improve tolerability, minimizing interruptions, dose reductions, and AEs vs SD. However, formal protocols are needed to ensure full dose escalation. Future studies should assess long-term outcomes (iDFS) with DE vs. SD abemaciclib schedules.

P1-10-11: Preliminary Analysis of Sociodemographic and Clinical Characteristics of Patients with Early Breast Cancer in the Patient-Centered, Real-World EVOLVE Registry

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Background: Despite improvement in survival rates over time, recurrence and treatment-related adverse events remain concerning risks for patients with early (stage I-III) breast cancer (eBC). The EVOLVE Registry, a patient-centered United States (US)-based registry was created to address the limitations of existing eBC databases and better understand real-world sociodemographics, clinical characteristics, diagnostic and treatment pathways, clinical outcomes, and patient experience in eBC. This is the first analysis of the ongoing EVOLVE Registry.

Methods: The EVOLVE Registry, created through a collaboration between PicnicHealth and AstraZeneca, consists of the de-identified medical record data, along with patient-reported social determinants of health (SDoH) and patient-reported outcomes (PROs) data. Inclusion criteria were defined as eBC (invasive, non-metastatic) diagnosed ≤3 years prior to enrollment and ≥18 years old at consent. Patient enrollment began May 2023. Using PicnicHealth's platform, all available retrospective medical records prior to enrollment, including pre-diagnosis, were retrieved. Following enrollment, medical records and survey/PRO were prospectively collected. eBC-specific data elements were abstracted from structured and narrative text. Rural/urban categorization was derived from Rural-Urban Commuting Area (RUCA) codes. While enrollment (target: 3,000 patients) and data collection is ongoing, data from medical records collected and completed surveys for patients enrolled in the EVOLVE Registry up to June 2024 were analyzed. Patient demographics, receptor status, and self-reported SDoH data are described.

Results: A total of 1,428 patients with eBC enrolled as of June 2024 with a median of 7 years of visits pre-diagnosis and 2 years of visits post-diagnosis. Mean (range) age at diagnosis was 55 (24 - >89) years, with 76% diagnosed between 40-69 years and 35% diagnosed ≤1 year of enrollment. Almost all patients (n=1,427) were female; 77% were White, 12% Black/African American, 2% Asian, 4% mixed race, and 5% other race; 13% were Hispanic/Latino; 93% resided in a metropolitan/micropolitan area and 7% small town/rural. Most patients had hormone receptor (HR)+/human epidermal growth factor receptor 2 (HER2)- eBC (70%), followed by 15% HR-/HER2-, 11% HR+/HER2+, 4% HR-/HER2+, and 1% unknown. About half (55%) were diagnosed at stage I, 32% at stage II, and 13% at stage III. Of the 58% of patients who responded to the SDoH survey, 98% had some form of medical insurance; 20% had a high school education or less; 31% had full time employment, 10% part-time employment, and 27% were retired; 62% reported a

household annual income of <$75k; 57% owned their own housing; 25% were concerned about losing their housing; 16% reported lack of transportation access kept them from medical appointments. 47% reported current or former use of nicotine-based products, 89% of whom reported cigarette use and 25% reported e-cigarette/vape use.

Conclusions: The EVOLVE Registry to date has enrolled a population broadly representative of patients with eBC in the US with regards to race, ethnicity, and eBC characteristics. The patient-reported SDoH indicated a highly insured population, however, this may not reflect financial burden and unmet needs, including for transportation and housing security, as those were still apparent in this population. With ongoing enrollment, this registry will help further the understanding of the evolving real-world treatment of eBC, existing disparities, and patient needs across diagnostic and treatment pathways that affect breast cancer outcomes.

P1-10-12: Evaluating the Impact of Prosigna® Testing on Adjuvant Chemotherapy Decisions in Early-Stage ER-Positive, HER2-Negative Breast Cancer: A UK Study

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Multi-gene assays are recommended for guiding adjuvant chemotherapy decisions in early-stage ER-positive, HER2-negative breast cancer. This study aimed to evaluate the impact of Prosigna® testing on clinical decisions and to identify factors that predict the Prosigna® risk stratification outcomes. Women with ER-positive, HER2-negative breast cancer, eligible for chemotherapy based on intermediate risk of distant recurrence (using tools such as Predict or the Nottingham Prognostic Index), were retrospectively identified from three UK hospitals. Patient and tumor characteristics, Prosigna® test results, and final treatment decisions were collected. Outcome parameters included disease progression and overall survival. Multinomial logistic regression identified key predictors of low, intermediate, and high-risk categories of Prosigna® test results.
Among 363 patients with a median age of 61, 71% were post-menopausal, and 48% had grade 3 tumors. Based on Prosigna® results, 60% (n=216) avoided chemotherapy despite clinical indications, while 14% (n=52) without initial indications were escalated to receive chemotherapy. Anthracycline and Taxane-based regimens were most common (76%), followed by Docetaxel and Cyclophosphamide (24%). At a median follow-up of 2 years, 2.4% (n=9) had disease progression, all of whom received chemotherapy. High-risk and intermediate-risk (Luminal B subtype) patients were primarily offered chemotherapy. Tumor grade emerged as the most significant predictor across all Prosigna® risk categories, with a positive coefficient for high risk and negative coefficients for intermediate and low risk. NPI and Predict score also influenced risk stratification, positively impacting high and intermediate risk categories and negatively impacting low risk. Age, tumor size, and LVSI had smaller, less consistent influences. The model achieved an overall accuracy of 41%,

with the highest precision and recall in predicting the high-risk category.
This study presents the first real-world data on the implementation of Prosigna® in the UK, potentially optimizing future national guidelines. These findings highlight the importance of tumor grade and prognostic indices in risk stratification, offering valuable insights for personalized breast cancer management.

P1-10-13: Real-world outcomes of physician's choice treatment (PCT) for hormone receptor-positive (HR+)/HER2-0 and HER2-low advanced breast cancer (ABC) patients (pts) after endocrine therapy (ET) and CDK4/6 inhibitors (CDK4/6i)

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Background: Treatment for HR+/HER2-0 or HER2-low (Immunohistochemistry (IHC) 1+ or 2+/in situ hybridization, ISH-negative) ABC pts after ET and CDK4/6i depends on disease dynamics, prior chemotherapy (CT), performance status and the presence of actionable genomic alterations. Currently, trastuzumab deruxtecan is approved for HER2-low ABC after ≥1 line of CT, but is not reimbursed in Poland.
Methods: We retrospectively analyzed treatment outcomes of HR+/HER2-0 or HER2-low ABC pts after progression on ET and CDK4/6: palbociclib (PAL), ribociclib (RIB) or abemaciclib (ABM). We analyzed second progression-free survival (PFS2), overall survival (OS) and duration of the subsequent line treatment after ET/CDK4/6i (DoT).
Results: A total of 472 pts (PAL=127; RIB=222; ABM=78) from 12 Polish cancer centers treated between Sep 2017 and Dec 2023 were analyzed. Median follow-up was 33.3 (95%CI 31.2 to 36.3) months (m). Mean age was 59.8 ± 12.4 years. 200 patients (42%) had visceral and 358 (76%) bone metastases. 296 (63%) and 176 (37%) received CDK4/6i with aromatase inhibitor (AI) and fulvestrant (FUL), respectively. HER2 IHC: 0, 1+, 2+/ISH-negative subsets were:228 (48%), 153 (32%), 78 (17%), 13 (2.8%), respectively. PCT included fulvestrant 103 (22%), capecitabine 88 (19%), taxane 51 (11%), anthracycline 47 (10%), vinorelbine 43 (9.1%), anthracycline and cyclophosphamide 33 (7%), aromatase inhibitor 29 (6.1%), platinum compounds 24 (5.1%), alpelisib and fulvestrant 18 (3.8%), and other 36 (7.7%). Median DoT for HER2 0 and HER2 low was 4.2 (3.7-5.6) and 4.9 (4.1-6.0) m, respectively, HR= 0.79 (95%CI: 0.64-0.97), p=0.026; PFS2: 18 (15-20) and 20 m (18-23), HR=0.81 (0.65-0.99), p=0.042; OS: 26 (23-31) and 35 (30-39), HR=0.71 (0.55-0.91), p=0.007.
Conclusions: HR+/HER2-negative ABC constitutes a heterogeneous population that vary in

prognosis and sensitivity to systemic treatments. Compared to HER2-0, HER2-low subgroup has better outcomes following ET/CDK4/6i therapy. The outcomes of PTC and prognostic significance of the HER2-ultralow subtype warrants evaluation.

P1-10-14: Bridging the gap between real-world studies (RWSs) and randomized controlled trials (RCTs) of 1st-line palbociclib+aromatase inhibitors (P+AI) in hormone receptor-positive(HR+)/HER2-negative(-) metastatic breast cancer (mBC)

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Background: RCTs remain the gold standard to assess the efficacy of a treatment seeking regulatory approval. However, RWSs provide insights into patients (pts)' outcomes in routine clinical practice. We assessed the real-world (RW) efficacy of 1st-line P+AI and compared it to RCTs' results.
Methods: A systematic review was performed to identify RWSs published from 2019 to 2023 including pts with HR+/HER2- mBC treated with 1st-line P+AI, with available median progression-free survival (mPFS) and/or overall survival (mOS). A meta-analysis was performed to estimate the pooled mPFS/OS using the median of the medians (MM) and weighted median of medians (WM). The RW estimates were deemed comparable to PALOMA1/2 RCTs' mPFS/OS, if MMPFS/OS or WMPFS/OS were in RCTs' mPFS/OS 95% confidence intervals (CI). Similar criteria applied to pooled hazard ratios (HR) of PFS/OS for P+AI vs. AI in visceral/non-visceral subgroups.
Results: Among 8 included RWSs (n=4624 pts) a MMPFS of 22.5 months (95%CI: 19.5-31.8) and WMPFS of 20.0 (95%CI: 19.3-31.8) were observed with 1st-line P+AI. The MMPFS was comparable to those of PALOMA1 (20.2, 95%CI: 13.8-27.5) and PALOMA2 (27.6 months, 95%CI: 22.4-30.3). The WMPFS was inferior to that of PALOMA2. A RW MMOS of 51.2 months (95%CI: 49.1-53.3) and WMOS of 49.1 (95%CI: 49.1-53.3) were observed, outperforming the PALOMA1 mOS of 37.5 months (95%CI: 37.5-47.8) and being comparable to PALOMA2 mOS of 53.8 (95%CI: 49.8-59.2). P+AI vs. AI in RW in visceral disease had superior PFS (HR: 0.56, 95%CI: 0.46-0.68) and OS (HR: 0.61, 95%CI: 0.49-0.77), similarly to the PALOMA2 PFS (HR: 0.62, 95%CI: 0.47-0.81) and better than PALOMA1 (HR: 1.13, 95%CI: 0.68-1.88) and PALOMA2 (HR: 0.86, 95%CI: 0.65-1.13) OS. In RW non-visceral disease, P+AI vs. AI had lower PFS benefit (HR: 0.76, 95%CI: 0.65-0.88) than PALOMA2 (HR: 0.50, 95%CI: 0.37-0.62). RW OS (HR: 0.82, 95%CI: 0.69-0.99) was similar than that of PALOMA2 (HR: 0.86, 95%CI: 0.65-1.13).
Conclusions: Our findings add to the understanding of the generalisability of RCTs results to RW and confirm the role of 1st-line P+AI in clinical practice.

P1-10-15: Treatment patterns and clinical outcomes according to PD-L1 status in more than 2000 patients with early-stage or metastatic triple-negative breast cancer (eTNBC/mTNBC) treated in the real-world setting: VANESSA study results

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Background: Clinical trials in unselected populations of patients with TNBC suggest that positive PD-L1 status is associated with more favorable outcomes in both the early-stage and metastatic settings and predicts benefit from immune checkpoint inhibition in mTNBC; however, additional studies are needed to confirm these findings, and little is known about PD-L1+ prevalence and outcomes in the real-world setting.
Patients and methods: The multicenter retrospective observational VANESSA study evaluated the prevalence and impact of PD-L1 status in consecutively and uniformly enrolled patients diagnosed with eTNBC/mTNBC between Jan 1, 2014, and Dec 31, 2017, and treated with systemic therapy. PD-L1 expression was assessed locally and centrally on archival samples using the Ventana PD-L1 (SP142) immunohistochemistry assay. Data for medical/treatment history and clinical outcomes were extracted from patients' medical records. Primary and secondary objectives focused on diagnostic parameters (reported elsewhere). Exploratory objectives included description of demographic and clinicopathologic characteristics, treatment patterns, and clinical outcomes according to PD-L1 status (PD-L1+ defined as PD-L1 expression on tumor-infiltrating immune cells covering ≥1% of the tumor area).
Results: Overall, 2233 patients from 19 countries were screened, of whom 2054 were eligible and 1967 had centrally assessed PD-L1 status. Chart review showed that among 1902 eligible patients with eTNBC, 681 (36%) received neoadjuvant chemotherapy (CT; predominantly anthracycline- and taxane-based) and 1261 (66%) received adjuvant CT. The prevalence of PD-L1 positivity by central testing was 1007/1822 (55%) in eTNBC and 38/145 (26%) in mTNBC. In the eTNBC cohort, demographic and tumor characteristics were generally similar in the PD-L1+ and PD-L1– subgroups (respectively, median age: 52 vs 52 years; stage I/II at diagnosis: 56% vs 49%; stage III at diagnosis: 26% vs 33%; Asian race: 22% vs 22%; Black/African American race: 5% vs 5%; BRCA mutation: 68/147 [46%] vs 34/84 [40%]). Pathologic complete response (defined as eradication of invasive disease in the breast and lymph nodes after neoadjuvant CT in evaluable patients) was observed in 27/168 patients (16%, 95% CI 11–23%) with PD-L1+ tumors and 31/182 (17%, 95% CI 12–23%) with PD-L1– tumors. Among patients evaluable for invasive disease-free survival (iDFS), annual rates were more favorable in patients with PD-L1+ eTNBC (n=681) than PD-L1– eTNBC (n=549) (1-year iDFS: 80% vs 71%, respectively; 3-year iDFS: 62% vs 47%; 5-year iDFS: 56% vs 40%). Overall survival (OS) rates in the PD-L1+ vs PD-L1– cohorts were 98% vs 97% at 1 year; 87% vs 82% at 3 years, and 82% vs 73% at 5 years. In the mTNBC

cohort, 120 patients had de novo mTNBC (30/38 [79%] with PD-L1+ TNBC, 90/107 [84%] with PD-L1– TNBC). Median age was 61 vs 56 years in patients with PD-L1+ vs PD-L1– mTNBC. There were no Black/African American patients in the group with PD-L1+ mTNBC and only 2 (5%) Asian patients. In patients evaluable for progression-free survival, the median was 7.6 (95% CI 4.1–15.0) months in 30 patients with PD-L1+ mTNBC and 4.9 (95% CI 3.6–6.1) months in 83 patients with PD-L1– mTNBC; 1-year OS rates were 81% vs 62%, respectively.
Conclusion: In eTNBC and mTNBC, PD-L1+ status was associated with more favorable long-term outcomes, possibly due to tumor-intrinsic characteristics and/or the host immune response. The vast majority of patients in the mTNBC cohort were diagnosed with de novo mTNBC; the temporal lack of PD-L1 expression and/or the lack of prior CT may contribute to diagnostic differences compared with clinical trial datasets.

P1-10-16: HER 2 + metastatic breast cancer with central nervous involvement, response to Trastuzumab-Deruxtecan in a heavily treated patient: A case report

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Introduction: Breast cancer (BC) is the leading cause of cancer deaths in women worldwide. Human Epidermal Growth Factor receptor 2 (HER 2) is a tyrosine kinase that shows overexpression in 15-20% of breast cancers. This represents a poor prognostic factor and hassles in disease management. Antibody drug conjugates (ADC's) are emerging new cancer drugs that are gaining accelerated approval as newer cancer treatment lines due to their outstanding results in clinical trials on later lines, improving patient survival and acceptable toxicity.
Case Description: A 39-year-old woman with negative family or personal relevant medical history, presented with stage IIIA grade 3 infiltrative ductal breast adenocarcinoma. IHQ: estrogen receptors 0%, progesterone receptors 0%, HER 2 3+.Informed consent for publication was obtained by the patient.
Neoadjuvant chemotherapy with Doxorubicin/ Cyclophosphamide x 4 cycles was administered, followed by Paclitaxel x 12 cycles plus Trastuzumab (TZB) presenting minor toxicity. Following neoadjuvant chemotherapy, modified radical mastectomy was performed with histopathologic results: ypT1c, ypN2a. Adjuvant TZB was continued to complete 1 year of treatment, and received 50 Gy/25 fx adjuvant radiotherapy (RT) was delivered.
On April 2019, the patient presented disease recurrence in the central nervous system (CNS) documented by Magnetic Resonance (MRI). Two intra axial lesions in right parietal and left cerebellum regions with vasogenic edema, left midline shift and right subfalcine herniation were observed. Oral steroids and palliative holocranial RT with 30 Gy/10 Fx were started with good clinical response.
First line treatment with Trastuzumab Emtansine was indicated due to disease recurrence

while receiving TZB, with partial response measured by RECIST 1.0. Nausea and hypertransaminasemia G1 were reported and treatment continued without modifications. After 45 cycles disease progression on CNS was reported by MRI and treated with CyberKnife, 10Gy/5Fx was administered. Second line treatment with Capecitabine was started, after 4 cycles local progression on breast and axilla was documented. New biopsy was obtained with the same immunochemistry results as previous.
Third line treatment with TZB /Pertuzumab/Docetaxel was given with progression after cycle 9. Then, 4th line treatment with Lapatinib was started and after 5 cycles, disease progression with CNS involvement was documented by MRI. Finally, approval for Ttrastuzumab- Deruxtecan was obtained in Mexico and was started as the 5th line treatment in this patient. At present, she has received 9 cycles with transaminasemia G1 as the only toxicity, with good quality of life preserved. Complete response in axilla and breast and partial response on CNS lesions has been reached.
Discussion: Despite poor prognosis associated with HER2+ breast cancer, seeking for treatment alternatives as health caregivers is essential to improve survival, guarding adverse events drug related and preserving quality of life remains imperative.
Conclusion: This case highlights the advances in clinical oncologic research and its beneficial influence improving survival outcomes in management of metastatic HER2 + breast cancer in a patient with CNS involvement in a heavily treated scenario. As well, it underlines the benefits of sequencing treatments and the efficacy of an ADC, highlighting the acceptable toxicity and guarding patient's quality life.

P1-10-17: HER2+ breast cancer diagnosed in the 2nd trimester of pregnancy

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Introduction: Breast cancer diagnosed during pregnancy is rare. However, given the increasing incidence of breast cancer in young women, and the delayed age of first pregnancy in the US, knowledge of treating breast cancers during different stages of pregnancy is critical.
Case: A 34-year-old woman at 19 weeks' gestation felt a left breast lump. At 22 weeks, she was evaluated by her obstetrician who identified a 2.6 cm mass. Ultrasound guided biopsy revealed an ER low positive (1%), PR negative (<1%), HER2 positive (IHC2+, FISH+), poorly differentiated invasive ductal carcinoma with Ki67 of 90%. Given her pregnancy, cancer staging was performed using whole body diffusion weighted MRI (WB DWI), without contrast, at 24 weeks which revealed a 4.8cm left sided biopsy proven malignancy, nonspecific mildly enlarged left axillary lymph nodes and no distant metastasis. Patient had a normal echocardiogram, and a Port-a-Cath was placed. Her case was thoroughly discussed at multidisciplinary conference during which neoadjuvant chemotherapy was recommended including 4 cycles of dose dense doxorubicin/cyclophosphamide (AC) during pregnancy followed by delivery at term (37 weeks) and then Taxol/Herceptin/Perjeta (THP) after delivery, followed by breast surgery. The patient continued to note growth of

her breast mass and associated pain, and she received C1D1 of AC at 25 weeks' gestation with significant clinical improvement of her tumor size to 3 cm after one cycle. She also had resolution of her breast pain. The patient was referred to a maternal fetal medicine (MFM) specialist at 23 weeks with plan for serial growth scans and high-risk obstetric care.
Discussion: Breast cancer diagnosed during pregnancy merits multidisciplinary management given the significant complexities of optimizing treatment and outcomes for both mother and baby. The priorities of breast cancer treatment during pregnancy are to maximize maternal health and survival outcomes as well as to minimize risk of harm to the fetus. This careful calculation of risk versus benefit begins at diagnosis when the clinician must consider the most appropriate screening modality. In general, PET/CT is avoided in pregnancy. MRI scans (without gadolinium) are preferable over CT scans, however if CT scans are necessary, diagnostic imaging which exposes the fetus to less than 50mGy are acceptable. In our patient's case, we were able to obtain MRI WB DWI scan.
Specifically, for HER2+ breast cancers, standard neoadjuvant chemotherapy includes taxanes, anthracyclines, cyclophosphamide, and anti-HER2 agents. Timing of chemotherapy in pregnancy is crucial as it is contraindicated in the first trimester. Breast cancer diagnosed in pregnancy, and particularly in the first trimester, requires careful consideration of the risks and benefits of continuation of pregnancy. In the second and third trimesters, agents such as doxorubicin and cyclophosphamide are generally considered safe. Dosing weight should be based on actual body weight rather than pre-pregnancy or ideal body weight. Taxanes have been associated with oligohydramnios, and notably, serum taxane levels can be significantly decreased during pregnancy, in particular for paclitaxel, which make administration difficult. Trastuzumab is contraindicated during pregnancy as it has been associated with oligohydramnios and anhydramnios. In this patient's case, the chosen regimen is AC in the second and third trimester, followed by THP post-partum, followed by surgery. In deliberation with the patient's MFM specialist, there is a plan to stop chemotherapy 3-4 weeks prior to delivery to avoid risks of myelosuppression. In the post-partum management, the placenta should be evaluated by pathology as there have been cases of metastasis to the placenta and breast feeding would be contraindicated in the setting of the patient's receipt of chemotherapy or anti-HER2 therapy.
Conclusion: Ultimately, this case underscores the importance of personalized, multidisciplinary care in optimizing outcomes for both mother and fetus when managing HER2+ breast cancer diagnosed during pregnancy.

P1-10-19: Hypophysitis and Haziness in HER2: A Case of Breast Cancer with Leptomeningeal Carcinomatosis

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Background: HER2 is overexpressed in up to 30% of breast cancers. These patients have increased risk for development of CNS metastases, including leptomeningeal carcinomatosis despite use of HER2 targeted therapy. Historically the development of leptomeningeal

disease has carried a poor prognosis, with studies showing overall survival of 3 to 4 months.
Case Presentation: 41-year-old female with Lynch syndrome originally presented to an outside facility with ER+/HER2+ de novo breast cancer metastatic to liver and brain treated with trastuzumab, pertuzumab, and weekly paclitaxel for one year. There was no evidence of disease on follow-up imaging. Following TAH/BSO, she started daily anastrozole and continued this for 2 years. She presented 2 years later with multiple metastatic brain lesions. She underwent definitive stereotactic radiosurgery (SRS) and was enrolled in a clinical trial using pembrolizumab for 3 cycles following SRS treatment. She presented to our institution 3 months after SRS with fatigue, nausea/vomiting, left eye blurred vision and mydriasis. MRI imaging of the brain showed changes consistent with leptomeningeal carcinomatosis and thickening of the infundibulum, consistent with hypophysitis. CSF cytology revealed abnormal appearing cells, suspicious for mammary carcinoma. Abdominal imaging showed rapid development of numerous hepatic metastases not observed on abdominal imaging two weeks prior. Liver biopsy revealed HER2+ (3+) metastatic breast cancer. She began systemic and intrathecal (IT) trastuzumab, in addition to capecitabine and tucatinib per HER2 CLIMB. IT trastuzumab was given 80mg twice weekly for 4 weeks, followed by once weekly for 4 weeks, followed by 80mg every 2 weeks thereafter. Hypophysitis was treated with steroids. Follow-up after 4 cycles of systemic and IT treatment revealed clinical improvement with return of vision in left eye; imaging showed improvement in leptomeningeal and hepatic disease burden. Most recent imaging seven months after initiation of current treatment course showed stable leptomeningial disease, no abnormal cells in the CSF and improved systemic disease. Nine months following initiation of therapy she remains with stable symptoms.
Conclusion: As demonstrated in this case, treatment of HER2+ breast cancer with leptomeningeal metastases with intrathecal trastuzumab can extend survival above historically reported data, with limited toxicity.

P1-10-20: Idiopathic granulomatous mastitis with superimposed tuberculosis mastitis

Melissa Rangel, Julie Wecsler, Julia Alexevia

Idiopathic granulomatous mastitis (IGM) is a rare condition and exact prevalence is unknown. It may mimic infectious and malignant breast disease and is a diagnosis of exclusion with unknown etiology. Though not life threatening, the pain and disfigurement can be debilitating. In the United States association of IGM with childbearing, lactation, Corynebacterium and Hispanic ethnicity have been reported. Among small retrospective and prospective reviews Hispanic ethnicity accounts for 80-97% of patients. There is no agreed upon treatment and different modalities include a combination of antibiotics, steroids and surgical debridement. We present here a case of a 42-year old woman with a history of pituitary macroadenoma, hyperprolactinemia s/p cabergoline treatment and chronic galactorrhea who presented to breast clinic with chronic breast pain, abscesses and

repeated biopsies over 6 years before eventual conformation of IGM diagnosis and TB mastitis.
The patient initially presented to an outside hospital with complaints or a right breast abscess that was treated over 2 years with multiple aspirations, I&Ds and oral antibiotics. She had no personal or family history of cancer. Mammogram obtained on presentation to our clinic was BIRADS 2 and multiple biopsies showed chronic and acute inflammation consistent with abscess cavity. The IGM, AFB, fungal and TB testing were all negative and remained negative despite relapsing and remitting symptoms of the right breast. After several months of failed management with antibiotics and aspiration she was started on a prednisone empirically. Multiple biopsies obtained and repeat aspirations remained negative for IGM or infectious etiology. Over 4 months symptoms improved, and steroids were weaned. Symptoms recurred again however after stopping steroids and this time biopsy and culture showed corynebacterium. She was started on doxycyline and kenalog injections into the breast with eventual improvement though not full resolution of her symptoms. Over this entire clinical course lasting 3 years repeat screening mammograms remained normal.
She eventually returned with severe bilateral breast pain accompanied by large left breast abscesses. After in office incision and drainage did not provide adequate relief intraoperative drainage of the left breast was planned. A periareolar incision was made and on deeper dissection multiple loculated areas were broken up releasing purulent drainage. The cavity was packed with iodoform gauze. Pathology showed breast tissue with chronic inflammation, granulation tissue and fat necrosis. PAS-F, Gram, and AFB special stains are negative for fungal elements, bacterial organisms, and acid-fast bacilli. The patient slowly improved until 3 months post op when she returns with recurrent left breast symptoms as well as subcutaneous skin nodules on her legs and arms. Pathology at this time was positive for IGM showing granulomatous mastitis and lymphocytic lobulitis. There was some mild improvement with continued kenalog and doxycyline. During a particularly bad recurrent flare a repeat TB quantiferon became positive. Patient was sent to infectious disease to initiate anti-TB treatment and had marked improvement of symptoms.
IGM remains poorly understood and though relatively rare can be incapacitating. Some cases, as the one described here, may have superimposed infections exacerbating patient symptoms. Though there has been an association with the Hispanic community there has been little meaningful research investigating etiology or predisposing factors. Understanding these factors may ultimately lead to improved treatments and outcomes.

P1-10-21: Intrathecal therapy in invasive lobular carcinoma with non-bulky leptomeningeal disease: case report

Daniel Sobral Filho, Aniceto Lopes da Silva Neto, Giulia Mazaro de Oliveira, Rafaela Lopes da Silva Naves, Raelson Rodrigues Miranda, Laura Testa

Introduction: Leptomeningeal disease (LMD) is a challenging complication of breast cancer (BC) with limited treatment options and a prognosis of three to four months in most

cohorts. This case report describes the treatment of a patient with LMD at her initial diagnosis, evaluating its indication and clinical evolution.
Case report: Female, 68y at BC diagnosis, post-menopausal. Previous history: hysterectomy at 43y; hormone implants replacement therapy until diagnosis; pleuropulmonary fibrolastosis. Family history: a sister died of ovarian cancer at 40y, NGS for 25 germline mutations negative. In 2018, the patient experienced bloating. Upper digestive endoscopy: normal. In April 2018, she was hospitalized due to ascites, with positive oncotic cytology. A biopsy confirmed peritoneal carcinomatosis (GATA3 and estrogen/progesterone receptors [ER/PR] positive; CDX-2 and HER2 negative). At initial staging, diffuse gastric thickening compatible with carcinomatosis. In the right breast, nodule measuring 1.6x1.0x0.8cm, biopsy confirmed invasive lobular carcinoma (ER 95%, PR 95%, HER2 negative, Ki67 15%). In May 2018, she received 5 cycles of Carboplatin + Paclitaxel with reduction in ascites and partial response at restaging. She then came to our institution and was started on Tamoxifen (as her estradiol levels were high due to hormone implants that were not able to be removed) and Palbociclib in August 2018. However, at this time, diplopia was present, and she reported it had started before chemotherapy. Cerebrospinal fluid (CSF) cytology was positive for neoplastic cells, and there were suspicious findings on cranial MRI: heterogeneous contrast uptake in the right cingulate gyrus with no parenchymal brain metastases (BM). She received 9 cycles of intrathecal (IT) methotrexate (12mg) from August 2018 until January 2019, with improvement of diplopia. CSF after 8 weeks of IT therapy was still positive, but it was negative in February 2019, with no suspicious findings on new cranial MRI. She remained on Tamoxifen and Palbociclib with stable disease (SD) in the breast, with no other measurable site. In January 2021, she developed epigastric pain. An endoscopy showed an area of infiltration in the duodenum, biopsy confirmed lobular BC. She underwent radiotherapy with resolution of her symptoms. Palbociclib was maintained and Tamoxifen was switched to Letrozole as her estradiol levels were indetectable. In October 2021, she presented a new progression of duodenal disease, and liposomal doxorubicin was administered for 6 cycles until April 2022 with partial response. Afterwards, she continued hormonal treatment with Fulvestrant and Alpelisib (PIK3Ca mutation on NGS). Due to pneumonitis, Alpelisib was suspended in June 2022. In November, Fulvestrant was also suspended due to continued worsening of pneumonitis. She was maintained on treatment holiday due to previous toxicities. In April 2023, she had SD, measurable only in the breast, being maintained with no oncological treatment. However, she continued to worsen her previous pulmonary condition (pleuropulmonary fibrolastosis) and died in August 2023.
Discussion: The patient above presented non-bulky LMD, with no hydrocephalus, and with no parenchymal BM, deriving clinical benefit with improvement in diplopia and survival of 59 months after the diagnosis of LMD. No relapse was observed in the central nervous system after IT therapy. The patient died from non-oncological causes with no disease activity while on drug holiday.
Conclusion: This patient with invasive lobular carcinoma and non-bulky LMD, no hydrocephalus and no parenchymal BM had a longer survival than most patients with LMD.

She received both IT and systemic therapy. This case reinforces the importance of seeking appropriate criteria for selecting patients with potential benefit from treatment.

P1-10-23: Management of Malignant Adenomyoepithelioma of the Breast

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Introduction: Adenomyoepithelioma (AME) of the breast is a biphasic tumor comprised of epithelial and myoepithelial components. AME is typically benign, but rarely can be malignant, with malignant cells arising from either the epithelial or myoepithelial components of the tumor. Malignant AME typically presents in post-menopausal women as a single palpable mass or is detected on screening mammography. Because malignant AME is very uncommon and there are few published case reports, there is limited data available to inform treatment guidelines. While surgical excision with negative margins is currently the accepted management for malignant AME, the role of adjuvant chemotherapy and radiation therapy remains unclear.

Case Description We present a case of a 57-year-old woman with screen detected malignant AME of the left breast. Screening mammography revealed a high density 2 cm left breast mass with obscured margins. On ultrasound, the mass was hypoechoic with microlobulated margins and posterior acoustic enhancement, BI-RADS 4, measuring 2.0 x 1.3 x 1.9 cm. Ultrasound-guided core needle biopsy revealed a myoepithelial tumor with atypia. The patient underwent a left breast excisional biopsy, which revealed malignant AME with necrosis, ER 7%, PR-, Her2-, and Ki-67 of 45%. The plan was for re-excision and sentinel node biopsy, after multidisciplinary discussion. Post-operative MRI revealed no residual disease. Re-excision and sentinel lymph node biopsy revealed clear margins and two benign lymph nodes. The patient is currently receiving adjuvant dose dense doxorubicin/cyclophosphamide, followed by paclitaxel for 12 weeks. She will undergo adjuvant radiation therapy. Endocrine therapy was excluded given the weak hormone receptor positivity.

Discussion We present a case of a woman with malignant AME. This tumor is rare – to the best of our knowledge, there are fewer than 100 cases reported in the literature. A three-tier classification of AME has been proposed – benign, atypical, and malignant AME (with malignant encompassing in situ and invasive tumors) – to help guide further management. PIK3CA and HRAS hot spot mutations are found in both benign and malignant AME and vary according to ER status, with HRAS found only in ER negative AMEs. Malignant AME tumors have the potential to metastasize to the lung, brain and liver. Since this tumor was essentially triple negative with a high Ki-67, adjuvant chemotherapy and radiation were recommended. Since the treatment recommendations for malignant AMEs are not standard, we propose starting an international case registry to help further characterize this rare tumor, and to identify the ideal treatment recommendations to maximize benefit and minimize harm.

P1-10-24: Managing Heterogeneous Metastatic Breast Cancer: A Clinical Case Vignette

Sharonlin Bhardwaj, Fang Fan, Joanne Mortimer

The behavior of metastatic breast cancer (MBC) is diverse and frequently unpredictable, posing considerable therapeutic challenges. This clinical vignette describes the path of a 42-year-old woman diagnosed with de novo metastatic breast cancer, whose single tumor population likely evolved into a heterogeneous disease over four years.

In October 2020, the patient was diagnosed with de novo invasive ductal carcinoma (IDC) of the left breast. Imaging revealed symptomatic bone metastases in the cervical (C7) and thoracic (T1, T2, T5, T6) spine, necessitating surgery (anterior cervical corpectomy and fusion C5-T1) followed by adjuvant radiation to the spine. A left breast 2:00 biopsy at diagnosis indicated estrogen receptor-positive (ER+ 95%), progesterone receptor-negative (PR- 5-10%), HER2/neu score 0, and a 50% proliferative index (Ki-67) via immunohistochemistry. HER2/neu FISH showed no amplification, with an average of 1.6 ERBB2 signals per cell and an ERBB2 to D17Z1 ratio of 0.7.

In November 2020, the patient was initiated on palbociclib, letrozole, and pembrolizumab as part of a clinical trial for first-line treatment of her metastatic breast cancer, which she tolerated well with manageable side effects. Additional left breast biopsies taken in December 2020 for research purposes, as part of the clinical trial, confirmed IDC with ER+ 100%, PR- 0%, HER2/neu score 2+, and >20% Ki-67. These samples also tested negative for HER2/neu amplification (ERBB2 signals 1.5, ratio 1.1).

By August 2021, imaging showed the left breast mass and associated left axillary lymphadenopathy had resolved with treatment. However, a recurrence was detected in September 2023 in the prior tumor bed, marked by a biopsy clip. This mass gradually grew, producing satellite lesions and causing ipsilateral left axillary lymph node re-growth. By May 2024, disease progression was evident with an enlarging left breast mass and left axillary lymphadenopathy, and she was taken off the clinical trial. Despite the local progression, her bony lesions remained sclerotic with stable uptake since at least 2022.

On June 14, 2024, the patient underwent a repeat biopsy of the left breast mass as part of the end-of-study requirements. The ultrasound-guided biopsy of the left breast 2:00 mass revealed two distinct tumor populations. IHC staining for both ER and Her2 showed a clear linear demarcation between the two tumor populations. Population 1 was ER 5%, PR 0%, HER2 3+, FISH positive (ERBB2 copy number 18.2, ratio 9.8), Ki-67 30-40%, while Population 2 was ER >95%, PR 0%, HER2 1+, FISH negative (ERBB2 copy number 1.5, ratio 1.1), Ki-67 30-40%. Left axillary lymph nodes were not biopsied.

Given the dynamic nature of her illness, we reviewed her case in a multidisciplinary tumor board. We reached a consensus for local control through lumpectomy and targeted lymph node dissection. Meanwhile, the patient will receive perioperative trastuzumab infusions and will continue letrozole. She will also be initiated on an alternative CDK4/6 inhibitor, ribociclib.

This case underlines the significance of individualized treatment programs for the management of metastatic breast cancer. The coexistence of different tumor types within a

single lesion presents therapy challenges, necessitating regular disease reassessment and therapy adjustment to maintain effective disease control. Additionally, this case raises the question of whether treatment-related pressure from this patient's unique first-line treatment combination with immunotherapy may have influenced her development of HER2/neu gain. This is otherwise uncommon in the general population of hormone-positive metastatic breast cancer.

P1-10-25: Metachronous triple negative breast cancer in a young patient with aggressive presentation of metastatic HER2 positive breast cancer: treatment considerations and management

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Background: Breast cancer (BC) is a heterogenous disease with 3 major subtypes which can be further classified into different molecular subtypes with differing treatment responses. Patients may present with more than one of these subtypes simultaneously and executing a cohesive treatment plan to address the heterogenous nature of this disease is essential.
Clinical Case: This is a case of a 40 year old female who presented with a large left breast fungating mass. She underwent a biopsy that revealed IDC, grade 3 with necrosis, Ki67 40%, estrogen receptor negative (ER- [<1%]), progesterone receptor negative (PR- [<1%]), HER2+ (immunohistochemistry [IHC] 3+). Imaging noted a 17cm x 8.8 cm left breast mass extending to inferior axilla, subpectoral region and skin surface with edema; left supraclavicular (SCV) nodes up to 2.9cm. Bone scan was negative. She was found to have a DVT in her left upper extremity due to disease burden causing lymphatic obstruction. Prior to start of chemotherapy, she developed dysarthria with right facial droop and right upper extremity drift. She received IV thrombolytic therapy due to CTA showing distal LM2 vs M3 occlusion with improvement of symptoms. MRI brain showed small left MCA territory strokes with an enhancing mass in the left cerebral peduncle consistent with metastasis. She completed gamma knife radiosurgery (GKRS) to a left brainstem lesion in 1/2023.
She started 1st line therapy for HER2+ metastatic breast cancer (MBC) using Docetaxel, Trastuzumab and Pertuzumab in 1/2023. Repeat brain MRI in 5/2023 noted 5mm x 2mm left cerebral peduncle lesion, decreased from prior, with no new lesions. Chest CT reported progression in the left breast primary tumor. She received palliative RT to the left breast to 40.05Gy in 15 fractions. Her course was complicated by recurrent DVT and anticoagulant failure, now on a low molecular weight heparin.
She was doing well until she presented to the ER in 7/2023 with fever and right axillary pain. Imaging revealed new right large SCV and right axillary lymphadenopathy. The known left breast/chest wall disease was improved. She underwent a right axillary biopsy which revealed IDC, grade 3, ER-(0%), PR- (0%), HER2- (IHC 1+), Ki-67 70%. Restaging scans remained free of distant disease. However, brain MRI showed development of numerous superficial enhancing lesions in addition to a lesion within the right basal ganglia and persistence of the lesion in the left cerebral peduncle. She was neurologically intact and

subsequently underwent GKRS to 9 lesions.
Given the diagnosis of right sided triple negative breast cancer (TNBC), treatment involved determination of therapeutic options that would simultaneously treat her TNBC and her HER2+ BC. Tumor genomic profile testing was performed which yielded no actionable targets.
Treatment was switched to Trastuzumab Deruxtecan (T-DXd) in 9/2023. She has remained on this regimen to date with resolution of right SCV and right axillary disease, resolution of left breast fungating mass with significantly improved residual left axillary wound defect and continued disease control in the brain.
Discussion: The decision to initiate T-DXd was made due to benefit of T-DXd in the treatment of both HER2+ and HER2 low MBC. In the DESTINY 02 clinical trial in HER2 + MBC, T-DXd showed a longer median overall survival (OS) of 39.2 months compared to 26.5 months with physician's choice therapy. In DESTINY 04 clinical trial in HER2 low MBC, T-DXd led to OS of 23.4 months compared to 16.8 months with physician's choice therapy. In addition, the TUXEDO-I trial demonstrated that T-DXd showed efficacy in patients with active brain metastases, yielding intracranial responses in 73.3% of the population with median progression-free survival of 14 months. In conclusion, this unique case highlights the temporal heterogeneity of breast cancer and supports the use of T-DXd in the treatment of this patient with metachronous TNBC (HER2 low) and HER2+ MBC to the brain.

P1-10-26: NSTI of the breast ; a rare and potentially fatal surgical emergency

Melissa Rangel, Elizabeth Marcus, Micheal Stover

Necrotizing soft tissue infections (NSTI) are true surgical emergencies. When diagnosed quickly and correctly lifesaving surgery is the standard of care. NSTI of the breast is a rare presentation of the disease, increasing the risk for a delay in diagnosis. We present a case of a 63 year-old female presenting with septic shock in the setting of a necrotizing soft tissue infection with actinomyces of the left breast requiring emergent surgical debridement.
Patient was a 63 year old female with insulin dependent diabetes (A1C of 11) who presented with several weeks of increasing pain and overlying skin changes. On admission to the ER she hypotensive and tachycardic, which improved with some volume resuscitation. WBC was 22, sodium 125, blood glucose was 366. She was stated on vancomycin, clindamycin and zosyn in the emergency department. CT scan was obtained showing extensive subcutaneous emphysema of the left breast as well as soft tissue thickening.
The patient was taken to the OR for debridement and essentially central mastectomy of the breast. Thick eschar was encountered over the nipple areolar complex with underlying necrosis. On exam in the OR, it was clear that a standard I&D was not possible due to extent of the necrosis and involvement of the nipple areolar complex. Instead, the cavity was incorporated in the excision without entering it, which involved taking the entire nipple areolar complex. Flaps were raised with some superior breast tissue preserved, essentially

making a thick flap. All inferior tissue was necrotic and was excised down to the inframammary crease. The breast was reflected off the underlying pectoralis major muscle, which appeared pink and reasonably healthy. The wound was packed. Subsequently, the patient returned to the OR for skin edge debridement and eventual wound vac placement and closure with plastic surgery. Once the wound bed appeared healthy and her hyperglycemia had been corrected, the patient underwent closure with drain placement. Final pathology showed necrotic skin and soft tissue. Wound cultures grew out actinomyces and the patient was started on Augmentin for a 4 month course, per recommendations of infectious disease. She followed closely post operatively with her PCP for close hyperglycemic control.

Management of NSTI of the breast requires a high index of suspicion, quick diagnosis and aggressive surgical management. Other authors have described a similar approach with mastectomy and delayed closure with multidisciplinary collaboration in patient care.

P1-10-27: NTHL-1 Syndrome in a Breast Cancer Survivor: Malignant Pleural Effusion Unveils Metastatic Colon Adenocarcinoma

Marian Varda, Lauren K Lewis, Charity A Huang

NTHL1-associated tumor syndrome is an autosomal recessive disorder stemming from mutations in the NTHL1 gene, initially linked with colorectal cancer but now recognized to elevate risks for various malignancies like breast, bladder, and endometrial cancers. This syndrome, characterized by NTHL1-associated polyposis and multiple adenoma development in adults, affects an estimated 0.014% of the population. NTHL1 encodes a DNA glycosidase crucial in repairing oxidatively damaged DNA via the base excision repair pathway.

We present a case which highlights the complex and diverse nature of NTHL1-associated tumor syndrome in a 40 year old premenopausal woman who developed ER+PR+HER2- invasive ductal carcinoma of the left breast in 2021. Subsequently, she underwent a left mastectomy and sentinel lymph node biopsy in May 2021, with one out of three sentinel lymph nodes positive for metastatic carcinoma. Post-operatively, she completed four cycles of docetaxel and cyclophosphamide chemotherapy followed by radiation therapy and tamoxifen. Post-surgical staging was determined as (m)pT2pN1a(sn)Mx. Two years after her breast cancer diagnosis, she developed new-onset dyspnea. Further investigation revealed a pleural effusion with pathology consistent with colon adenocarcinoma, despite no primary lesions being identified during subsequent EGD and colonoscopy. Genetic testing uncovered a heterozygous NTHL1 recessive mutation. The patient was initiated on first-line therapy for metastatic colon cancer and eventually passed within 6 months of therapy.

Heterozygous carriers of NTHL1 mutations face cancer risks, with emerging evidence suggesting increased breast cancer susceptibility, though precise risk estimates and management changes remain unclear. Recognition of NTHL1 syndrome's clinical heterogeneity and overlaps with other hereditary cancer syndromes underscores the

importance of early genetic testing for accurate diagnosis and management. Despite its rarity, increasing awareness and research are vital to comprehending and managing this complex syndrome.
Recent studies highlight that heterozygous NTHL1 carriers may confront notable cancer risks, such as colorectal and breast cancers. However, current literature lacks surveillance guidelines for these individuals. Understanding the prevalence and clinical implications of both heterozygous and homozygous NTHL1 mutations is critical for effective risk assessment, genetic counseling, and clinical management. Further research into molecular mechanisms and gene-environment interactions is essential to delineate how these mutations influence disease progression.

P1-10-28: Pembrolizumab Monotherapy for a Patient with Lynch Syndrome (LS) and Stage 2 Triple-negative Breast Cancer (TNBC): A Clinical Vignette

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Introduction: LS is an inherited cancer syndrome associated with germline variants in genes responsible for repair of specific errors in the DNA [mismatch repair (MMR)]. LS confers an increased risk of several types of cancer, including colorectal (CRC), endometrial, and other GI malignancies; breast cancer has been controversial. Defects in mismatch repair lead to microsatellite instability (MSI), a condition of genetic hypermutability. Tumors with high MSI (MSI-H) tend to exhibit extensive mutations, which can lead to the production of neoantigens that, in turn, respond to immunotherapy. Thus, patients with LS and MSI-H CRC often have excellent responses to immune checkpoint inhibitor (ICI) monotherapy. Breast cancer is less common in patients with Lynch syndrome. Data on the response of LS-associated or MSI-H TNBC are limited. Biomarkers predictive of response to ICI represent an unmet need in TNBC care.
Case presentation: 69-year-old woman with a prior history of an unspecified gynecologic malignancy in the early 2000s (treated in China, records unavailable) and locally invasive rectal adenocarcinoma in 2022. Germline genetic testing revealed pathogenic variants of MSH6 and MSH3, confirming the diagnosis of LS. In August 2023, the patient was diagnosed with a left breast TNBC, measuring 4.5 x 5.0 cm on ultrasound (US). Concurrently, she was found to have recurrent metastatic rectal cancer with presacral disease and peritoneal implants, chronic sacral osteomyelitis, and a pre-sacral abscess, ultimately requiring long-term antibiotics. Immunohistochemistry performed on peritoneal implant tissue revealed loss of nuclear expression of MSH2 and MSH6.
Given her advanced CRC and increased risk for infections with chemotherapy, she began treatment with pembrolizumab monotherapy. After one cycle, the breast mass measured 3.0 x 2.5 cm; after two cycles, the mass was no longer palpable. Repeat PET/CT and breast US in January 2024 showed complete radiographic resolution of the breast mass, decreased avidity and size of the presacral tumor, and no avid adenopathy in the pelvis or abdomen,

indicating excellent treatment response. In July of 2024, she continues to have no evidence of disease in the breast, and the CRC remains stable. Molecular studies of the TNBC from Foundation®CDx (Foundation Medicine, Inc) demonstrated an extremely high tumor mutational burden (TMB) of 794 Muts/Mb, with 66 distinct genetic mutations, including BRCA2 R2336H/E1455/E292, PIK3CA R108H, and POLE L424P.
Conclusion: This is a unique case of a patient who carries two pathogenic variants in genes associated with LS (MSH6 and MSH3), who developed metastatic recurrent rectal adenocarcinoma, as well as synchronously diagnosed stage 2 TNBC, with a complete clinical response in breast cancer to pembrolizumab.
This case highlights the significant relationship between TMB and its utility as a predictive indicator of response to immunotherapy. It confirms prior studies suggesting that it is a promising biomarker, with the limitation that high TMB is rare in TNBC. It is notable that a complete response with pembrolizumab monotherapy was attained, thereby circumventing the toxicities associated with chemotherapy. This prompts us to consider if there may be value in testing breast cancers for MSI-H status, as this can suggest an increased likelihood of response to ICI. Furthermore, a question is raised about the possibility of avoiding chemotherapy for patients with MSI-H tumors, as has been demonstrated for patients with CRC.
Of note, the patient was found to have somatic pathogenic variants in BRCA2, which were likely a result of MMR deficiency in the setting of LS. However, the presence of these targetable mutations suggests that the tumor may respond to PARP inhibitors, which we will consider as a subsequent line of therapy.

P1-10-29: Periprosthetic breast implant seroma: a case report and discussion on BIA-ALCL

Dominic Collins, Diane M. Krutzler-Berry

Background: Late sequelae of breast implants, such as anaplastic large cell lymphoma, seroma and double capsules are rare. Breast implant associated anaplastic large cell lymphoma (BIA-ALCL) is an especially rare adverse outcome of breast implants that present late and can lead to poorer prognosis. This report outlines a case of late periprosthetic breast implant seroma while focusing on the diagnosis and management of BIA-ALCL.
Case Presentation: A 49-year-old Caucasian female presented to the clinic with significant pain and bluish discoloration of her left breast. Past medical history is significant for diagnosis of stage IIA T2N0M0 ER+/PR-/HER2- left breast cancer and stage IA T1N0M0 ER+/PR+/HER2- right breast cancer without axillary lymph node metastasis in 2019. The patient underwent bilateral mastectomy with placement of textured implants followed by adjuvant endocrine therapy with letrozole. Subsequent revision of the left breast implant was performed in 2020 due to grade 3 capsular contracture. Given her prior history and continued capsular contracture with the left breast implant, concern was present for breast cancer recurrence. Patient underwent MRI evaluation of the left breast which noted a hyperintense peri-implant signal on T1 images circumferentially. A thickening of 2.5cm was

noted inferiorly and posteriorly – BIRADs 4 designation was assigned to the breast following these findings. Due to the distinct clinical presentation of the patient, there was suspicion for BIA-ALCL. CT guided 17-gauge needle biopsy was performed allowing for multiple core samples of the lesion. Analysis of the core needle biopsy revealed degenerated acellular material that was consistent with fibrin. The patient then underwent subsequent removal of the left breast implant with a complete left breast capsulectomy. Pathology report following the complete breast capsulectomy indicated a paucicellular specimen with necrotic cells revealing bilateral fibroadipose tissue with chronic lymphoplasmacytic inflammation and fat necrosis without malignancy. Flow cytometric analysis with CD45 and side scatter properties of the lymphocytes of the left chest wall capsule and fluid revealed no significant findings, thus indicating no presence of BIA-ALCL. B-lymphocyte markers such as CD19, CD20, CD23 were negative in the sample. An elevated CD5 ratio was present in the sample most likely indicating a reactive T-lymphocyte mediated inflammatory process.
Conclusion: Although the patient presented in this case was negative for malignancy, it is important to outline the significance of her presentation regarding suspicion of breast implant associated anaplastic large cell lymphoma and the subsequent work up which led to her diagnosis of chronic lymphoplasmacytic inflammation and fat necrosis. While BIA-ALCL is a rare complication of breast implants with only 134 reported cases, it is essential to distribute information pertaining to cases like this to help better refine the clinical presentation for the disease. Given the multidisciplinary nature of breast care, reported cases such as this one will help aid physicians across various fields in gaining a better understanding of what signs and symptoms to be aware of in their patient populations; potentially leading to a diagnosis of an exceptionally rare, but life threatening, condition.

P1-10-30: Pulmonary vein thrombi in metastatic breast cancer: a management dilemma

Kriti Ahuja, Riya Patel, Jayasree Krishnan, Shipra Gandhi

Background: Pulmonary Vein thrombosis (PVT) is an infrequently reported complication in oncology, particularly so in breast cancer, with potentially devastating complications particularly due to the risk for intracardiac extension, systemic embolization and potential stroke. We present what we believe to be the second reported case in literature. PVT may be asymptomatic or present with cough, hemoptysis, dyspnea, weight loss, pleuritic chest pain or rarely with transient ischemic attack/stroke, end organ infarction or sudden cardiac death. It is diagnosed by imaging including CT Chest, echocardiogram, or occasionally cardiac MRI. There is no clear consensus on management strategy and it usually entails a multidisciplinary approach with surgical resection, systemic or radiation therapy to reduce tumor burden, as well as anticoagulation.
Case Presentation: Here, we share our experience with extensive PVT with cardiac extension in a patient with metastatic triple negative breast cancer and a systematic review of similar cases reported to date. Our patient was a 63 year old female with ER/PR negative

HER2 low (1+ by IHC) breast cancer initially diagnosed as pT3 N0 Mx, managed surgically, who subsequently developed recurrence with metastatic disease to left upper lobe of the lung). She progressed on multiple systemic treatments, including atezolizumab with nab paclitaxel, eribulin, capecitabine, sacituzumab govitecan, and a clinical trial. She was also initiated on therapeutic anticoagulation with apixaban for a PICC line thrombus. While on trastuzumab deruxtecan, a restaging scan demonstrated development of tumor thrombus in the left superior pulmonary vein which progressed to involve the left atrium. At the time, anticoagulation was continued and surgical management deferred. Given the development of tumor thrombus, in order to control further invasion into the heart, the thrombus was irradiated and systemic therapy was switched to carboplatin and gemcitabine, with resultant partial response. To prevent further growth and invasion of the tumor thrombus, a complete sternotomy, left atrial thrombectomy, left upper lobectomy and ligation of superior pulmonary vein was performed. Pathology of the left atrial mass was consistent with metastatic breast cancer. She recovered well from the surgery with a well healed surgical site. Later, due to infection at the surgical site, she developed sepsis which excluded her from active treatment.
Discussion: We performed an extensive literature search which revealed that most cases occurred in lung cancer, or in metastasis from choriocarcinoma, leiomyosarcoma, hepatocellular cancer, or renal cancer among others. Only one such case in breast cancer seems to reported thus far. We evaluated management strategies in 21 cases which included resection occasionally followed by chemotherapy/radiation (11) with 1 mortality from perioperative stroke, chemoradiation (2), chemotherapy (4), gamma knife radiation therapy (1). Two patients died without any cancer-directed treatment. The patient with breast cancer underwent embolectomy, thereafter, opting for domiciliary care. Some patients received anticoagulation[RP1] in combination with cancer directed care. Overall, surgical resection appears to be the most common approach, followed by chemotherapy, chemoradiation and radiation, respectively with similar success rates in the cases we reviewed. Anticoagulation is usually utilized as a temporizing measure when employed.
Conclusion: PVT in metastatic breast cancer is an extremely rare but lethal complication. Early diagnosis and multimodal approach, focusing on treatment of underlying malignancy to reduce the disease burden with careful consideration of risks and benefits of surgery is essential in treating these patients.

P1-11-01: Immune Infiltration Correlates with Transcriptomic Subtypes in Primary ER+ Invasive Lobular Breast Cancer

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Background: Understanding the interplay between breast cancer and its microenvironment is crucial for improving treatment strategies. While immunotherapy has shown promise in triple-negative breast cancer, its role in estrogen receptor-positive, HER2-negative (ER+/HER2-) breast cancer, particularly invasive lobular carcinoma (ILC), remains

understudied. The immune landscape in ILC and its prognostic implications need further exploration.

Methods: We conducted a comprehensive analysis of RNA sequencing (RNA-seq) and multiplex immunohistochemistry (mIHC) data from 21 primary ER+/HER2- ILC tumors. RNA-seq was performed on all samples, and mIHC was conducted on 13 tumors from the same formalin-fixed, paraffin-embedded (FFPE) blocks. Transcriptomic subtypes were identified using unsupervised clustering and TCGA classifiers, while immune infiltration patterns were derived using non-negative matrix factorization (NMF) clustering.

Results: Our analysis revealed two distinct transcriptomic subtypes: proliferative and non-proliferative. The proliferative subtype, characterized by higher expression of cell-cycle pathways, showed increased immune infiltration compared to the non-proliferative subtype, which exhibited higher stromal scores. Five unique immune infiltration patterns were identified, with the proliferative subtype predominantly associated with immunosuppressive regulatory T-cells and macrophages.

We further defined a tumor-associated macrophage (TAM)-Low signature, comprising genes negatively correlated with macrophage infiltration. This signature was enriched in extracellular matrix pathways and showed an inverse correlation with proliferative, pro-inflammatory TAMs (Prolif-TAMs). In public datasets, the TAM-Low signature predicted improved relapse-free and overall survival in ER+ breast cancer. Analysis of the POETIC trial data indicated that the lower Prolif-TAM signature expression was associated with better response to neoadjuvant aromatase inhibitor therapy.

Conclusion: This study provides a comprehensive characterization of transcriptomic subtypes and immune infiltration patterns in ER+/HER2- ILC. The proliferative subtype is associated with a more immunosuppressive microenvironment, while the TAM-Low signature emerges as a promising biomarker for predicting reduced infiltration of pro-inflammatory macrophages and improved patient outcomes. These findings highlight the need for further research to validate the TAM-Low signature and to explore its potential in guiding immunotherapy and endocrine treatment strategies in ER+ breast cancer.

P1-11-02: Risk of Recurrence by Nodal Status and High-Risk Features in Patients with HR+, HER2-, Early Breast Cancer: An Analysis of Real-world Data

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Background: Tumor involvement of axillary lymph nodes (ALN) is the most significant prognostic marker for recurrence for HR+, HER2- early breast cancer (EBC). For node-

positive EBC, most patients (76%) present with 1-3 ALN (N1) disease; however, outcomes for N1 disease are variable. The monarchE trial selected patients at high risk of recurrence based on nodal status and included patients with 4-9 ALN (N2) or ≥10 ALN (N3); however, patients with N1 disease were required to have additional clinicopathological risk features including tumor size ≥5 cm and/or grade 3 disease. To further evaluate the risk of recurrence in patients with N1 disease, real-world data were used to describe outcomes for patients with N1 disease with and without high-risk clinicopathological features. Outcomes for patients with node-negative (N0) disease and N2 and N3 disease were also described for additional context. Methods: This study used the US nationwide Flatiron Health electronic health record (EHR)-derived de-identified database, predominantly with patients from community oncology settings. The database included >15,000 patients diagnosed with EBC from Jan 2011. Data cut-off was September 2020. Eligible patients had: (1) pathological stage I-III, HR+, HER2- disease, (2) undergone definitive surgery for primary breast tumor, and (3) initiated adjuvant ET by March 2020. Patients with N1 disease and high-risk clinicopathological features (i.e., tumor size ≥5 cm and/or grade 3) were assigned to the high-risk group (N1-HRG) and patients with N1 disease who did not meet monarchE criteria were assigned to the non-high-risk group (N1-NHRG). IDFS was defined as time from adjuvant ET initiation to recurrence or death; patients without events were censored at last structured EHR activity date prior to data cut-off. IDFS was estimated using the Kaplan-Meier (KM) method. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for N1-HRG vs N1-NHRG (and vs N0), adjusting for patient and disease characteristics. Additional models evaluated N2, N3, and N1-HRG+N2+N3 each vs patients who did not meet monarchE criteria (NHRG). Patient and disease characteristics were summarized descriptively for all (sub)groups.
Results: 4658 patients met eligibility criteria, with N1-HRG (n=286), N2 (n=161), N3 (n=99), and NHRG (n=3999; N1-NHRG [n=548], N0 [n=3293]). Median follow-up was 43.5 months for N1-HRG+N2+N3 and 42.2 months for NHRG. For N1-HRG, 67% of patients had stage II, 82% had grade 3, and 25% had tumor size ≥5 cm. Five-year IDFS rates were 74% for N1-HRG, 89% for N1-NHRG, and 93% for N0. Recurrence risk for N1-HRG was >2-fold as high as N1-NHRG and N0 with adjusted HR (95% CI) of 2.18 (1.45, 3.29; nominal p-value = 0.0002) and 2.97 (2.19, 4.02; nominal p-value <0.0001), respectively. Five-year IDFS rates were 66% for N2, 65% for N3, and 71% for N1-HRG+N2+N3. Relative to NHRG, HR (95% CI) was 3.68 (2.63, 5.15) for N2, 4.03 (2.75, 5.91) for N3, and 3.25 (2.62, 4.03) for N1-HRG+N2+N3. Additional characteristics and KM curves for (sub)groups will be presented.
Conclusion: These real-world data demonstrate that patients with N1 EBC and high-risk clinicopathological features had a recurrence risk of 26% at 5 years which is nearly as high as recurrence risk in patients with N2/N3 disease. In contrast, patients with N1 EBC without high-risk features had a recurrence risk of 11% at 5 years which is similar to N0 disease. Patients that met monarchE criteria (N1-HRG, N2, N3 EBC) had a 5-year risk of recurrence of 29%, supporting the use of adjuvant abemaciclib plus ET in these patients with high-risk EBC.

P1-11-03: The Benefits and Burdens of Adjuvant Endocrine Therapy for Older Individuals with Hormone Receptor Positive Breast Cancer

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Purpose: Adjuvant endocrine therapy (ET) is associated with significant morbidity amongst older women with breast cancer, but evidence on health service utilization is limited. The purpose of this study is to: 1) determine the difference in health care utilization between women who were adherent to ET, non-adherent to ET, and those who never initiated ET, and 2) estimate the risk of recurrence and death.
Methods: We identified a population-based cohort of individuals age >65 years, diagnosed with stage T1N0 HR+, HER negative EBC, treated with breast-conserving surgery (BCS) +/- radiotherapy (RT) between 2012 and 2021. Individuals with prior malignancy or who received chemotherapy were excluded. Exposure to ET was ascertained and proportion of time adherent to ET was calculated for a 24 month exposure period beginning 3 months after diagnosis date. For each case >65% adherent to ET, we matched 1 case <65% adherent and 1 control who did not receive ET on age at diagnosis, stage, type of nodal surgery, receipt of RT, history of diabetes and socioeconomic status. We used hospital records, billing claims and the cancer registry to identify the development of ipsilateral local recurrence (LR), contralateral breast cancer (CBC), breast cancer mortality (BCM) and all-cause mortality. We calculated the cumulative incidence of LR, CBC and BCM adjusting for competing risk of death from other causes and stratified by receipt of RT. We identified primary care and specialist visits, imaging tests, and visits with diagnostic codes for venothromboembolism (VTE), fracture, cardiovascular disease and diabetes to examine health care utilization from 5 years to 1 year prior to diagnosis date, and 3 years following the exposure period. We computed rates per 100 person-years to conduct a difference-in-differences analysis and used negative binomial regression to test change in rates of health services usage between cases and controls.
Results: The study cohort includes 3486 individuals (median age 72; 690 received RT); 1162 were >65% adherent, 1162 were <65% adherent and 1162 did not receive ET. Among individuals treated with BCS alone and were adherent (>65%) to ET, ET was associated with a 5% absolute reduction in 7-year LR risk compared to those who did not receive ET (1.7% (95% CI: 0.3, 5.7) vs 6.7% (95%CI: 3.4, 11.5); p=.09), with no difference in BCM (0% vs 3.1% (95% CI: 1.1, 6.8) p=0.08). Individuals treated with BCS+RT who were adherent (>65%) to ET, had a 2.6% absolute reduction in the 7-year cumulative LR risk compared to those who received no ET (0.5% (95%CI: 0.1, 1.5) vs 3.1% (95%CI: 1.9,4.8); p=.01) with no difference in BCM (0.4% (95% CI: 0.1, 1.2) vs 1.4% (95% CI: 0.7, 2.7); p=0.09). There was no significant difference in 7-year cumulative incidence of CBC between those >65% adherent versus those who did not receive ET (2.0% (95%CI: 1.1, 3.5) vs 4.2% (95%CI: 2.9, 5.7); p=0.22).
Individuals who received ET and were >65% adherent had significantly more visits to primary care (RR=1.1 95%CI: 1.04, 1.1; p<.001), specialists (RR=1.25 95%CI: 1.2, 1.3; p<.001) and gynecologists (R=1.2 95%CI: 1.02, 1.5; p=.03), more visits with a diagnosis of

VTE (RR=17 95%CI: 1.4, 2.0; p<.001), received more CT scans (RR=1.16 95%CI: 1.05, 1.29; p<.001), MRI (RR= 1.15 95%CI: 1.02, 1.3; p=.02), bone mineral density exams (RR=2.0 95%CI: 1.8, 2.2; p<.001) and more ambulatory visits (RR= 1.2 95%CI: 1.1, 1.3; p<.001) compared to those who did not receive ET. Individuals who were <65% adherent to ET had smaller absolute benefits but similar increased burdens.
Conclusions: For older individuals with HR+EBC treated with BCS, adjuvant ET is associated with small absolute reductions in recurrence risk, no difference in BCM and significantly more physician visits and imaging tests. This suggests that for some individuals treated with BCS+RT, the burdens of ET may outweigh the benefits.

P1-11-04: Neoadjuvant Z-endoxifen for Premenopausal Estrogen Receptor (ER)+, Human Epidermal Growth Factor Receptor (HER2)- Breast Cancer (BC): Evaluation of the Pharmacokinetic (PK) Run-in for the EVANGELINE Study.

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Background: Aromatase inhibition (AI) with ovarian function suppression (OFS) is standard for premenopausal women (PrW) with ER+/HER2- primary breast cancer (BC). However, > 40% are intolerant of OFS leaving tamoxifen (TAM) as their only option. In the neoadjuvant endocrine setting, PrW with endocrine sensitive disease (ESD) (week 4 Ki-67 ≤ 10%) have 5-year disease free survival > 97%; however, only 35% achieve ESD with TAM vs 76% with AI+OFS (Gluz SABCS 2023). Z-endoxifen (ENDX) is a selective estrogen receptor modulator targeting ERα at 3-5 ng/ml and PKCβ1 ≥ 500 ng/ml, the latter resulting in AKT inhibition and apoptosis (Jayaraman NPJ Breast Cancer). EVANGELINE (NCT05607004) is an open label, randomized neoadjuvant phase II study with a PK Run-in to evaluate whether ENDX is non-inferior to exemestane plus goserelin in terms of ESD rate. The PK Run-in was designed to identify an ENDX dose that targets both ERα and PKCβ1, defined as day 28-day ENDX steady state concentration (Css) > 500 ng/ml in ≥ 5/6 patients (pts), and to assess antitumor activity and toxicity. In the PK Run-in, pts received either ENDX 40 mg/day (as monotherapy) or 80 mg/day (+/- goserelin). Pts with ESD remained on ENDX for 6 cycles (24 weeks) followed by surgery. We reported (Goetz AACR 2024) that among 7 pts (age 28-51; median 46) enrolled at the 40 mg/day dose, the median ENDX 28-day Css was 264 ng/mL (range:180-377), and ESD rate was 86% (6/7 pts), with either week 4 Ki-67 remaining ≤ 10% (n=3) or decreasing to ≤ 10% (n=3) with 1 pt remaining >10%. Grade 2 or higher AEs included grade 2 amenorrhea (n=2), grade 2 hot flashes (n=1) and grade 2 hypertension (n=1). The 6 pts with ESD completed 24 weeks of ENDX followed by surgery. Here we present the surgical findings of these 6 pts including Cell Cycle Arrest (CCA),

Residual Cancer Burden (RCB), as well as the preliminary 4 week ESD rate for the 80 mg/day +/- goserelin cohorts.
Methods: All pts who started protocol treatment are included in the summarized results. Results: The 6 pts treated with 24 weeks of ENDX 40 mg/day underwent surgery with no complications. cT stage was T2 in 5 pts and T3 in 1 pt. Central review of pre- and week 24 breast MRIs using RECIST criteria found tumor diameter reductions of 15 to 100% resulting in 1 CR, 1 PR and 4 SD. Median pre-Ki67 was 11 (range: 4-33%). Surgical CCA (Ki67 ≤ 2.7%) rate was 67% (4/6). The remaining 2 pts had Ki-67 of 3% (n=1) or in the patient with a near pCR, too few cells to quantitate (n=1). RCB was 1 (n=1), II (n=3), and III (n=2). PEPI scores will be reported at the meeting. At the 80 mg/day dose, ten pts were randomized to either ENDX alone (n=5, age 44-53; median 46) or ENDX + goserelin (n=5, age 37-52; median 44) with similar ER expression (all > 80%), cT, cN category and tumor grade in each arm. Ki-67 testing was completed pre- and week 4 for 9 pts where pre Ki-67 was ≤ 10% in 3 pts, > 10% in 4 pts, and not available in 2 pts (inadequate tumor cellularity). Following drug treatment, week 4 Ki-67 was maintained or fell to ≤ 10% in 6 pts, remained > 10% (1 pt), was indeterminate in 2 pts (inadequate tumor cellularity). PK data (80 mg/day) was available from the first 9 pts, and demonstrated a median 28 day ENDX Css of 344 ng/ml (range 161-959) with 3/9 pts > 500 ng/ml. At the 80 mg/day dose, there were no grade 3-4 toxicities. Grade 2 toxicities included hot flashes (2 pts), dyspepsia (1 pt), amenorrhea (1 pt), oligomenorrhea (1 pt), and nausea (1 pt). No VTE events were reported. Conclusions: Neoadjuvant ENDX, when administered to PrW, demonstrated substantial antitumor activity when administered as monotherapy (40 or 80 mg/day) or with OFS (80 mg/day). Increasing the ENDX dose from 40 to 80 mg improved the likelihood of achieving an Endx Css > 500 ng/ml without significantly increasing toxicity. We will present the final results from the 80 mg/day cohort at the meeting.

P1-11-05: Molecular and tumor microenvironment (TME) dynamics and correlations with response of estrogen receptor (ER)-positive breast cancer treated with endocrine therapy (ET), avelumab with or without palbociclib

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Background: Cyclin-D kinase 4/6 inhibitors (CDK4/6i) have been shown to improve antigen presentation and recruit activated T cells into the TME, with pre-clinical and clinical data suggesting synergy when combined with immune-checkpoint inhibitors (ICIs). We previously reported the clinical responses of patients (pts) with stage II/III ER+ breast cancer treated with neoadjuvant ET with a programmed cell death ligand 1 (PD-L1) inhibitor (avelumab) with or without a CDK4/6i (palbociclib, palbo) on the ImmunoADAPT trial (NCT03573648). We hereby present our correlative work to identify pts most likely to

respond.
Methods: This is a phase 2 pilot study including pts with stage II/III ER-positive/HER2-negative breast cancer who were randomized 2:1 to receive ET (aromatase inhibitors for post-menopausal pts; tamoxifen+/-ovarian function suppression for pre-menopausal pts) with avelumab, with or without palbo (palbo vs. control arms). We investigated molecular and pathologic characteristics, including baseline stromal tumor infiltrating lymphocytes (sTILs), HER2 scoring, histologic type and grade, ER and progesterone receptor (PR) % expression as well as genetic score (Oncotype Dx [ODx] or Mammaprint [MMP]) testing in association with response to treatment by RECIST 1.1 on MRI (overall response rate). A breast pathologist evaluated histology, grade, and sTILs (0-100%). Standard immunohistochemistry was used for ER, PR, and HER2 scoring; and in situ hybridization (ISH) as indicated by ASCO/CAP for HER2. Genetic scores are reported as either high-risk (ODx ≥26 or MMP high) or low-risk (ODx <26 or MMP low). Descriptive statistics and results from univariate logistic regression are reported. Additional translational correlatives, including imaging mass cytometry (IMC) and spatial transcriptomic (ST) analysis, will be reported at the meeting.
Results: From 2018-2023, 30 pts were enrolled and eligible for the primary analysis, including 20 pts on the palbo arm (1 pt with bilateral breast cancer, response to each breast cancer evaluated separately) and 10 on the control arm. Ten pts achieved an objective response (9/21, 42.9% from the palbo arm and 1/9, 11.1% from the control arm; 1 pt did not receive the final MRI). We observed a numerical trend towards improved responses with lobular/mixed vs. ductal histology, OR 2.0, p=0.402, 95% CI 0.4 – 10.42, and also in lower grade tumors; grade 3 vs 1/2, OR 0.44, p=0.497, 95% CI, 0.02-3.61. Baseline sTILs were calculated in 16 of the samples; 50% of the samples had sTILs <1%. There was a trend towards better responses with low baseline sTILs; sTILs ≥1% vs <1%, OR 0.24, p=0.199, 95% CI 0.02 – 1.93. Genetic testing was performed in 27/30 pts. None of the pts with a high-risk ODx/MMP (n=5) achieved an objective response. All pts with high-risk vs. only 36% of those with low-risk genetic testing had baseline sTILs ≥1%. There were no statistically significant associations between level of ER/PR/HER2 expression and response to treatment.
Conclusions: Responses in the ImmunoADAPT study were largely restricted to the ET+avelumab+palbo arm; lobular histology, lower tumor grade and sTILs <1% demonstrated a trend towards improved responses. None of the pts with high ODx/MMP responded. These observations require further investigation to understand the individual contributions of treatment and biological changes to the TME; serial IMC and ST data will be presented at the meeting.

P1-11-06: Impact of OncotypeDx Risk Categorization & Receipt of Chemotherapy on Survival Outcomes Among Patients with Small (T1mi/a/b) Node-Negative (N0/N0(i+)/N1mi) Hormone Receptor Positive (HR+) Breast Cancer

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Background: Application of genomic assays in clinically low-risk hormone receptor positive breast cancer (HR+BC) is understudied as patients with small (T1mi/a/b) node-negative (N0/N1mi) disease were often excluded from prospective trials. However, use of these tests in real world clinical practice, including OncotypeDx, occurs not infrequently, leading clinicians to question the reliability of the results produced whenever they are performed.
Methods: We aimed to help address this question by conducting a large, retrospective analysis of available survival data within the National Cancer Database (NCDB) for patients with small, node-negative disease. Where OncotypeDx recurrence score (RS) results were available, we categorized patients into low (RS <11), intermediate (RS 11-25), & high risk (RS 26-100) groupings. Additionally, we categorized patients based receipt of adjuvant chemotherapy. The primary outcome of Overall survival (OS) was explored for patients with high-risk disease via univariate analysis (cox proportional hazard models & Kaplan Meier survival estimates). Secondary outcomes included univariate analysis of OS based on OncotypeDx testing (regardless of chemotherapy receipt or risk group) & OS between low, intermediate, & high-risk patients, independent of chemotherapy use.
Results: In total, of the 308513 patients with T1mi/a/b N0/N0(i+)/N1mi HR+BC identified within the NCDB between the years 2010-2020, 18372 (6.0%) had received chemotherapy. Among those chemotherapy recipients who underwent OncotypeDx testing (n=8700), 363 were low risk (4.2%), 3475 were intermediate risk (39.9%), & 4862 were high risk (55.9%). Conversely, 81223 patients with T1mi/a/b N0 HR+BC underwent OncotypeDx testing without receipt of chemotherapy during this period. Of those, 29954 were low risk (36.9%), 48569 were intermediate risk (59.8%), & 2700 were high risk (3.3%). When comparing OS among high-risk patients where chemotherapy was omitted versus administered, a significant reduction in OS was noted with omission (HR 1.73, 95% CI 1.44-2.10, p<0.001), with 5-year OS being 94.9% vs 96.8%, respectively. When comparing OS for patients who underwent OncotypeDx testing versus those who did not, regardless of chemotherapy receipt, there was a significant improvement in OS for those tested (HR 0.45, 95% CI 0.43-0.47, p<0.001), with 5-year OS being 97.2% versus 93.7%, respectively. Furthermore, when comparing OS among low, intermediate, & high-risk patients, regardless of chemotherapy receipt, a significant difference between groups was noted (p<0.001), with high-risk being least favorable.
Conclusions: The findings above suggest that risk stratification may be advantageous, even among otherwise clinically low-risk individuals with HR+BC. Multivariable analysis is

further planned to better examine the association between OncotypeDx risk categories & pathologic features such as tumor size, nodal findings, tumor grade, estrogen receptor expression levels, progesterone receptor expression levels, & the presence of lymphovascular invasion. Clinical factors, such as age, race, ethnicity, & receipt of endocrine therapy will also be examined.

P1-11-07: Associations between Breast Cancer Index Classifications and MSK-IMPACT Genomic Profiles in HR+/HER2- Breast Cancer

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Background: The Breast Cancer Index (BCI) is a gene expression-based signature that stratifies patients based on the risk of overall (0-10 years) and late (post-5 years) distant recurrence (DR). BCI also predicts the likelihood of benefit from extended endocrine therapy in early-stage, HR+ breast cancer (BC). This study aims to explore the correlation between BCI and disease-free interval and possible associations between gene-expression-based classification by BCI and the genomic features of primary breast cancers in patients with HR+/HER2- breast cancer who developed metastatic relapse.
Methods: Primary tumor samples from HR+/HER2- metastatic/recurrent BC patients underwent both BCI and MSK-IMPACT targeted sequencing. MSK-IMPACT identifies somatic mutations, rearrangements, and copy-number alterations in up to 505 cancer genes. Time to DR (TTDR) defined as the time between date of surgery and distant recurrence. Kaplan-Meier survival analysis and Cox proportional hazards regression were used to evaluate BCI's prognostic performance. Pearson's correlation (R) was used for correlation between BCI score and TTDR. Fisher's exact tests were used to identify genomic alterations with significant differences comparing BCI prognostic groups. Due to small sample size, comparisons were not adjusted for multiple testing.
Results: The study includes 107 HR+/HER2- metastatic patients with primary tumors tested with both MSK-IMPACT and BCI: 44% post-menopausal, 60% T2/3, 64% grade 3, 56% lymph node positive (LN+).
Consistent with the nature of this cohort (all experienced DR), BCI classified the majority of the patients (n=94, 88%) as high-risk for DR. The BCI score was negatively correlated with TTDR (R=-0.24, p=0.011). Patients classified as high-risk by BCI tended to have DR earlier than low-risk patients (median TTDR: 2.8y vs. 3.4y; HR=1.95 with 95% CI: 1.05-3.62; p=0.031).
Overall, the genomic profile of the cohort was consistent with high-risk luminal primary tumors, with higher than expected rate of TP53 mutations (35%). Analysis of somatic genomic alterations by MSK-IMPACT revealed a trend that there are more mutations in PIK3CA and TBX3 in the BCI low-risk group compared to the high-risk group (77% vs 46%, p=0.038) and (38 vs 3%, p=0.020), respectively.

Conclusion: In this cohort of high-risk BC patients who all had a DR event, BCI remained prognostic with lower BCI scores associated with longer TTDR. Initial findings from the genomic correlative analysis suggested an association of BCI with certain genomic features. Further analyses with additional samples are ongoing to substantiate these findings.

P1-11-08: Practice patterns and uptake of adjuvant abemaciclib in high risk localized hormone receptor positive, HER2-negative breast cancer

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Introduction: In MonarchE, two years of adjuvant abemaciclib combined with endocrine therapy (ET) improved invasive disease-free survival and distant relapse free survival, with recent data confirming continued benefit beyond the two-year treatment period. The FDA approved abemaciclib in October 2021 in patients (pts) with HR+, HER2- early breast cancer (EBC) with node positive and Ki-67 ≥20%, based on an unplanned MonarchE analysis. The approval was amended in March 2023 to revert back to the intention to treat (ITT) protocol, including ≥4 axillary lymph nodes (ALN) or 1-3 ALN with size >50 mm or grade 3. There is a paucity of real-world data surrounding abemaciclib adoption in the eligible patient (pt) population. In this study we aimed to define practice patterns at Mayo Clinic, a large academic enterprise, to understand adjuvant abemaciclib Electronic Medical Record (EMR) documentation, physician recommendations and pt preferences in abemaciclib utilization.
Methods: As part of an IRB exempt quality improvement project, we retrospectively analyzed a registry database of pts meeting MonarchE eligibility criteria at Mayo Clinic. We analyzed if pts met criteria for adjuvant abemaciclib based on the October 2021 approval and the amended March 2023 approval along with diagnostic and staging information. EMR notes were reviewed for documented abemaciclib discussion, discussion outcome with pts, oncologist prescriptions and timing of drug start. Time dependent analysis related to FDA label indication were based on abemaciclib start within 6 months of adjuvant ET initiation.
Results: 400 pts met MonarchE criteria and initial analysis included 123 consecutive pts in Rochester, MN who started ET between April 2021 and October 2023. The median age at diagnosis was 56 years. Race/ethnicity included 104 (85%) White, 11 (9%) Asian, 7 (6%) Black, and 2 (1.6%) Hispanic. A total of 65 (53%) pts received neoadjuvant systemic therapy including 14 (11%) with neoadjuvant ET. 85/123 (69%) pts had EMR indicated abemaciclib discussion. Of these, 53 met both early and late FDA criteria, 25 pts were eligible only for the original FDA-label criteria and 45 were eligible only based on the March 2023 indication. The proportion of pts meeting each specific criteria included N2/N3 disease (60/123) or N1 with ≥5 cm (32/123), G3 (28/123) or elevated Ki-67 (58/123). Adjusting for time dependence of the FDA approval, 64/87 (73%) pts had a documented

adjuvant abemaciclib discussion in the EMR. Of the 36 pts that met the IIT population but prior to the recent FDA approval, 21 (58%) had a documented abemaciclib discussion. Of the 85 documented discussions, 73 pts were recommended adjuvant abemaciclib. Of the 12 pts that were not recommended abemaciclib, main reasons included adjuvant olaparib in gBRCA1/2 carriers (n=3), comorbidity concerns (n=2), and ET tolerance difficulties (n=2). Of the 73 pts recommended to take abemaciclib, 13 (18%) pts declined. The most common EMR documented reasons for declining included concerns for side effects (n=4), perceived benefit (n=1), cost (n=1) or unknown/other (n=7). 4 pts (3.3%) had a breast cancer recurrence, 3 of these pts had EMR abemaciclib documentation. Of these, all 3 pts developed distant recurrence, 1 was prior to abemaciclib start and 2 were 22-24 months after surgery with intermittent abemaciclib use due to side effects.
Conclusions: In this retrospective study of high-risk HR-positive EBC, an adjuvant abemaciclib discussion was documented in the EMR 69% of the time. Most pts who were recommended abemaciclib did start it, though nearly 20% declined. With the possible approval of a second adjuvant CDK 4/6 inhibitor, prospective real-time capture of practice patterns from the EMR may inform pt management. Data for the full Mayo Clinic Enterprise will be available at the time of the meeting.

P1-11-09: Clinical Outcomes and the 21-Gene Recurrence Score Assay in Early-Stage Breast Cancer Associated with CHEK2, ATM and PALB2 Germline Pathogenic Variants

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Background: The 21-gene recurrence score assay (Oncotype DX Breast Recurrence Score) is widely used to predict the risk of recurrence and guide adjuvant therapy decisions in early-stage hormone receptor-positive, HER2-negative breast cancer (BC). Despite its widespread use, the impact of CHEK2, ATM, and PALB2 germline mutations on clinical outcomes and Oncotype DX recurrence score (RS) remains unclear. This study aims to investigate the relationship between these germline pathogenic variants and Oncotype RS, and to assess their combined impact on clinical outcomes in early-stage BC.Methods:Patients with invasive hormone-positive, HER2-negative BC who underwent the 21-gene RS assay and genetic testing in the Breast Medical Oncology and the Breast Clinical Cancer Genetics Clinic at the University of Texas MD Anderson Cancer Center were identified from the prospectively maintained research database. Clinical and tumor characteristics were analyzed using descriptive statistics. The Chi-square test, Fisher exact test, and Wilcoxon rank-sum test were used to compare the groups where appropriate. Univariate and multivariate Cox regression models were employed to evaluate the relationship between covariates and overall survival (OS) and relapse-free survival (RFS).Results: Between 1996 and June 2023, 5652 patients with Oncotype RS were included in this analysis. Among 796 estrogen receptor-positive, HER2-negative BC patients with Oncotype RS and genetic testing, 22 (2.8%) had an ATM mutation, 18 (2.3%) had a CHEK2 mutation, and 17 (2.1%)

had a PALB2 mutation. The median age at diagnosis was 51 years (22-80), with no statistically significant differences in baseline patient characteristics among the three groups. The overall median Oncotype RS was 17 (0-67). Tumors that were progesterone receptor-negative or with higher nuclear grade had higher Oncotype RS (p <.0001). The distribution of Oncotype RS differed among the three groups, with significantly higher Oncotype RS higher in PALB2 group compared to without pathogenic variants (p =.0047). The PALB2 group also demonstrated a trend towards higher median Oncotype RS in patients aged over 50 (p=.009). In the univariate analysis, older age (>50 years) (HR 0.68, 95% CI, 0.45-1.02; p=.0492), adjuvant endocrine therapy (ET) (HR 0.24, 95% CI, 0.14-0.41; p<.0001), and adjuvant radiotherapy (XRT) (HR 0.49, 95% CI, 0.33-0.74; p<.0001) were associated with better RFS. In contrast, poorer RFS was associated with presence of a CHEK2 mutation (HR 3.02, 95% CI, 1.14-8.01; p=.0263), high Oncotype RS (HR 1.53, 95% CI, 1.01-2.31; p=.0432), lymphatic invasion (HR 2.16, 95% CI, 1.35-3.46; p=.0011) , vascular invasion (HR 2.22, 95% CI, 1.39-3.55; p<.0001), and high Ki67 levels (HR 2.05, 95% CI, 1.37-3.08; p<.0001).Only adjuvant ET significantly improved OS (HR 0.30, 95% CI, 0.13-0.71; p=.006). In multivariate analysis, high Ki67 levels (HR 2.52, 95% CI, 1.45-4.58; p=.0018) were associated with poor RFS, whereas adjuvant ET (HR 0.17, 95% CI, 0.07-3.35; p<.0001) and adjuvant XRT (HR 0.53, 95% CI, 0.30-0.90; p=.0259) were associated with better RFS. The presence of the mutation did not show a statistically significant effect on OS (p=0.6).Conclusion: Our data demonstrate that higher Oncotype RS, CHEK2 mutations, lymphatic and vascular invasion, and higher Ki67 levels were identified as predictors of poorer RFS. Conversely, older age (>50 years), adjuvant ET, and adjuvant XRT were associated with improved RFS. Importantly, the presence of ATM, CHEK2, or PALB2 mutations did not significantly impact OS. These findings highlight the importance of individualized treatment strategies that integrate genetic profiling and clinical factors to optimize outcomes in BC management.

P1-11-10: Current Endocrine/Systemic Treatment Landscape of ER+ HER2 negative early-stage Breast Cancer: First Data from the RESCUE trial

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Background: RESCUE is a prospective, international, multicenter health care study on risk assessment by the clinicomolecular test EndoPredict® and long-term patient outcome in early luminal breast cancer. For patients (pt) with early luminal breast cancer, precise national and international guidelines on (neo)adjuvant systemic therapy exist. Nevertheless, especially regarding endocrine therapies, treatment landscape has been shown to be heterogeneous in real world.

Objective: By analyzing baseline and follow up data from RESCUE, we describe the current (neo)adjuvant treatment landscape for pt with ER+ HER2neg early-stage breast cancer including: type of endocrine agents used, addition of GnRH analogs (GnRHAa)/ovarian function suppression (OFS) to ET in premenopausal pt, use of chemotherapy and use of bone modifying agents (Bisphoshonates or Denosumab) in the adjuvant setting.
Methods: Data from pt, who entered the RESCUE trial between July 2018 and September 2022 at 45 (42 German/3 Swiss) breast centers were collected via electronic case report forms. Pt were eligible, if they had been diagnosed with HR+/HER2- primary invasive breast cancer stage I/II, T1 to T3 with 0 to 3 positive lymph nodes and if they had an EndoPredict®-test within six months before inclusion.
Results: 1143 pt were analyzed. Median age was 57,0 years (range26,0-82,0). 614 pt(53,7%) were classified as pN0. Tumor-Grading was reported as follows: G1 (137pt, 12,0%), G2 (893pt, 78,1%), G3 (111pt, 9,7%). Distribution of Ki-67 was recorded as follows: £10% (228pt, 20,0%), 11-24% (162pt, 14,2%), ^{3}25% (753pt,65,9%). Of the 1134 female pt, 394 were premenopausal and 734 postmenopausal. Systemic treatment recommendations given by the centers´ documented tumor board decisions were as follows: 62,9% (n=248) of premenopausal pt and 61,0% (n=448) of postmenopausal pt were recommended to undergo (neo)adjuvant Chemotherapy (CTX). In the premenopausal cohort, the choice of ET recommendation included an GnRHa/OFS in 14,5% of cases (57pt). In these 57 pt, GnRHa/OFS was recommended to be combined with an aromatase inhibitor as initial ET in 15 cases (3,8% of premenopausal pt) and with tamoxifen in 41 cases (10,4% of premenopausal pt). 47 pt (82,5%) of the 57 pt with GnRHa/OFS-recommendation and 183 pt (54,3%) of the 337 pt without GnRHa/OFS-recommendation were recommended to undergo (neo)adjuvant CTX. Bone modifying agents (Bisphosphonates or Denosumab) were recommended in 10,2% of premenopausal and 32,7% postmenopausal pt. Follow up data on actually received ET (including +/- GnRH) and CTX (including +/- anthracyclines) will be presented in detail.
Conclusion: These real world data show that in spite of existing guideline recommendations for the use of GnRHa/OFS in premenopausal women with early luminal breast cancer at higher risk for relapse, this is not routinely implemented. Therefore, regarding the ET recommendation, this patient cohort might be undertreated. Follow up data on actually received ET (including +/- GnRH) and CTX (including +/- anthracyclines) will be presented in detail.

Sponsor: The study is sponsored by the North-Eastern-German Society of Gynecological Oncology (NOGGO) e.V.

P1-11-11: Pathological Complete Response and Survival Outcomes of Single Hormone Receptor-Positive/HER2-Negative Breast Cancer after Neoadjuvant Treatment and Further analysis of its Intrinsic Biological Features and Immune Landscape

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Background: Prior research has predominantly concentrated on the overarching hormone receptor-positive (HR+)/HER2-negative (HER2-) classification, frequently amalgamating double HR-positive (dHR+) and single HR-positive (sHR+) tumors, consequently neglecting the distinctiveness of the sHR+ subgroup, particularly in the neoadjuvant context. The objective of this study was to evaluate the rates of pathological complete response (pCR) and survival in sHR+/HER2- breast cancer following neoadjuvant therapy, as well as to delve into its inherent biological characteristics and immune profile.
Methods: This study incorporated six cohorts. The first and fourth cohorts were from the Cancer Hospital, Chinese Academy of Medical Sciences (CHCAMS, n=1049), and the Surveillance, Epidemiology, and End Results (SEER, n=21092) database for analyzing neoadjuvant chemosensitivity and survival outcomes. Clinicopathological and subtype data from CHCAMS, SEER, the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC, n=1052), and Fudan University Shanghai Cancer Center (FUSCC, n=570) were utilized to explore intrinsic biological features correlating with the pCR rate and prognosis of sHR+/HER2- breast cancer. Genomic and transcriptomic profiles from METABRIC, The Cancer Genome Atlas (TCGA, n=741), and MSK-IMPCAT (n=1535) were also examined to explore potential associations with endocrine and immunotherapy responsiveness.
Results: Compared with double HR-positive (dHR+, ER+ and PR+)/HER2- breast cancer, sHR+ (ER+/PR- or ER-/PR+)/HER2- disease exhibited a higher pCR rate (20.2% vs. 3.2%, P <0.001) but significantly worse survival (hazard ratio, 2.97; 95% confidence interval, 1.62–5.43, P <0.001) in the CHCAMS neoadjuvant cohort. Clinically, sHR+/HER2- tumors showed higher histological grade and proliferation rate than did dHR+/HER2- tumors, along with a higher HR-low positivity rate (50.9% vs. 3.0%, P <0.001) in primary tumor and showing a preference towards changing to triple-negative tumors in residual disease (42.7% vs. 1.8%, P <0.001). Additionally, patients with sHR+/HER2- breast cancer manifested lower endocrine sensitivity scores, with nearly 20% of them belonging to the PAM50-defined basal-like subgroup. Immunologically, sHR+/HER2- tumors showed higher tumor mutation burden (TMB), expression of immune checkpoint-related genes (PD-1, PD-L1, CTLA4 etc.), and infiltration of tumor-infiltrating lymphocytes (TILs), especially CD8+ T cells, than did dHR+/HER2- tumors.
Conclusion: Patients with sHR+/HER2- breast cancer were characterized by relative sensitivity to neoadjuvant chemotherapy but worse prognosis, resembling triple-negative breast cancer in biological behavior. Immune relatively hot phenotypes of sHR+/HER2- breast cancer revealed a potential to benefit from immunotherapy.

P1-11-12: Early-stage HR+/HER2- breast cancer patients under 50 years old with a low-risk identified by the 70-gene signature (MammaPrintTM) could benefit from ovarian function suppression: A real-world study in China.

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Background: The introduction of multigene assays has significantly altered the indications for adjuvant chemotherapy in hormone receptor positive/human epidermal growth factor receptor 2 negative (HR+/HER2-) early breast cancer. The 70-gene signature (MammaPrintTM) test has demonstrated its ability to identify prognostic capability. Patients with high clinical risk classified as low risk by MammaPrintTM (MP) can be safely spared adjuvant chemotherapy. However, it is worth noting that the majority of clinical studies conducted on MP have predominantly involved Caucasian populations. Breast cancer is known to exhibit biological and clinical differences across different ethnicities and races. In China, the onset of breast cancer occurs a decade earlier than in Western populations, with an average age of diagnosis at 48.7 years and a peak incidence between 45 to 49 years. The prevalence of breast cancer among patients under 50 is significantly higher than in Western countries. Consequently, it is crucial to validate the performance of MP across diverse populations to ensure its generalizability and reliability. This study aimed to explore the real-world utilization of MP among Chinese patients.
Method: From March 2018 to June 2022, genomic analysis utilizing the 70-gene platform, MammaPrintTM, was conducted on a consecutive series of 637 patients. An evaluation was performed to assess the distribution of clinicopathological characteristics across various risk groups as determined by the MP assay. This assessment was further compared with the findings from the pivotal MINDACT trial to indentify any notable differences. A robust analysis was conducted using the Kaplan-Meier method for survival curves and the Cox proportional hazards model to estimate hazard ratios.
Results: Among the 637 patients enrolled in the study, 261 (41.0%) were identified as high risk, with a substantial majority, 214 (82.0%), undergoing chemotherapy. In contrast, 376 (59.0%) were categorized as low risk, with a significantly smaller proportion, 46 (12.2%), receiving chemotherapy. A stark contrast was observed in the distribution of risk categories between the two groups, with a statistical significance ($p < 0.001$). Patients characterized by tumor grade 1, Ki67<30%, and PR ≥ 20% were predominantly classified as low risk. Compared to the MINDACT study population, the patients in this cohort were notably younger, with 33.3% versus 56.2% being under 50 years of age, and had larger tumors with higher tumor grades ($p < 0.001$). Notably, younger patients, those with Ki67≥ 30%, and lymph node-positive patients were more inclined to receive chemotherapy, even when classified as low risk. Conversely, older patients in the high-risk group were less likely to be treated with chemotherapy.
During a median follow-up period of 33 months (8 -66 months), 17 events were recorded. No significant difference in breast cancer free interval (BCFI) was observed between genetically high and low-risk groups for all patients (96.5% vs 97.3%, P=0.28), including

those under 50 years of age (95.5% vs 97.5%, P=0.863). However, among patients aged 50 or older, those classified as genetically low risk exhibited a superior BCFI compared to their high-risk counterparts (98.9% vs. 97.1%, P=0.047).
It was interesting to observe that the BCFI significantly improved following ovarian function suppression in patients under 50 years of age identified as low risk, compared to those without such suppression (P=0.035). Nonetheless, no significant difference was noted between the two groups for high-risk patients (P=0.115).
Conclusion: The real-world data clearly illustrate the benefits of the MP assay in decreasing the necessity for adjuvant chemotherapy in Chinese patients with low genomic risk in HR+/HER2- early breast cancer. The recurrence rates were similar in the high and low risk groups, which might be due to the favorable prognosis of the study population, the effectiveness of tailored treatments in managing breast cancer across different risk profiles and the short follow-up period. The poorer prognosis observed in high-risk patients aged 50 or older might be attributed to the fact that a significant number older patients did not receive chemotherapy.
Our data showed that low-risk patients under the age of 50 could benefit from ovarian function suppression. This finding warrants further investigation.

P1-11-13: Development and Validation of a Deep Learning Model for HR+/HER2- Early-stage Breast Cancer Recurrence Prediction (HERPAI) using Conventional Clinical and Pathological Data: A Real-world Study in Chinese Population

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Background: Escalation of adjuvant endocrine therapy for selected hormone receptor-positive (HR+) human epidermal growth factor receptor 2-negative (HER2-) early-stage breast cancer patients has been a standard-of-care treatment. Effective prognostic model is crucial for identifying patients who have unmet treatment needs and supporting personalized decision-making to improve the patients' outcomes. However, existing prognostic models fallen short when applied to these patients with low recurrence risk, especially among Chinese population. Thus, we sought to develop and validate a deep learning model for HR+ early-stage recurrence prediction (HERPAI) based on conventional clinical and pathological data.
Method: HR+/HER2- early-stage (T1-2N0-1) invasive breast cancer patients who received definitive surgery and followed by endocrine therapy from four independent medical centers were included in this retrospective study. Patients from center 1 were used as derivation cohort (90% in the development and 10% in the internal test cohort), while those from other centers were combined as an external test cohort. A deep learning prognostic model, HERPAI, was developed based on Transformer to predict risk of invasive disease-free survival (iDFS) utilizing clinical and pathological predictors (age, menopausal status, body mass index, family history, breast and lymph node surgery modality, T stage, N stage, histology, grade, expression levels of ER, PR, HER2, and Ki67). Hyper-parameter

random searching was performed using five-fold cross-validation strategy in the development cohort. The model performance was evaluated using Concordance index (C-index) for all of the cohorts, as well as the subgroups defined by menopausal status, N stage, Ki67 expression, adjuvant chemotherapy, and radiation therapy status. Cut-off value for selecting patients with 5-year recurrence risk > 10% was determined in the validation cohort for risk stratification. Hazard ratio (HR) was estimated between risk groups for iDFS. The association between HERPAI score and selected gene were explored using linear regression in the patients with genetic data in the derivation cohort to provide insights into the biological foundation of HERPAI.
Results: A total of 5,424 patients were included in the derivation cohort, of who 4,882 were used as development and 542 were used as internal test cohort, while 942 patients were included in the external cohort. HERPAI yielded a C-index of 0.73 (95% CI, 0.65 to 0.81) 0.73 (95% CI, 0.62 to 0.85), and 0.68 (95% CI, 0.60 to 0.77), in the validation, internal, and external test cohort, respectively. Consistent performances were observed for all pre-specified subgroups in three cohorts. High-risk patients (approximately 25% of the overall population) were associated with an increased risk of iDFS for validation (HR 2.56 [95% CI 1.25 to 5.22], P = 0.01), internal (HR 2.52 [95% CI 0.97 to 6.57], P = 0.06) and external test (HR 1.94 [95% CI, 1.00 to 3.74], P = 0.049) cohort, respectively. Moreover, HERPAI could predict distant recurrence and overall survival with a C-index of 0.68 (95% CI, 0.49 to 0.86) and 0.71 (95% CI, 0.47 to 0.94) in the external test cohort, respectively. In addition, HERPAI outperformed other prognostic factors in predicting iDFS, including T stage, N stage and Ki67 (C index ranged 0.54 to 0.59 in external test cohort). The HERPAI score was significantly associated with GRB7, GSTM1, Ki67, and RPLPO gene expression, after adjusted for age and family history (all P < 0.05).
Conclusions: HERPAI is a promising tool for selecting vulnerable HR+/HER2- early-stage BC patients who are at high-risk of recurrence and may benefit from escalating adjuvant endocrine therapy.

P1-11-14: Hormone receptor positive (ER+) HER2+ Lobular Breast Carcinoma: Molecular Response to Perioperative Endocrine Therapy and Clinical Outcomes in the POETIC Trial

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Background: Invasive Lobular Carcinoma (ILC) represents about 15% of all invasive breast cancers (BC). ILC seldom exhibits overexpression of human epidermal growth factor 2 (HER2+), and majority of research focuses on ER+ HER2-negative phenotypes. There is limited data on response of ER+HER2+ ILC to endocrine therapy (ET) and the therapeutic efficacy of combined chemotherapy and anti-HER2 treatments (C+T). This study aims to report the biological responsiveness of ER+HER2+ ILC to perioperative aromatase inhibitors (POAI) and their clinical outcome within POETIC trial, one of the most

comprehensive studies of the ER+HER2+ BC.
Methods: POETIC trial was a phase III study of post-menopausal patients with ER+ BC, randomized 2:1 to 2-weeks of POAI or control, followed by standard-of-care. This study focused on ER+HER2+ BC (n = 342), 27 of whom had ILC at baseline (B) (17 POAI and 10 control). Ki67 levels were assessed at B and on-treatment (2wk) (low ≤10%; high >10%). Biological response to AI were categorized to LowB -Low2wK (LL); HighB –Low2wk (HL) or HighB–High2wk (HH). Baseline and on-treatment samples were gene expression profiled (NanoString BC360TM) for BC intrinsic subtypes (IS) (Luminal A: LumA, Luminal B: LumB, HER2-enriched: HER2E, Basal) and 46 gene signatures. T-tests were used to compare gene expression profiles (GEP) between ILC and invasive ductal carcinoma (IDC). P-values were adjusted using false discovery rate (FDR). Time to recurrence (TTR) was estimated using Kaplan-Meier method.
Results: Among the 27 ILC cases, the clinicopathological characteristics were similar to IDC. At the molecular level, the frequencies of IS in ILC were 22% (n = 6) LumA, 37% (n = 10) LumB and 41% (n =11) HER2E, compared to IDC being 17% (51/301) LumA, 37% (112/301) LumB, 44% (133/301) HER2E, and 2% (5/301) Basal.
In terms of early response to POAI in ILC, 33% (5/15) were HH, 53% (8/15) were HL and 14% (2/15) were LL. In comparison in IDC, 53% (100/188) were HH, 36% (68/188) were HL and 9% (17/188) were LL.
The 5 HH ILCs were mainly HER2E (1 LumB, 4 HER2E), while most HL were Luminals (1 LumA, 5 LumB, 2 HER2E), and all LL were Luminals, showing the poor anti-proliferative effect conferred by HER2E.
There were no distinct GEP associated with ILC compared to IDC in the hierarchical clustering of gene signature scores. IS and immune signatures drove the distinct expression patterns across the population of ILC and IDC. ILC had lower expression of cell adhesion than IDC (T-test FDR < 0.001). The impact of POAI to molecular changes were comparable based on histological tumor types and were driven by their intrinsics subtypes.
The 5-year TTR survival probabilities of ILC and IDC were 88% and 89.5% respectively. Fifty-two percent (14/27) of ILC received C+T and all remained recurrence free. Out of the 48% (13/27) ILC that did not receive adjuvant C+T and were only treated with ET, three (23%) had recurrence at 0.7, 2.1 and 4.1 years. Sixty-one percent (183/301) of IDC received C+T, of which 8% (14/183) had a recurrence event. Forty-nine percent (118/301) of IDC did not receive adjuvant C+T, from which 17% (20/118) recurred.
Conclusions: This study reported comprehensive study on the molecular features and their clinical behavior of the understudied ER+HER2+ ILC. Our findings suggested there were no distinct molecular profiles between ILC and IDC phenotypes of ER+HER2+ at baseline; their response and molecular changes after POAI were driven by IS as expected. While there was an enrichment of early sensitive tumors to POAI, the combined C+T treatment and ET might represent a good option to preventing recurrence in clinical high risk ILC. However, the small sample size precludes definitive conclusions.

P1-11-15: Enhancing Risk Stratification in HR+ Early Breast Cancer: A Real-World Evaluation of Oncotype Dx and Prosigna

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Introduction: Oncotype Dx (ODX) is the most widely used genomic test for adjuvant treatment decision-making in patients with hormone receptor (HR)+ early breast cancer (EBC). Risk stratification in this clinical setting is crucial and often challenging, thus additional molecular information could significantly enhance patients' management. We hypothesized that Prosigna, a prognostic assay estimating distant relapse-free survival in postmenopausal women with HR+ EBC, could serve as a complementary test to ODX for risk stratification. This study aims to evaluate the concordance between ODX and Prosigna in a real-world scenario.
Methods: A total of 30 postmenopausal HR+ EBC patients, who were previously tested with ODX and classified as low (n=12), intermediate (n=5), and high (n=13) genomic risk, were included. The three risk categories were assigned considering the recurrence score (RS) and lymph node status: low (RS 0-10), intermediate (RS 11-25), and high risk (RS 26-100) for node-negative (pN0), as reported in TAILORx trial; low (RS ≤ 25) and high risk (RS>25) for 1-3 node-positive (N1-3), as reported in RxPONDER trial. For each case, RNA was extracted from the same formalin-fixed paraffin-embedded (FFPE) tumor block that was used for ODX, and subjected to Prosigna testing on a NanoString nCounter® DX Analysis. The Prosigna risk of recurrence (ROR) score also considers lymph node status: for pN0, 0-40 indicates low, 41-60 intermediate, and 61-100 high risk; for N1-3, 0-15 indicates low, 16-40 intermediate, and 41-100 high risk. Descriptive analyses of clinicopathological features were performed. Cohen's Kappa and Spearman (rs) correlation analyses were calculated between RS, ROR, and clinicopathological factors.
Results: The overall agreement between the two platforms was the same as in the TransATAC study (κ=0.32; p<0.001). Considering ODX as the gold standard, a low-high disagreement was found in n=6 (20.0%) cases, while a low-intermediate or intermediate-high disagreement was observed in n=7 (23.3%) cases. The lowest concordance was seen in the ODX low (1/12) and intermediate (3/5) risk groups, with Prosigna tending to assign a higher risk. This tendency was confirmed by the 100% concordance (13/13) in the high-risk group. Prosigna assigned luminal A, luminal B, and HER2-enriched molecular subtypes to n=7, 23.3%; n=22, 73.3%; and n=1, 3.3% cases, respectively. Most luminal B (n=20; 90.9%) cases had high ROR scores, whereas n=12 (54.5%) cases were classified as high RS. A significant proportion of luminal A (6/7; 85.7%) cases were classified as intermediate ROR, while ODX classified n=1 (14.3%) of them as intermediate and n=6 (85.7%) as low RS. Tumor samples with high ROR were more likely G3 (p=0.002), pT2 (p=0.021), and Ki67>20% (p=0.01) in comparison with the intermediate risk ones. ROR showed a strong correlation with Ki67 expression (rs=0.72, p<0.001), whereas RS demonstrated a moderate correlation (rs=0.57, p<0.001). According to available follow-up (F/U) data (median 6

months; range 1-36), recurrence was detected in only one case (G2; Ki67=40%; F/U=14 months), which both assays classified as intermediate risk.
Conclusions: Our study confirms that ODX and Prosigna provide different types of clinical information for postmenopausal HR+ EBC patients, as they analyze distinct genes and pathways. In our real-world cohort, Prosigna generally assigned a higher risk category compared to ODX. Therefore, integrating multiple molecular assays could potentially refine risk assessment and enhance personalized treatment strategies in this patient population. However, it remains to be defined which specific subpopulation might benefit from this integrated approach for molecular testing. Further investigations with larger cohorts and long-term F/U data are needed to validate these preliminary findings.

P1-11-16: Impact of ribociclib dose reduction on efficacy in patients with hormone receptor– "positive/human epidermal growth factor receptor 2–negative (HR+/HER2–) early breast cancer (EBC) in NATALEE”

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Background: The phase III NATALEE trial demonstrated statistically significant and clinically meaningful invasive disease–free survival (iDFS) benefit with ribociclib + a nonsteroidal aromatase inhibitor (NSAI) vs NSAI alone in patients with stage II/III HR+/HER2− EBC that deepened even after all patients stopped ribociclib (HR, 0.715; 95% CI, 0.609-0.840; 4-year absolute benefit, 4.9%; median follow-up, 44.2 months). Here, we present an exploratory analysis of efficacy in patients with and without dose reduction in the NATALEE trial.

Methods: Men and pre- and postmenopausal women with HR+/HER2− EBC were randomized 1:1 to receive ribociclib (400 mg/day; 3 weeks on/1 week off for 36 months) + NSAI or NSAI alone (men and premenopausal women also received goserelin). Key inclusion criteria were stage II or III disease (per AJCC 8th edition); patients with node-negative stage IIA disease (T2N0) were required to have additional high-risk criteria (grade 2 with Ki-67≥20% or high genomic risk; or grade 3). Only one dose reduction of ribociclib (400 mg/day to 200 mg/day) was allowed for the management of adverse events (AEs). Relative dose intensity (RDI; defined as the actual cumulative dose per duration of exposure [adjusted for the 3-weeks-on/1-week-off schedule] divided by the planned dose intensity of 400 mg/day) was analyzed by grouping patients into low, medium, or high RDI tertiles and summarized using Kaplan-Meier methods. A stratified Cox proportional hazards model was used to compare iDFS rates with ribociclib across these tertiles. The data cutoff date was April 29, 2024 (median follow-up, 44.2 months). Additional analyses on efficacy in patients who discontinued ribociclib earlier than the planned 3 years of treatment and additional analysis considering the immortal time bias will be included in the presentation.

Results: Among 2526 patients who received treatment in the ribociclib + NSAI arm, 687 (27.2%) had a ribociclib dose reduction, and 1839 (72.8%) did not. Median time to the dose reduction was 3.3 months. The most common reason for dose reduction was an AE (23.0% [582/2526]), and the most common AEs leading to dose reduction included neutropenia (14.0% [353/2526]), alanine aminotransferase increased (1.9% [48/2526]), and fatigue (1.0% [26/2526]). Baseline characteristics were balanced between those with and without dose reduction. Duration of ribociclib exposure was similar among patients with and without a dose reduction (median, 35.7 months in both groups). RDI did not impact iDFS rates. Patients with low (0 to <82.27%), medium (82.27% to <97.44%), and high (≥97.44%) RDI had similar iDFS rates (low vs high HR, 0.931; 95% CI, 0.690-1.254; medium vs high HR, 0.985; 95% CI, 0.736-1.319).

Conclusions: This post hoc exploratory analysis of NATALEE demonstrated that iDFS benefit was maintained among patients who had a dose reduction of ribociclib and was not impacted by ribociclib RDI. These data suggest that it may be possible to implement a dose reduction of ribociclib to 200 mg/day when necessary to manage AEs without compromising treatment efficacy for patients with HR+/HER2− EBC.

P1-11-17: High Tumor-Infiltrating Lymphocyte Levels Correlate with High MammaPrint® Recurrence Risk in Early-Stage Breast Carcinomas

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Introduction: Tumor-infiltrating lymphocytes (TIL) are linked to responses to chemotherapy and immunotherapy and clinical outcomes, especially in high-risk breast carcinomas. MammaPrint® (MP) and BluePrint® (BP) are genomic tests designed to provide risk stratification and molecular classification for early-stage hormone receptor (HR)-positive breast carcinomas, which could include tumors with HER2-low expression. We investigated correlations between TIL measurements, HER2 status, and MP/BP assays in early-stage HR-positive breast carcinomas.
Materials and Methods: 167 early-stage HR-positive breast carcinomas with known MP/BP risk categorization were evaluated for TIL using whole slide scanned images according to the International TILs Working Group 2014 guidelines. HER2-low breast cancers were identified by IHC scores of 1+ and 2+ without HER2 amplification. A subset of high-TIL, high-risk cases underwent TSO500 (Illumina) next-generation sequencing (NGS).
Results: The patients had a mean age of 51 years, ranging from 26 to 75 years. Among the profiled cases, 97% were either luminal A (96/167) or luminal B (66/167) breast carcinomas, with only five cases classified as HER2-enriched (n = 2) or basal-like (n = 3) carcinomas. Tumor grade was strongly associated with recurrence risk ($p<0.001$). The prevalence of the HER2-low phenotype was 65%, including 46/69 (67%) high-risk cases. TIL levels ranged from 0 to 70% and were low (≤10%) in the majority (75%) of cases in the cohort. However, high TIL levels were more frequently observed in cases with high recurrence risk (56% vs. 39%, $p = 0.03$). Additionally, TIL-enriched high-recurrence risk

carcinomas contained targetable genomic alterations, including PIK3CA, BRCA1, BRCA2, and HER2 mutations.
Conclusions: TIL levels are higher in early-stage HR-positive breast carcinomas with a high recurrence risk. These tumors also harbor targetable genomic alterations, suggesting that TIL measurement and genomic profiling could enhance risk stratification and identify patients who might benefit from targeted therapies. Her-2 low expression in high-risk patients provides a consideration for including novel ADC therapies in this subset of patients.

P1-11-18: Detrimental effect on survival of neoadjuvant (NACT) versus adjuvant (ACT) chemotherapy in stage II-III hormone receptor-positive & HER2-neg (HR+/HER2-) breast cancer: A propensity score-matched (PSM) analysis of a retrospective patient series.

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Background: The use of NACT in stage II-III HR+/HER2- breast cancer are controversial for low rate of pathological complete response (pCR) and is an ongoing debate whether NACT or ACT provides better survival outcomes for these patients.
Patients and Method: Seven hundred sixty-one patients (pts) with HR+/HER2- local advanced breast cancer at stage II-III undergoing NACT (503 pts) or ACT (258 pts) chemotherapy between 2005 and 2021 were identified from our center Fondazione Policlinico Universitario A. Gemelli IRCCS of Rome.
A PSM was employed to create cohorts with balanced characteristics at the baseline, across different categories (age, menopausal status, grading, stage, Ki67, ER, PgR, HER2). The efficacy of NACT and ACT in terms of overall survival (OS) and real-word invasive disease free-survival (rwiDFS) was evaluated using Kaplan-Meier analysis and the Cox proportional hazards model.
Results: Using PSM, a total of 492 pts was ultimately included in the study (246 vs 246). The OS was significantly longer for ACT versus NACT (80.6% vs 98.3% survival rate at 5 years, HR 0.10, 95%CI 0.04-0.23, p <0.0001). The rwiDFS was longer for ACT versus NACT (NR vs 127 months, HR 0.67, 95%IC 0.45-1.0,1 p 0.054).
Pts underwent ACT had significantly better OS in comparison to those achieved pCR (HR 0.24, 95%IC 0.09-0.61) or not-pCR (HR 0.09, 95%IC 0.05-0.15) after NACT.
Conclusion: For patients with stage II-III and HR+/HER2- OS and rwiDFS were found to be worse with NACT compared ACT. NACT in HR+/HER2- should be used only in selected local advanced case and when other strategy is not possible. We need new treatments in neoadjuvant setting for local advanced HR+\HER2-.

P1-11-19: Evaluation and Management of Incomplete Ovarian Function Suppression in Premenopausal Breast Cancer Patients Receiving Anti-Hormone Therapy

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Background: Breast cancer is the most common malignancy among females. The Tamoxifen and Exemestane Trial (TEXT) and the Suppression of Ovarian Function Trial (SOFT) were two landmark studies, which showed adjuvant therapy with the aromatase inhibitor (AI), exemestane, or tamoxifen combined with ovarian function suppression improved disease-free survival among premenopausal women. However, data has since emerged regarding incomplete OFS with Gonadotropin-releasing hormone (GnRH) agonists in some premenopausal patients. The SOFT Estrogen Substudy (SOFT-EST) was a prospective substudy of SOFT, which evaluated estradiol (E2) levels to determine if patients on exemestane and triptorelin experienced suboptimal ovarian function suppression (OFS). In this study, 17% of patients had an E2 level greater than 2.72 pg/mL at each time point, which was determined to be incomplete OFS. This study called into question whether E2 levels should routinely be monitored in women on GnRH agonists. The National Comprehensive Cancer Network (NCCN) and the American Society of Clinical Oncology (ASCO) provide recommendations to monitor E2 levels in premenopausal patients on an aromatase inhibitor (AI) due to the concern of incomplete OFS for patients receiving GnRH agonist therapy. The purpose of this study is to develop and implement a protocol for monitoring E2 levels at Froedtert & MCW based on the NCCN and ASCO recommendations.

Methods: This study implemented an OFS monitoring guideline in combination with a collaborative practice agreement at Froedtert & MCW. Pharmacists were responsible for ordering and monitoring E2 levels on eligible patients based on a specified protocol. An E2 level ≤ 2.72 pg/mL while receiving an AI or ≤ --------21 pg/mL while receiving tamoxifen in addition to a GnRH agonist injection was defined as complete OFS. E2 levels were retrospectively analyzed to evaluate if patients had incomplete OFS. The primary outcome was proportion of patients identified per the E2 monitoring protocol that fail to achieve OFS for two consecutive levels over an 18-month period.

Results: A total of 85 patients were reviewed at the time of analysis. Fifty-three patients (62.4%) achieved complete OFS (three consecutive E2 levels within goal), compared to 17 patients (20%) demonstrating incomplete OFS. Seven patients (8.2%) have labs that are still pending. Patients with labs pending include those who have not had enough consecutive levels demonstrating complete or incomplete OFS. Eight patients (9.4%) declined lab monitoring. Of the 17 patients with incomplete OFS, six patients (35.3%) achieved OFS after switching their GnRH agonist agent and one patient (5.8%) achieved OFS with continued monitoring without switching agents. Five patients continue to have incomplete OFS despite switching GnRH agonists (29.4%). More patients were

chemotherapy naïve in the continued incomplete OFS group compared to those who had complete OFS after intervention (p = 0.010).

Conclusion: Our study has shown a trend toward the need for checking E2 levels to assess for incomplete OFS and that changing GnRH agonist agents may improve rates of complete OFS for some patients. Additionally, our study has shown that chemotherapy naïve patients experience more incomplete OFS; however, further data is needed to validate these results.

P1-11-20: Chemotherapy use in patients with early-stage high-risk ER+/HER2- breast cancer in the United States (US) community setting: A retrospective observational cohort study

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Background: Novel options for perioperative systemic anticancer immunotherapy are currently being evaluated for early-stage high-risk ER+/HER2– breast cancer, for example, neoadjuvant pembrolizumab plus chemotherapy (chemo) followed by adjuvant pembrolizumab plus endocrine therapy in the phase 3 KEYNOTE-756 trial (NCT03725059). This retrospective cohort study aimed to describe patient characteristics and chemo treatment patterns in this patient population overall and based on chemo receipt status.
Methods: This study used the Syapse Learning Health Network, a database capturing the continuum of care for patients treated at US community practices, to select adults (≥18 years) with initial diagnosis (1/1/2016 – 1/30/2023) of high-risk (grade 3 AND T1c–T2/cN1–cN2 or T3–T4/cN0–cN2) ER+/HER2– breast cancer who had first surgery at the primary site, as in KEYNOTE-756, with latest data up to 05/31/2024. Patients enrolled in a clinical trial or with lobular carcinoma in situ, lymphoma, or other primary cancer were excluded. Using descriptive statistical tests, we compared baseline characteristics between patients who received chemo in the neoadjuvant and/or adjuvant setting and those who did not receive chemo. Real-world time on treatment (rwToT) with neoadjuvant and adjuvant chemo was estimated using Kaplan-Meier curves.
Results: Of 16,998 adults with HR+/HER2– nonmetastatic breast cancer at diagnosis, 441 patients (2.6%) with high-risk ER+/HER2– breast cancer (clinical stage I/II/III: 4%/55%/41%) underwent surgery and were eligible for the analyses. Median age was 55 years (range 23–89); 99% were women (60% postmenopausal); 68% were White; 95% (n=160) with known performance status had ECOG 0/1. Ductal carcinoma was most common (91%). Overall, 7% had ER-low positivity; 89% had positive clinical nodal status (cN1/N2: 81%/8%); and 69% were PR+. Of the 16% (n=71) with available Oncotype DX score, 42% scored high (>25), 31% intermediate (18–25), and 27% low (<18). Of the 44% (n=192) with available data, 7% were documented to have a germline BRCA mutation. Of 441 patients, 400 (91%) received systemic therapy in the neoadjuvant and/or adjuvant setting. About 78% of the cohort (346/441) received chemo, including 71% (n=244) in neoadjuvant, 23% (n=78) in adjuvant, and 7% (n=24) in both settings. Chemo recipients,

compared with chemo non-recipients (22%; n=95), were relatively younger (median [range]: 53 years [23–84] vs. 68 years [30–89]; p<0.001) and less often postmenopausal women (55% vs. 78%; p<0.001) with Medicare insurance (19% vs. 53%). Several baseline clinical characteristics differed significantly between chemo recipients and chemo non-recipients, including clinical stage III (44% vs. 29%; p=0.03), node-positive status (92% vs. 79%; p<0.001), ductal carcinoma tumor histology (93% vs. 84%; p=0.02), and Oncotype Dx high RS (61% vs. 10%; p<0.005). Median follow-up time was 26.2 months (range: 1.1–87.9). Among patients who received neoadjuvant only (n=69) or adjuvant only chemo (n=19), rwToT was 4.1 months (95% CI, 3.7–4.4) and 2.5 months (2.1–4.5), respectively. Conclusions: In this real-world study among patients with early-stage high-risk ER+/HER2– breast cancer, approximately 3 out of every 4 patients received chemo in the neoadjuvant and/or adjuvant setting. Younger patients with more advanced tumor stage and node-positive status were more likely to receive chemo than older patients with less advanced tumor stage and node-negative status. Our future work will describe more details of chemotherapy use and its impact on clinical outcomes, characterizing the unmet need in this high-risk patient population.

P1-11-21: Dose Reduction Impacts Persistence on Abemaciclib: A Retrospective Analysis of Real-World Data From the IntegraConnect PrecisionQ De-Identified Database

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Background: In the phase III monarchE study, patients treated with the CDK4/6 inhibitor abemaciclib in the adjuvant setting had significantly improved invasive disease-free survival and distant relapse-free survival which were not compromised by dose reductions.1 This study explored the impact of dose reduction on time to treatment discontinuation (TTD) in the real-world setting. Methods: This was a retrospective, observational analysis of data from patients in the IntegraConnect PrecisionQ real-world de-identified database. The study included patients with breast cancer who initiated adjuvant treatment with abemaciclib after October 1, 2021, with follow-up through May 15, 2024. The primary outcome was TTD among patients with and without a dose reduction (with a starting dose as per the label); also included were patient demographics, clinical characteristics, starting dose, and rates of dose reduction. Data are presented using descriptive statistics. Results: Of the 1,326 included patients, most were female (98.9%) and white (53.6%), and the average (standard deviation) age at diagnosis was 58.8 (13.3) years. The median (interquartile range) follow-up from diagnosis was 19.8 (12.9-27.2) months. Most patients (79%) started at the 300 mg (150 mg BID) dose, followed by 200 (100 mg BID) mg (15%) and 100 mg (50 mg BID) (6%). The median (95% confidence interval [CI]) TTD for the 100 mg, 200 mg, and 300 mg starting dose groups were 24.49 (22.20-not reached [NR]) months, 25.67 (22.92-28.98) months, and 23.18 (22.26-26.10)

months, respectively. Across all patients, median (95% CI) TTD was 28.98 (22.20-NR) in those with dose increases (n=53), was 27.34 (25.93-NR) in those with dose reductions (n=490), and was 19.34 (18.49-22.30) in those with no dose changes (n=783). Among patients who started at the 300 mg dose as per the label (n=1047), 436 (42%) had a dose reduction. The median (95% CI) TTD was 27.34 (25.93-not reached) months and 19.08 (18.10-21.64) months for patients with and without dose reductions, respectively. The treatment probability (95% CI) at 6 months was 97.6% (95.6, 98.7) and 84.7% (81.3, 87.6) in patients with and without dose reductions, respectively; at 18 months, these values were 78.6% (73.3, 83.1) and 55.9% (50.4, 61.0), and at 24 months, they were 62.4% (54.5, 69.4) and 36.4% (29.6, 43.3). Demographics were similar between patients with and without dose reductions. Conclusion: In the real-world setting, 21% of patients treated with abemaciclib in the adjuvant setting had a starting dose below the dose recommended by the label, and 42% of those who started at the 300 mg dose underwent dose reduction. Patients who had dose reductions spent a longer time on treatment than those who did not. Further analysis would need to be performed to understand why patients are treated for more than two years with abemaciclib. Reference 1 Goetz MP et al. NPJ Breast Cancer. 2024;10(1):34.

P1-11-22: Adjuvant Endocrine Therapy in HR+ Breast Cancer: HCPs Report on Real-World Barriers to Treatment Adherence

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Background: Endocrine therapy remains the treatment backbone for patients with hormone receptor-positive (HR+) breast cancer. However, many patients with early-stage HR+ breast cancer struggle with treatment adherence due to treatment-related adverse events (TRAEs) and menopausal symptoms that affect quality of life. Research shows that nonadherence is especially prevalent among Black patients and patients younger than 40. In premenopausal patients, ovarian function suppression (OFS) is recommended in addition to standard endocrine therapy. OFS can increase the incidence and severity of TRAEs, but also significantly reduces the risk of recurrence of breast cancer. Use of a GnRH agonist during chemotherapy can also reduce the incidence of ovarian failure. Therefore, it is critical that providers understand the importance of selecting the appropriate adjuvant therapy, managing TRAEs, and promoting treatment adherence so that patients can stay on their treatment plan reliably and achieve the best disease-related outcomes. This study aimed to identify real-world provider barriers and knowledge gaps in managing AEs and enhancing patient adherence to adjuvant endocrine therapy.
Methods: In January-February 2024, 118 healthcare providers (HCPs) completed surveys on practice gaps and challenges related to care delivery, treatment selection, AE management, and shared decision-making in HR+ Breast Cancer.
Results: Over 70% of HCPs reported high or very high confidence in differentiating available endocrine therapy regimens for premenopausal versus postmenopausal HR+ breast cancer patients. However, significant challenges remain, as providers estimated that less than 50%

of their early HR+ breast cancer patients adhere to their endocrine therapy for the full 5 years. HCPs reported individualizing treatment plans, engaging patients in shared decision-making, and recognizing and managing treatment-related adverse events as their top 3 challenges. Sixty-three percent of providers cited side effects of endocrine therapy as the top reason for inconsistent adherence to treatment by patients. They reported that patients struggle the most with vaginal dryness (40%), arthralgias and bone pain (39%), and nausea and vomiting (32%). Providers' perceived top barriers to patients' communication about side effects were that patients are not sure which symptoms are important or relevant to share (66%), there is not enough time during their appointment (36%), or they do not wish to complain so they do not mention their symptoms (31%). Around half of HCPs reported utilizing patient navigators to support individuals from diverse backgrounds (54%), regularly assessing and updating cultural competence and implicit bias training (45%), and providing education materials in multiple languages (44%) as strategies to address health care disparities and promote equitable access to shared decision-making to support patient engagement and adherence. However, only 15% reported that they display affirming messaging in waiting rooms and patient rooms, and only 12% reported that their institution or leadership has made a public statement of support in advocating for health equity. When asked about strategies that would most improve adherence to endocrine therapy, HCPs selected improved patient education on the importance of treatment adherence (54%), tools to support adherence monitoring (52%), and improved patient education on expectations for treatment and when/how to reach out to their health care team (47%). Conclusions: Oncologists face multiple challenges that limit sustained adherence to endocrine therapy in their patients with HR+ breast cancer, especially in diverse patients and patients younger than 40 years old. Further research and implementation of strategies to address healthcare disparities and improve sustained adherence to endocrine therapy are needed to improve outcomes for all HR+ breast cancer patients.

P1-11-23: Risk of Disease Recurrence Among Patients with Stage II and III HR-positive, HER2-negative Breast Cancer in Colombia: A Retrospective Cohort Study

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Background: In HR+/HER2- breast cancer (BC), most patients are diagnosed in early stages (ES) when treatment (tx) is administered with curative intent; however, cancer recurrence remains a significant problem in this population. In Colombia, regardless of this disease being a public health problem, there is no data regarding disease recurrence (DR) and few data on the natural history of the disease. We conducted a study to assess risk of DR and better understand clinical characteristics of stage II-III HR+HER2- BC patients.
Methods: A retrospective analytical cohort study was conducted. Patients were selected from Suramericana database, one of the main health insurance companies in the country. Adults with a diagnosis (dx) of stage II-III HR+/HER2- BC between January 2017 and December 2022 were selected. Primary endpoint was local, regional, or distant recurrence

after primary surgery. We calculated cumulative incidence and incidence rates (IR) of recurrence per 1000 person-years stratified by stage. Kaplan-Meier method was used to estimate cumulative probabilities during follow-up.
Results: A total of 2142 patients were included. Median age at dx was 56 years, 99.3% of the patients were women and 57.7% were postmenopausal. Most pts (68.7%) were stage II, 43.9% had no nodal involvement at dx, and 54.7% had a Ki67 greater than 20%. A total of 1876 patients had surgical resection with median follow-up time of 24.7 months. Cumulative incidence of DR was 11.1% at 5 years, while the IR was 43.0 and 61.1 per 1000 person-years in stages II and III, respectively. Of the patients with DR, 56.6% were still receiving tx with endocrine therapy. Risk of DR was 5.9%, 13.1%, and 24.1% at 1, 3 and 5 years respectively. A significant increase of cumulative incidence of DR was observed in stage III (p=0.012, HR 1.43, 95%CI 1.08 - 1.91) and patients with high nodal involvement (p=0.004, HR 2.86 95%CI 1.56 - 5.24).
Conclusions: Real world data from this cohort show a similar incidence of DR in Colombia to the reported in the international literature. Regardless of adjuvant tx with endocrine therapy, patients persist at risk of DR and more effective therapies are needed to provide better outcomes for patients in this potentially curative scenario.

P1-11-24: Neoadjuvant Chemotherapy for T3 tumors in the Era of Precision Medicine - Biology is Still King

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Background: Clinical T3 (cT3) breast cancer is a vexing problem due to the challenge of cosmetically acceptable breast conservation leading NCCN and ASCO to recommend neoadjuvant chemotherapy (NCT). However, MammaPrint® risk of recurrence and BluePrint® molecular subtyping genomic signatures have demonstrated high accuracy in predicting chemotherapy response. Thus, genomic profiling can potentially enable Choosing Wisely® informed treatment choices and reduced toxicity for patients unlikely to benefit from NCT, despite larger size at presentation. In this study, we examined the utility of MammaPrint and BluePrint for identifying cT3 tumors that respond to NCT.
Methods: A pooled analysis from NBRST (NCT01479101), FLEX (NCT03053193) and MINT (NCT01501487) trials was conducted on all cT3 patients who received NCT, had MammaPrint and BluePrint results, and post-surgical pathological Complete Response (pCR) data. MammaPrint risk was characterized as Low or High Risk. BluePrint subtype classified tumors as Luminal-Type, HER2-Type, or Basal-Type. Luminal-Type tumors were further classified as Luminal A (Low Risk) or Luminal B (High Risk). Tumor pCR rates were analyzed as an outcome measure. The association of genomic subtype and clinical features with likelihood of pCR was evaluated by multivariate logistic regression. Differences in pCR rates between genomic risk categories were evaluated by two-sided proportional z-test and stratified by nodal status.

Results: A total of 404 patients (MINT, n=67; NBRST, n=214; FLEX, n=123) with cT3 breast cancer underwent NCT followed by resection and 87 (21.5%) achieved pCR. The mean (SD) age was 52 (±12) years; 186 (51.7%) were premenopausal; 287 (71%) were node positive. Logistic regression revealed that MammaPrint/BluePrint subtyping showed significantly higher odds ratios for pCR in High Risk Basal-Type (OR= 3.06, 95% CI: 1.15-8.19, p=0.025) and HER2-Type (OR=6.27, 95% CI: 2.19-19.38, p=0.001) compared to the reference category (Luminal-Type), indicating strong positive associations. Only clinical subtype hormone receptor-positive (HR+), human epidermal growth factor-positive (HER2+) exhibited a higher likelihood of pCR (OR = 2.91, 95% CI: 0.97-8.23, p=0.048). Menopausal status, nodal status, and grade were not significantly associated with likelihood of pCR. Of the 209 (51.7%) patients with HR+, HER2- disease, 6.7% (14) achieved pCR. Among patients with HR+HER2-, cT3 MammaPrint/BluePrint Low Risk, Luminal A tumors (n=58), no (0%) pCR was achieved regardless of nodal involvement (n=37 node positive Low Risk). In contrast, MammaPrint High Risk (n=151) had significantly higher rates of pCR compared to Low Risk (p=0.036). By molecular subtype, pCR was achieved for 7 (5.8%) of the 120 Luminal B, and 7 (23.3%) of the 30 Basal Type, cT3 tumors.
Conclusion: These data suggest that patients with MammaPrint Low Risk, cT3 tumors are unlikely to respond to NCT. These data are in alignment with long-term follow-up, level 1A evidence from MINDACT showing that patients with MammaPrint Low Risk HR+HER2- tumors may safely omit chemotherapy, regardless of nodal involvement. Intuitively, testing newer agents or neoadjuvant endocrine therapy for downstaging or proceeding to definitive surgery should be considered for genomically low-risk, cT3 cancers.

P1-11-25: Real-World Data on Adjuvant Abemaciclib in High-Risk Hormone Receptor-Positive Early Breast Cancer: A Retrospective Study

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Background: The addition of Abemaciclib to adjuvant endocrine therapy (ET) in high-risk hormone receptor positive (HR+) early breast cancer (EBC) was approved based on the monarchE trial results. This retrospective study aims to evaluate real-world clinical outcomes of ET combined with abemaciclib in the curative setting.
Methods: A total of 97 patients with HR+ EBC receiving adjuvant abemaciclib (as per the monarchE study) were identified at a single institution between 2021- 2024. Clinical and pathological data, including demographics, treatment details, adverse events and outcomes were retrospectively reviewed. Descriptive statistics were used to analyze and report patients' characteristics. Continuous and categorical variables were analyzed with Fisher's exact test, Wilcoxon rank sum test and Pearson's Chi-squared test as appropriate. Statistical analyses were performed with R Studio v.4 at a significance level of 0.05.
Results: Patients had a median age of 51 years (interquartile range (IQR) 43-62). The majority of patients were White (71%), and Black women comprised 20%; 51% of the patients were post-menopausal. Initial clinical stages were predominantly stage II (46%),

followed by stage I (36%) and stage III (18%). Approximately 40% had received neoadjuvant chemotherapy and 50% of patients received adjuvant chemotherapy.
The most common indication for adjuvant Abemaciclib was the presence of ≥4 involved lymph nodes, accounting for 42% of patients. Among those with 1-3 involved nodes, the most frequent associated characteristic for adding Abemaciclib was a high Ki67 level (34%), followed by grade 3 tumors (16%) and tumor size ≥5 cm (14%). Aromatase inhibitors were the most common choice of adjuvant ET and 41% of patients were also on ovarian suppression. 12% of the patients received tamoxifen as the choice of ET. One patient harbored a BRCA2 mutation and received adjuvant olaparib for three months (interrupted for toxicity) before starting abemaciclib. The median duration of adjuvant Abemaciclib treatment was 14 months (IQR 9-23), with a median relative dose intensity (RDI) of 80% (IQR 33-100). Grade 3 adverse events (AEs) occurred in 60% of patients, with 53% requiring dose reductions. The most common grade 3 AEs were diarrhea (29%), fatigue (11%), neutropenia (8%), and abdominal pain (6%). 3% of patients experienced interstitial lung disease and one patient had G3 hepatotoxicity.
Patients who required dose reductions due to AEs were older than patients who did not require a dose reduction (median age 55 years, IQR 46-64 vs 47 years, IQR 43- 53; p=0.024). Patients with dose reductions had a longer duration of Abemaciclib treatment compared to patients who received the full dose (18 months, IQR 10-24, vs. 13 months, IQR 8-21; p=0.2).
Of the 14 patients who discontinued Abemaciclib, 8 had a previous dose reduction. No differences by age and race were observed amongst patients who discontinued abemaciclib vs who did not. Only 3% of the patients experienced disease recurrence (2 distant and 1 local recurrence).
Conclusion: In this retrospective study, adjuvant Abemaciclib combined with endocrine therapy demonstrated real-world applicability in treating high-risk hormone receptor-positive early breast cancer. However, our real-world data showed a higher incidence of grade 3 adverse events compared to the monarchE registration trial, indicating a need for careful management of toxicity in clinical practice.

P1-11-26: Impact of pre-operative MammaPrint/BluePrint use for final treatment decisions in patients with Stage II/IIIa HR+/HER2- early-stage breast cancer eligible for neoadjuvant chemotherapy: the DETERMIND study.

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Background: MammaPrint (MP) and BluePrint (BP) signatures have been validated in the adjuvant setting to identify patients for which adjuvant chemotherapy (CT) could be spare. Neoadjuvant Chemotherapy (NCT) is a common approach for patients (pts) with clinically

high-risk Early Breast Cancer (EBC). Other options include neoadjuvant endocrine therapy (NET) or primary surgery particularly for those patients with low-risk luminal tumors. In the DETERMIND study, we explore whether pre-operative use of MP/BP may help clinicians to reinforce the best treatment strategy for clinically high-risk patients.
Methods: DETERMIND is a prospective, open-label, multicenter study, assessing the utility of MP/BP signature on the decision-making process of optimal therapy for patients with operable clinically high-risk HR+/HER2- EBC, stage II-IIIA (up to N1) and recommendation for NCT. A total of 165 pts have been included in 13 centers. Patient data were collected at inclusion (baseline), at the time of MP/BP results and at 1- and 3-years follow-up.
Results: 165 patients with valid MP/BP results were included in 13 centers in Spain. A total of 13 cases (7%) were excluded due to the impossibility of determining MP/BP in the baseline biopsy. The median age was 57 years; 86% had clinical stage II and 45% were cN1. MP/BP classified 60 patients (37%) as luminal A, 97 (60%) as luminal B, and 4 cases with non-luminal phenotype (3 Basal, 1 HER2). In the initial decision, 70 patients (44%) did not receive NQT, being referred to NET (16%) or initial surgery (28%). Of the patients with luminal A MP/BP, 57 (95%) did not receive NQT. In the one-year follow-up results, 67% of luminal A MP/BP patients de-escalated QT, while the MP/BP test supported QT in 92% of patients with a high-risk result ($p<0.01$). The confidence of both the oncologist and the patients in the final therapeutic decision significantly increased with the MP/BP result.

Conclusion: The DETERMIND study indicates that the MP/BP test is a useful tool for establishing the indication of QT in high clinical risk HR[+]/HER2[-] EBC, as well as its feasibility in the initial tru-cut biopsy. These results are consistent with the data from the MINDACT study, supporting QT de-escalation in patients with low genomic risk and good prognosis.

P1-11-27: Comparative Evaluation of Digistain and Oncotype DX in Predicting Metastasis-Free Survival in Early Stage Hormone-Receptor Positive Breast Cancer: A Randomized Study at Charing Cross Hospital

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Background: Oncotype DX is an established genomic test for risk stratification in early-stage hormone-receptor positive, HER2-negative breast cancer. Digistain employs mid-infrared spectroscopy to assess tumor aneuploidy, offering a rapid, cost-effective alternative with potential for higher sensitivity. This study aims to compare the predictive accuracy of Digistain against Oncotype DX for metastasis-free survival.
Methods: In this double-blinded study, 233 randomly selected lymph node-negative patients from Charing Cross Hospital, previously scored by Oncotype DX, were reassessed using Digistain. The primary endpoint was metastasis-free survival, with a median follow-up of 6 years.

Results: The comparative analysis revealed a high degree of consistency in risk classification between Oncotype DX and Digistain. After adjusting for missing data, 50% of patients were classified as low-risk by Oncotype DX (defined as <10% risk of recurrence), while Digistain classified 44% of patients within the same risk category. Importantly all patients deemed low-risk by Oncotype DX were consistently categorized as low-risk by Digistain, confirming a robust concordance in risk stratification by both tests.
A noteworthy observation involved a single patient classified as low-risk by Oncotype DX but assessed as high-risk by Digistain, who subsequently developed metastatic breast cancer within five years. This case underscores a potentially heightened sensitivity of Digistain in identifying risks of metastasis, suggesting that Digistain may offer critical advantages in precise risk assessment in certain clinical scenarios.
Conclusions: These findings underscore Digistain's potential as a valid alternative to Oncotype DX, with a possible edge in sensitivity for identifying metastasis risk. The congruence in high-risk patient identification and the critical observation of an at-risk patient missed by Oncotype DX highlight Digistain's promise for enhancing clinical decision-making in breast cancer management.
Keywords: Breast cancer, Digistain, Oncotype DX, metastasis-free survival, risk stratification, hormone-receptor positive

P1-11-28: Time to Next Treatment of Abemaciclib Plus Endocrine Therapy in Early-Stage High Risk HR+/HER2- Breast Cancer: A Retrospective Analysis of Real-World Data From the IntegraConnect PrecisionQ De-Identified Database

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Background: In the phase III monarchE study, patients treated with the CDK4/6 inhibitor abemaciclib in the adjuvant setting had significantly improved invasive disease-free survival compared to those receiving endocrine therapy alone.1 This real-world study explored whether patients are receiving the new standard of care of abemaciclib plus endocrine therapy and assessed the time to next treatment (TTNT), as a surrogate for progression free survival, of abemaciclib plus endocrine therapy (A+E) versus endocrine therapy alone (E-mono). Methods: This was a retrospective, observational analysis of data from patients in the IntegraConnect PrecisionQ real-world de-identified database. The study included patients with breast cancer who were initiated on adjuvant treatment after January 1, 2022, with follow-up through January 31, 2024. Patients must not have been metastatic at time of treatment initiation, must have been HR positive, HER2 negative, and had met one of the following conditions: N2 or greater, N1 and T3 or greater, or N1 and Grade 3. Data are presented using descriptive statistics. Results: Of the 1,363 patients included, 515 (37.8%) received A+E and 848 (62.2%) received E-mono. Among the A+E patients, most were female (98.6%) and white (55.5%), and the average (standard

deviation) age at diagnosis was 58.6 (13.2) years. Among the E-mono patients, most were female (96.5%) and white (55.0%), and the average (standard deviation) age at diagnosis was 63.9 (14.1) years. The median (interquartile range) follow-up from treatment initiation in A+E was 12 (7.1, 17.3) months and in E-mono was 12.6 (6.8, 18.7) months. The median (95% CI) TTNT in the A+E cohort was not-reached (22.4-not reached) and in the E-mono cohort was 20.9 (19.10-21.9) months. The percentage of patients who discontinued in the A+E cohort was 17.1% vs. 36.9% in the E-mono cohort. The percentage (95% confidence interval) of patients who did not advance after 18 months in the A+E cohort was 77.3% (71.4-82.1) vs. the E-mono cohort which was 57% (52.6-61.1). Conclusion: In the real-world setting, just 38% of early-stage high risk HR+/HER2- breast cancer patients were treated with abemaciclib plus endocrine therapy in the adjuvant setting. Among patients treated with abemaciclib plus endocrine therapy they had greater likelihood of not advancing to the next line of therapy compared to endocrine therapy alone. This study suggests there is an opportunity to improve adherence to the guidelines in the treatment of patients with early-stage high risk HR+/HER2- breast cancer.
Reference 1 Johnston SRD, et al. Lancet Oncol. 2023;24:77-90.

P1-11-29: Clinical Characteristics and Treatment Persistence in US Patients with HR+/HER2-, Node Positive Early Breast Cancer Treated with Abemaciclib: Real-World Study from First Year After Approval

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Background: Abemaciclib in combination with endocrine therapy (ET) is approved for adjuvant treatment of adult patients with HR+/HER2-, node-positive, early breast cancer (EBC) at high risk of recurrence and this regimen falls under NCCN category 1 (preferred) recommendation. The monarchE trial established the efficacy of abemaciclib plus ET for EBC, with the highest rates of early discontinuations observed in the first few months. The utilization of abemaciclib in the real-world EBC setting after Oct 2021 approval can provide insights on the treatment patterns beyond the controlled clinical trial setting and help inform adverse event management strategies. This retrospective study describes clinical characteristics and treatment persistence in patients with HR+/HER2-, node positive EBC initiating abemaciclib.
Methods: Data were accessed from the US nationwide Flatiron Health electronic health records-derived de-identified database. Adult patients with node positive, stage I-III EBC initiating abemaciclib between Oct 2021 and Nov 2022 at 150mg twice daily (BID) were analyzed. Persistence rate was defined as the proportion of patients remaining on abemaciclib at 3 months allowing for up to 60-day medication gap. All results were summarized descriptively.
Results: A cohort of 354 patients were selected, with a median follow-up time from abemaciclib initiation of 8.8 months (interquartile range [IQR] 5.9–12.1). The median age

was 56 years (IQR 48–64), 25.4% were ≥65 years old, 12.7% were Black, 4.0% were Asian, 12.4% were other non-White race, and most patients (80.8%) received care in a community setting. Over half of the patients were postmenopausal (55.4%) and had an ECOG performance status (PS) 0 (57.9%), while 25.1% had ECOG PS 1 and 2.5% had ECOG PS 2. Approximately one-third of the patients (33.9%) had ≥1 comorbidity and 12.1% had ≥2 comorbidities with diabetes (14.1%) as the most frequent one. Most patients had stage II (41.8%) or III (38.4%) disease, nodal status N1 (45.2%) or N2 (35.3%), and tumor grade 2 (52.3%). Abemaciclib was initiated at a median of 11.1 months (IQR 9.3–13.5) after diagnosis. Prior to abemaciclib initiation, most patients received chemotherapy (83.1%), with 46.3% receiving neoadjuvant chemotherapy and 96.3% underwent radiotherapy. Most patients (74.0%) initiated ET prior to abemaciclib, with the median time from initiation of ET to abemaciclib initiation as 1.6 months (IQR 0.0–5.0). The median time to abemaciclib initiation from breast surgery was 6.7 months (IQR 4.2–9.9). The most frequent regimen was abemaciclib plus aromatase inhibitors (91.0%). At 3 months, 81.6% of patients were persistent; 5.6% resumed abemaciclib after >60-day interruption and 11.3% had discontinued due to adverse events. Additional information on dose modifications will be presented.
Conclusion: In this real-world study of utilization of abemaciclib in the first year after approval for EBC, an older, less fit, and more racially diverse population than participated in the monarchE trial, as well as a higher proportion of patients with lower nodal status was observed. The high rate of persistence at 3 months suggests that abemaciclib for EBC is tolerated in routine clinical practice.

P1-11-30: The Clinical Utility of NGS-based Multigene Assay in Optimizing Chemotherapy Decisions for Hormone Receptor-Positive Early Breast Cancer: A retrospective study

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Background: Multigene assays play a crucial role in guiding adjuvant chemotherapy decisions for hormone receptor (HR)-positive breast cancer. This study evaluates the clinical utility of the NGS-based multigene assay in determining the need for adjuvant chemotherapy in HR-positive early breast cancer patients in Korea.

Methods: A retrospective analysis was conducted on HR-positive, HER2-negative early breast cancer patients treated at Seoul National University Hospital (SNUH) from October 2019 to March 2024. A total of 1,700 patients with stage I-II (pT1-3, pN0-1), who underwent the NGS-based multigene assay for adjuvant therapy decision-making, were included. Exclusions comprised male patients, assay test failures, and referrals to other hospitals. Multivariate logistic regression analyzed factors influencing the adjuvant chemotherapy decisions. Patients were stratified by menopausal status to evaluate Decision Index (DI) distribution and treatment decisions. Clinical risk score (CRS) categorized

patients based on histologic grade, tumor size, and nodal status, similar to criteria from the MINDACT trial. DFS analysis was conducted according to DI and CRS subcategories.

Results: In total of 1700 patients, 65.2% (1,108/1,700) were categorized as low-risk DI, and 34.8% (592/1700) as high-risk DI risk. Factors significantly associated with high-risk DI included postmenopausal status, larger tumor size, higher histologic grade, nodal metastasis, lymphatic invasion, and high Ki-67 expression (all $p < 0.001$). Multivariate analysis identified high DI, high Ki-67, premenopausal status, histologic grades II and III, and pathologic N1 state as prognostic factors influencing chemotherapy decisions. In total, of the 662 patients in the high clinical risk group, 60.9% (403/622) avoided adjuvant chemotherapy. Among patients with low clinical risk but high DI, 64.6% (181/280) received chemotherapy, while 98.3% (344/350) of those with high clinical risk but low DI avoided chemotherapy. In the high DI risk group, 26.0% (154/592) patients did not receive adjuvant chemotherapy. Of the 154 patients, 38.3% (59 patients) refused to undergo chemotherapy, while 61.7% (95 patients) did not receive adjuvant chemotherapy based on the decision of surgical or medical oncologists. Among patients who did not receive chemotherapy according to surgical or medical oncologists' decision, the mean age was 60.2 years, with 81.1% (77/95) being postmenopausal. All premenopausal women (18.9%, 18/95) had a mean DI of 20.8 (range 20 – 22.2) and the adjuvant therapy included either tamoxifen or an aromatase inhibitor with ovarian function suppression. DFS (including local, regional, and distant metastasis) analysis with a median follow-up of 2.9 years showed a significant difference between the high and low DI risk groups ($p < 0.05$). Moreover, DFS analysis of stratified groups within the DI and clinical risk groups demonstrated significantly worse survival ($p < 0.001$) in the clinical high and high DI risk groups compared to other groups, with no significant differences among the other groups ($p = 0.868, 0.144, 0.506$).

Conclusion: This study demonstrates the potential of the NGS-based multigene assay to significantly reduce avoidable chemotherapy in clinically high-risk HR-positive early breast cancer patients in Korea. Factors affecting the treatment decisions irrespective of high-risk DI, include premenopausal status, high Ki-67, histologic grade, and nodal involvement. Further long-term studies are needed to validate the oncologic safety implications of utilizing the NGS-based multigene assay.

P1-12-01: HER2 categorical changes after neoadjuvant chemotherapy: A real world data of matched breast cancers with the inclusion of HER2-Low category

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Objectives: The aim of this study was to investigate the HER2 expression status in the initial pre-neoadjuvant chemotherapy (NAC) and residual post-NAC tumors, focusing on HER2

categorical changes after NAC treatment, including the HER2-low category.
Methods: This retrospective cohort study included female patients over 18 years of age diagnosed with non-metastatic breast cancer undergoing NAC from 2011 to 2023. Patients who did not achieve complete pathological responses were evaluated for changes in immunohistochemistry (IHC) before and after NAC. HER2 IHC was re-evaluated with consensus according to the current ASCO/CAP guidelines. Tumors were categorized into HER2-negative (IHC0), HER2-low (IHC1+ or IHC2+/ISH-), and HER2-positive (IHC3+ or IHC2+/ISH+) subgroups. Quantitative and qualitative factors related to changes in IHC were assessed. The study received approval from the research ethics committee (CAAE 80127724.1.0000.5463).
Results: We included 369 patients, most of whom (215/58.3%) did not change their IHC profile. Baseline statuses were HER2-negative (24/6.5%), HER2-low (256/69.4%), and HER2-positive (89/24.1%). Tumors with HR+ were observed in 227 patients (61.5%). HER2-positive tumors exhibited more changes in IHC (63/63.0%, $p<0.0001$) compared to HER2-negative (5/20.8%) and HER2-low tumors (92/35.9%). Significant differences were found in the changes: all HER2-negative tumors changed to HER2-low; HER2-positive tumors changed to HER2-low in 30 cases (43.7%) and to HER2-negative in 26 cases (41.2%). Most HER2-low tumors (51/55.4%) remained HER2-low or changed to HER2-negative (26/29.2%). The presence of HR+ was significantly associated with greater changes to HER2-negative (125/55.0%) and HER2-low (83/36.5%).
Conclusions: Our findings indicate that changes in IHC profiles post-NAC are common, particularly among HER2-positive and HER2-low tumors. Significant alterations in HER2 status were observed, with many HER2-positive tumors shifting to HER2-low or HER2-negative post-NAC. Additionally, the presence of hormone receptors (HR+) is associated with a higher likelihood of conversion to HER2-negative. These changes are clinically relevant, as they correlate with increased mortality rates, underscoring the importance of re-evaluating HER2 profiles post-NAC as potential prognostic indicators in breast cancer patients.

P1-12-02: Real-World Use of Gene Expression Tests in Swedish Breast Cancer Patients: A Multi-Institutional Study of 35 hospitals

Emelie Karlsson, Balazs Acs, Irma Fredriksson, Johan Hartman

Background: Gene expression profiling (GEP) tests are used to provide prognostic insights for breast cancer (BC) patients with intermediate risk in the adjuvant setting. GEPs provide guidance for the clinical decision-making on administering chemotherapy by stratifying the risk of relapse, aiming to avoid overtreatment or undertreatment. Since 2019, GEP tests are recommended in Swedish healthcare. The GEPs approved for clinical use in Sweden are the Prosigna/PAM50 and the Oncotype Dx tests. In addition, a non-CE-IVD certified laboratory-developed test called GEX is used at some hospitals. All GEP tests are based on different techniques which analyze different sets of breast cancer-related genes, hence a direct comparison between the test results will be deceptive for treatment guidance.According to

Swedish national guidelines, patients eligible for GEP tests are women with ER+/HER2- BC with up to 3 lymph node metastases with an intermediate risk of relapse. The distribution of BC phenotypes across Sweden is considered to be homogenous. This study aimed to compare and assess real-world use of GEPs, and their risk profiling results among 35 hospitals in Sweden in 2023.
Materials and methods: All Swedish BC cases diagnosed in 2023 were identified in the National Quality Register for Breast Cancer (NKBC). In accordance with Swedish national BC guidelines, all surgically resected specimen from patients with intermediate risk were identified for the study, resulting in 8483 patient cases from 35 investigated hospitals dispersed across six regions in Sweden. GEP tests were performed on 1815 of the 8483 identified patient cases. Data from six hospitals were excluded due to insufficient information reported to the NKBC registry.
Results: During 2023, GEP tests were performed on more than 20% of the eligible BC patients distributed over 29 hospitals in Sweden. The distribution of GEP usage in Sweden showed high variability between the hospitals, where the patients getting access to risk profiling ranged from 15% to 70% depending on what hospital the patient was admitted to and what GEP test was used for the risk assessment. Additionally, the distribution of GEPs risk results showed a high variability between the Swedish hospitals: low risk score ranged from 11.1% to 61.9%, intermediate risk score ranged from 0.0% to 77.8%, and high risk score ranged from 7.1% to 50.0%.
Conclusions: This study demonstrates a high variability in both usage and risk categorization by GEPs for BC patients in Sweden. The clinical utility of independent prognostic tests is valuable for eligible BC patients, but our study indicates a critical lack of standardization for the usage of GEP tests that may lead to discrepancy in treatment outcome. Even though the Swedish national guidelines have clearly defined indications for using GEPs on BC patients, our data imply that some hospitals may run GEP tests on BC tumor samples that do not meet these indications. The variability in risk score distribution among hospitals may also be due to the different GEPs being used. Further standardization of clinical practice guidelines is needed to decrease the large variability in the use of GEP in practice and to limit the associated risk for incorrect patient selection for breast cancer treatment.

P1-12-03: Clinical Efficacy and Outcomes of Astragalus Polysaccharides (PG2) toward Cancer-related Fatigue of Advanced Breast Cancer Patients

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Background: Cancer-related Fatigue (CRF) is the most prevalent and disturbing symptom among breast cancer patients. Astragalus Polysaccharides (PG2, PhytoHealth Corporation,

Taiwan) is a drug approved for CRF treatment and marketed in Taiwan. In the PG2 phase IV clinical trial demonstrated that PG2 improved CRF in over 60% of cancer patients, particularly significant in the breast cancer subgroup. Then PG2 got reimbursement from National Health Insurance of Taiwan. The aim of this study was to assess the real-world treatment efficacy of CRF by PG2 among advanced breast cancer patients.

Methods: This was a retrospective study. Enrolled patients were aged 20 years and above with stage IV breast cancer, experienced moderate to severe fatigue that fulfilled the diagnostic criteria defined in ICD-10, with other interventions proving ineffective, and had an Eastern Cooperative Oncology Group performance score of 0-2. Patients' demographic information, disease characteristics, cancer treatment, and visual analog fatigue scores (Fatigue-VAS) were collected. The efficacy on CRF treatment by PG2 were analyzed in intention-to-treat (ITT) population and subgroups based on accumulated doses, menopausal statuses (pre-menopause and post-menopause), BMI (< 25 kg/m^2 (normal weight) and ≥ 25 kg/m^2 (overweight) and sites of metastases (visceral metastases (VM) and non-visceral metastases (non-VM)).

Results: This study included a total of 204 evaluable breast cancer patients. After completion of pre-planned PG2 treatment, there were significant improvements from baseline in the worst fatigue scores whether assessed over the past 24 hours or during the last cancer treatment ranged from 2.6 to 3.5 across ITT population and all menopausal statuses, BMI, and metastases subgroups (both $p<0.001$). A numerically higher proportion of premenopausal patients experienced relief to mild or no fatigue over past 24 hours and during last cancer treatment compared to postmenopausal patients (92.86% vs. 60.71% and 92.86% vs. 58.57%), and the distribution of fatigue severity levels in the population between various menopausal status groups showed a statistically significant difference ($p=0.023$ and $p=0.014$). The categorization of fatigue severity during last cancer treatment results showed that patients with non-VM treated with PG2 had significantly better fatigue improvement outcomes compared to those with VM ($p=0.041$). Improvements in fatigue were similar between normal weight and overweight groups after PG2 treatment.

Conclusions: The retrospective study demonstrated that PG2 exhibits significant therapeutic efficacy in relieving CRF among advanced breast cancer patients. Premenopausal and non-VM patients might be more beneficial to PG2 treatment.

P1-12-04: Correlation between Immune-Related Adverse Events (irAE) and Survival Outcomes in Metastatic Breast Cancer Patients Treated with Immune Checkpoint Inhibitors (ICI): a multi-institutional study

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Background: There have been discrepant results between development of irAE, and their effect on overall survival (OS) in several cancer types treated with ICI. Some studies have shown a positive correlation between incidence of irAE and OS. While these effects are being explored in breast cancer, there is a paucity of data regarding patients (pts) with metastatic breast cancer (mBC) treated with ICI.

Methods: This multicenter retrospective study, involving four academic institutions, evaluates the incidence of irAE in pts with mBC who received ICI between 2014 and 2024. The presence and grading of irAE were determined by physician documentation. Pts demographic data and characteristics were summarized using descriptive statistics. OS was calculated from the date of treatment initiation to death from any cause. Pts who were still alive were censored at the last clinic encounter. Survival probabilities were estimated with Kaplan–Meier curves and log-rank tests. All other co-covariables were analyzed using multivariate logistic regression and cox proportional hazards model.

Results: There were a total of 252 evaluable cases, with median age at the start of ICI of 54. The study population consisted of 66% white, 9% black and 25% others. Median body mass index (BMI) was 26.4. Among the pts, 64% were triple negative breast cancer (TNBC), 30% hormone receptor positive (HR+), 5% human epidermal growth factor receptor 2 positive (HER2+) and 1% both HR/HER2+. Combination therapy was received by 78% of the patients, while 22% received ICI alone. Amongst pts who received ICI, 45% pts were enrolled in clinical trials. Pts were on ICI for an average of 138.38 days before their first irAE. irAE were noted in 47% pts, with 35% experiencing at least 1 irAE, 11% had 2 irAE and 1% with 3-4 irAE. Grade 1-2 irAE were noted in 34%, and grade 3-4 in 13.5%.

The median OS was 17 months amongst all pts. Pts with irAE had a longer median OS compared to those without irAE (23 vs 13 months; HR, 0.50; 95% CI 0.36-0.69; $p < 0.00001$). Pts with TNBC had improved overall survival (OS) with ICI in comparison to HR+ and HER2+ pts (OS 17 months; 95% CI 13-28; $p = 0.0074$). In addition, those with higher grade irAE had improved OS compared to those with low grade irAE (HR 0.41, 95% CI 0.15-1.16; $p=0.03$). Higher number of treatment cycles and higher baseline BMI were associated with a significantly lower risk of mortality ($p=0.006$ and $p=0.018$, respectively). Clinical factors such as age, smoking and other comorbidities, while potentially influential, did not reach statistical significance in this multivariable analysis.

Conclusion: Our analysis shows that almost half of the pts in the study (47%) had irAE with median OS of 17 months. Pts with irAE had a longer OS (23 months) compared to those without irAE (13 months). The median OS for TNBC was higher than HR+ and HER2+ breast cancer. The total number of ICI cycles and baseline BMI were shown to have a significant impact on the survival of mBC pts. The combined irAE grades show a trend towards a decreased risk of mortality, but the findings are not statistically significant.
Overall, while there are indications that specific side effects and treatment factors may influence patient outcomes, including increased OS among patients with irAE, further

research with larger datasets is needed to confirm these results. This study provides a foundation for understanding how various factors contribute to the survival of breast cancer patients undergoing ICI therapy.

P1-12-05: Improving Outcomes in Patients With High-risk Breast Cancer: Outcomes and Analysis From an Educational Program on HR+/HER2- Early and Metastatic Breast Cancer

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Background: Treatment of breast cancer has advanced rapidly over the past decade. However, patients with hormone receptor-positive (HR+), HER2-negative (HER2-) disease with high-risk features are particularly vulnerable to relapse or progression in both the early stage (EBC) and advanced/metastatic stage (MBC) settings, respectively. Here, we report results from formative educational surveys and post-education assessments on improving outcomes for patients with high-risk HR+/HER2- breast cancers.
Methods: A multidisciplinary curriculum was developed to provide CME/CE-certified education on identifying high-risk features, assessing for endocrine resistance mutations, and selecting the optimal therapy for each patient with high-risk HR+/HER2- breast cancer, as well as provide education on oral therapy adherence and toxicity management. From December 22, 2023, to May 28, 2024, data were collected from online interactivity software live and online healthcare professional (HCP) surveys administered during and after CME/CE education. Expert-endorsed patient resources and clinical guideline–based PowerPoint slidesets were provided for download to all program participants. Program follow-up survey data were also collected on the impact of the education on current clinical practice and other barriers to changing practice.
Results: Over 1000 learners participated in the live or enduring education program. From a total of 898 HCPs (physicians, nurses, pharmacists), 84% cared for patients with breast cancer. At baseline, approximately 19% of participants felt confident in their ability to assess high-risk features in patients with HR+/HER2- breast cancer, which increased to 43% following the education. Compared with 62% at baseline, post-education 92% of learners were able to identify patients with EBC with tumor ≥5 cm and positive axillary lymph nodes as a higher risk population; 61% at baseline vs 95% post-education correctly selected aromatase inhibitor (AI) plus abemaciclib as the optimal guideline-recommended/evidence-based treatment for patients with high-risk EBC; and 66% vs 90%, respectively, selected AI or fulvestrant with a CDK4/6 inhibitor in high-risk MBC. An absolute 50% and 65% value increase was achieved for learners' knowledge/competence in individualizing therapy for high-risk metastatic breast cancer and for recommending strategies to promote oral treatment adherence with CDK4/6 inhibitors, respectively.
The most common barriers cited (n = 364) to implementing the recommended changes to practice included acceptance of recommendations by peers and colleagues (34%) and insurance/formulary restrictions (24%). Of those completing post–CME education follow-

up surveys (n = 417), 48% indicated a shift from awareness to agreement with best practices, 13% increased adoption or application of best practices, and 21% stated the education helped in another way. In total, 860 downloaded PDF resources and 1866 downloaded slides tailored to improving patient–HCP communication and reinforcing takeaways and self-learning after event conclusion.
Conclusions: This large, expert-led, multidisciplinary, CME/CE-certified educational program on HR+/HER2- high-risk breast cancer showed substantial improvements in HCPs' confidence, knowledge, and competence in their ability to identify patients with high-risk features, recommend optimal guideline-based therapies, and promote oral therapy adherence by following best practices and strategies. There remain key but addressable barriers to implementing best practices. These results highlight the importance of ongoing education and the need for a multidisciplinary program to improve HCPs decisions for high-risk HR+/HER2- breast cancer.

P1-12-06: The Effectiveness of Trastuzumab, Pertuzumab and Docetaxel Combination as a First-Line Treatment of HER2-Positive Metastatic Breast Cancer Patients : A Real-World Evidence in Korea

Min Jeong Kim, Hyo Jung Kim, Jin Seok Ahn, Ji-Yeon Kim, Junghoon Shin, Yeon Hee Park

Background: The combination of trastuzumab, pertuzumab, and docetaxel (THP) has been established as a standard first-line treatment for HER2-positive metastatic breast cancer, following the CLEOPATRA study, which demonstrated the added efficacy of pertuzumab compared to placebo with trastuzumab and docetaxel. In the CLEOPATRA study and previous real-world evidence studies, patients who discontinue docetaxel due to adverse events after the induction period continue HER2 blocking treatment with trastuzumab and pertuzumab (HP). In the era of trastuzumab deruxtecan introduction in earlier treatment lines, it is important to evaluate the effectiveness of the THP treatment, especially long-term continuous HP without docetaxel, as a meaningful option for the quality of life of patients, by utilizing real-world evidence (RWE).
Methods: We conducted a retrospective analysis of 320 HER2-positive patients who began receiving THP treatment at Samsung Medical Center between April 2015 and December 2021. This analysis utilized de-identified and anonymous data from the Clinical Data Warehouse based on institutional real-world electronic medical data. Patients included in the study received at least one cycle of THP. The cutoff date for the analysis was 31st May 2024.
The primary outcomes of the study were Progression-Free Survival (PFS) and Overall Survival (OS). The secondary outcome assessed the OS differences between different subgroups.
Result: The median follow up was 46.8 months (range 0.35-109.1). Among 320 patients, 151 patients (47.2%) were hormone receptor (HR) negative, while 133 patients (41%) were HR positive either estrogen receptor or progesterone receptor. The median cycle of the THP was 22 cycles (range 1-158) and the median cycle of docetaxel was 8 cycles (range

1-34). Median PFS was 30.8 months and median OS was not yet reached.
In our study, the median number of cycles of HP after discontinuation of docetaxel was 12 cycles (range 0-144). Sixty-six patients (20.6%) maintained HP for more than 3 years, 37 patients (11.6%) received it for more than 4 years, and even 23 patients (7.2%) continued HP for more than 5 years. The median number of cycles of HP was 3 (range 0-103), 14 (range 0-128), and 17 (range 0-144) in patient groups who received fewer than 6 cycles of docetaxel (50 patients, 15.6%), 6-10 cycles (200 patients, 62.5%), and more than 10 cycles (70 patients, 21.9%), respectively. Patients who received fewer than 6 cycles of docetaxel showed significantly poorer survival outcomes compared to the other groups (6-10 cycles of docetaxel: HR = 0.39, 95% CI 0.24-0.64, p = 0.0001; more than 10 cycles of docetaxel: HR = 0.25, 95% CI 0.13-0.48, p < 0.0001). However, the overall survival between the patient group who received 6-10 cycles of docetaxel and the group who received more than 10 cycles of docetaxel showed no significant differences (HR = 0.62, 95% CI 0.34-1.15, p = 0.13).
Conclusion: Our findings are consistent with the survival benefit of the THP regimen in real-world practice, aligning with the results of the randomized clinical trial, CLEOPATRA. This study highlights that HP after the induction phase with docetaxel is a clinically meaningful treatment option, considering both patients' quality of life and survival benefit.
This study showed a relevantly longer survival outcome compared to CLEOPATRA, addressing diversity and healthcare system-specific variations in treatment outcomes that may not be fully captured by global clinical trials. While acknowledging the limitations in generalizability inherent in this single-center, retrospective analysis, the study provides valuable insights into the effectiveness of this treatment regimen in a Korean population, contributing to the broader understanding of its performance in Asian patients. Our results underscore the importance of RWE in complementing clinical trial findings and optimizing treatment strategies for HER2-positive metastatic breast cancer patients.

P1-12-07: First-line (1L) treatment decision patterns and survival in Peruvian patients (pts) with hormone receptor (HR)-positive HER2-negative advanced breast cancer (ABC)

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Background: Advanced breast cancer (ABC) is an incurable disease, with a median overall survival (OS) of 3 years, even in high-income countries. Endocrine therapy (ET) + cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) is recommended for pts with HR (+) HER2 (-) ABC. Although chemotherapy (CT) is reserved in some cases, it is still used in many countries as first-line (1L) therapy. The aim of our study is to evaluate the 1L available treatment choices in a Peruvian public oncologic institution and the factors that influence on therapy decision. In addition, we explore the OS of sequencing treatments.
Methods: Retrospective analysis of HR (+) /HER2 (-) ABC pts (de novo,

recurrent/progressive) classified in 3 groups according to the treatment received in 1L and second-line (2L): ET-CT, ET (aromatase inhibitor)-ET (fulvestrant) and CT-ET between January 2021 and December 2023. Clinicopathological data was retrieved from clinical files, and factors were evaluated to test the association of the choice of 1L (ET vs. CT) with outcomes. Survival curves were constructed with the Kaplan-Meier method.
Results: A total of 145 female pts were included. The median age was 53.9 years (24-85); 63.4% were post-menopausal, 29.2% were diagnosed as metastatic de novo, 93.1% had ECOG 0-1 and 69.0% were luminal B subtype. 5.7% of patients have visceral crisis at diagnosis and receive CT. Regarding available treatment options, 1L CT was chosen in 43.4% of pts. In univariate analysis, the presence of visceral metastases (most frequent lung metastases) (OR 2.31, 1.07-5.09, p=0.034) and de novo disease (OR 2.15, 1.04-4-52, p=0.039) were associated with a higher use of CT, while lymph node metastases were associated with the choice of 1L ET (OR 0.36, 0.12-0.92, p=0.044). In multivariate analysis, de novo disease was the prognostic factor for select 1L CT (OR 2.26, 1.02-5.07, p=0.046). 44.2% of our pts who received 1L CT met “aggressive disease” criteria from RIGHT Choice trial (which showed a benefit of CDK4/6i + aromatase inhibitor vs. CT in pts with aggresive disease), including: 78% met "rapid disease progression", 52% with "markedly symptomatic non-visceral metastases" and 30.4% “symptomatic visceral metastases” definition. At treatment sequencing, 42.5% pts were CT-ET, 40.8% ET-ET and 16.7% ET-CT. Progression-free survival (PFS) 1L ET (aromatase inhibitor) was 18 months. With a median follow-up of 41 months, the OS rates at 12, 36 and 60 months were 98.5%, 82.0% and 64.5%, respectively. No differences were found in PFS or OS according to sequencing groups.
Conclusions: The presence of de novo disease and visceral metastases were prognostic factor in Peruvian pts with ABC RH (+) /HER2 (-) for choosing 1L CT. Almost half of pts met the “aggressive disease” criteria of RIGHT Choice trial for select 1L CT. Treatment sequencing regimens (including CT) increases PFS and OS in Peruvian patients. There were no statistically differences in PFS or OS with sequential treatments groups.

P1-12-08: Cyclin-Dependent Kinase 4/6 Inhibitors in Metastatic Breast Cancer and Use of Concomitant Proton Pump Inhibitors: Real-World Experience from a Tertiary Care Center

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Introduction: Cyclin-dependent kinase 4/6 inhibitors (CDKI) are indicated for the treatment of women with hormone receptor (HR) positive, human epidermal growth factor receptor 2 (HER2) negative locally advanced or metastatic breast cancer in combination with an aromatase inhibitor or fulvestrant as initial endocrine-based therapy. Little is known about the concomitant use of other common drugs, such as proton pump inhibitors (PPI). The

present retrospective series analyzes the outcomes of patients receiving these drugs in a single institution.
Methods: Patients with HR-positive, HER2-negative metastatic breast cancer who received either palbociclib or ribociclib plus endocrine therapy between 2015 and 2021 were included. Clinicopathological variables and data on efficacy, toxicity, and the use of concomitant PPI were collected. Progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method. A Cox proportional hazards model was used to estimate the hazard ratio.
Results: A total of 154 patients were included: 71.4% of patients received palbociclib and 28.5% received ribociclib. There were more de novo metastatic patients in the ribociclib group (34.1%). Regarding the hormone therapy received, 64% of patients received concomitant treatment with aromatase inhibitors and 34.4% with fulvestrant. Letrozole was the most used in both groups; however, patients in the Palbociclib group received Fulvestrant (42.7%) more frequently than those in the Ribociclib group (13.6%). As for the concomitant use of proton pump inhibitors (PPIs), there were no differences in distribution between the two groups (Palbociclib 42.7% - Ribociclib 38.6%).
A higher rate of toxicity was recorded in patients who received ribociclib (40.9%), with hematological toxicity (65.6%) and asthenia (22.7%) being the most frequent. Severe grade 3 or 4 hematological toxicity, primarily neutropenia, was most common, with no statistically significant differences between palbociclib (61.8%) and ribociclib (63.6%).
After a median follow-up of 47.2 months, 54 deaths were recorded. It was found that patients who received ribociclib had a longer PFS than those who received palbociclib (50 months vs 29.49 months, p=0.025). Hazard Ratio (HR) 0.59, 95% Confidence Interval (CI95%) (0.37-0.96), p = 0.033.
Populations were stratified based on whether they received concomitant PPI treatment. It was evident that in patients who received PPI treatment, differences in PFS in favor of ribociclib persisted (median PFS 50 months vs 11.27 months; p = 0.002); however, in patients who did not receive PPI treatment, no differences in PFS were observed (median PFS 44.74 months vs 34.58 months; p= 0.650).
Regarding overall survival, no statistically significant differences were observed between the two treatment groups (p = 0.353). However, when stratifying the population, in patients who had received PPI treatment, statistically significant differences in overall survival were evident (Ribociclib median OS 71.44 months VS Palbociclib median OS 29.55 months, p= 0.025).
Conclusions: Our study suggests that there may be differences between ribociclib and palbociclib, particularly in terms of PFS. Additionally, the concomitant use of PPI treatment may have a detrimental effect on the outcome of patients treated with palbociclib. Further studies are needed to confirm these findings.

P1-12-09: Real-world prescribing patterns and outcomes of CDK4/6 inhibitors in elderly patients with metastatic HR+/HER2- breast cancer

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Background: Older patients are a heterogeneous group often underrepresented in clinical trials. Yet, a significant proportion of our patient population are elderly, with the median age of 68-74 years old at cancer diagnosis. Elderly patients are perceived to be less tolerant of cancer therapy with higher rates of adverse events, dose reductions, and discontinuation. In metastatic HR+/HER2- breast cancer, use of CDK4/6 inhibitor with endocrine therapy is often first line therapy. Our objective is to describe real-world prescribing patterns of CDK4/6 inhibitors in an elderly patient population with metastatic breast cancer, identify adverse events that contribute to dose adjustment and discontinuation, and determine progression free survival of patients based on the initial dose of a CDK4/6 inhibitor.
Methods: This is a retrospective study within the University of Maryland Medical System. All patients prescribed a CDK4/6 inhibitor between 11/8/2015-11/30/2022 were reviewed. Inclusion criteria included patients 75 years or older when initiated on a CDK4/6 inhibitor, metastatic breast cancer, and patients who received CDK4/6 inhibitors for at least one cycle. The analysis cutoff date was 2/8/2024. Analysis was done using the SAS computer program.
Results: Out of 397 patients reviewed, 45 patients met criteria for analysis. Palbociclib was used in 32 (70%) patients, abemaciclib in 8 (17%) patients, and ribociclib in 6 (13%) patients. At initiation, 24 (53%) of patients were started on full dose of CDK4/6 inhibitor. Of the other 21 (47%) of patients, initiation at a reduced dose was due to advanced age for 8 patients, however no reported reason was documented for 13 patients. Baseline characteristics of ECOG score, prior chemotherapy, brain metastases, and bone metastases were not statistically different between patients initiated at full or reduced dose. Of 45 patients, 26 patients had a subsequent dose reduction. Seventeen (65%) patients were reduced to dose level -1, and 9 (35%) patients were reduced to dose level -2. Two patients had a dose escalation. The median duration until first dose reduction was 2.6 months (0.6-35). The most common reasons for dose reduction were due to myelosuppression (50% vs 75%) and fatigue (16.7% vs 25%) whether initiated on a full dose or reduced dose of CDK4/6 inhibitor, respectively. However, 18 of 26 (69%) patients initiated at a full dose needed dose reduction due to adverse events compared to 8 of 26 (31%) patients initiated at a reduced dose (p=0.19). The most common reasons for discontinuation were due to progression of disease (47.1% vs 44.4%) and adverse events (29.4% vs 44.4%) whether initiated on a full dose or reduced dose of CDK4/6 inhibitor, respectively. Two patients who had a severe adverse event required hospitalization. The median total duration of a CDK4/6 inhibitor was 18.1 months (0.86-77). Ten patients did not experience any adverse events, and ten patients continued on a CDK4/6 inhibitor at data cutoff. Median progression-free survival if initiated at a full dose versus reduced dose was 18.6 months (3.5-75.1) and 19.2 months (1.2-77.0), respectively (p=0.51; 24 month PFS HR 1.04, 95% CI 0.85-1.27).
Conclusion: In this study, most older patients either initiated CDK4/6 inhibitors at a reduced dose or needed subsequent dose reductions. Study limitations include retrospective nature, small sample size, and larger proportion of patients on palbociclib as it was the first approved CDK4/6 inhibitor. This study supports initiating CDK4/6 inhibitors at a reduced dose for patients with metastatic HR+/HER2- breast cancer who are 75 years or older due to fewer incidence of adverse events with no difference in survival outcomes.

This aligns with pooled analyses of PALOMA-2, MONALESSA-2, and MONARCH-3 and another real-world study in Asia.

P1-12-10: Prediction and Survival Analysis of Regional Recurrence in Breast Cancer: Impact of Mode of Detection

Eun Sook Ko, Myoung Kyoung Kim

Purpose: To develop a predictive model for regional recurrence in breast cancer and to evaluate the survival outcomes based on the mode of detection.
Methods and Materials: This retrospective cohort study examined medical records of patients who underwent invasive breast cancer surgery at a single institution from January 2011 to December 2019. Surveillance breast ultrasound, covering the axilla, internal mammary, and supraclavicular areas, was conducted semiannually for five years, then annually for those with a personal history of breast cancer (PHBC). Regional recurrences were identified, and associated factors were analyzed. Interval regional recurrences were defined as those diagnosed after a negative screening but before the next scheduled screening. Overall survival (OS) rates were estimated using multiple Cox regression models, and the impact of the mode of detection on survival outcomes was evaluated using the multivariable Fine-Gray subdistribution hazard model.
Results: Among 13,406 women (mean age: 49.8 ± 10 years), 126 (0.9%) developed regional recurrence over a median follow-up of 6.5 years. Of these, 37 cases (0.3%) were interval regional recurrences. Patients with regional recurrence had worse overall survival (OS) compared to those without recurrence (all Ps < .001). Interval regional recurrence exhibited worse OS compared to surveillance-detected or non-regional recurrence, although the difference was not statistically significant (HR: 1.6 [0.6, 4.1]; P = .32; HR: 1.5 [0.8, 2.7]; P = .19). Factors associated with an increased risk for regional recurrence included high T stage (HR: 2.1 [1.5, 3.2]; P < .001), triple-negative breast cancer (TNBC) subtype (HR: 1.8 [1.2, 2.8]; P = .009), receipt of axillary lymph node dissection (ALND) (HR: 2.0 [1.2, 3.3]; P = .007), and lack of adjuvant radiation therapy (HR: 2.8 [2.0, 4.1]; P < .001). In the 126 patients with regional recurrence, factors increasing the hazard of death included shorter time to recurrence (HR: 3.3 [1.7, 5]; P < .001), older age (HR: 1.1 [1.0, 1.1]; P < .001), and the TNBC subtype (HR: 6.5 [2.5, 16.8]; P < .001).
Conclusions: In patients with a personal history of breast cancer, regional recurrence is associated with poorer overall survival. The mode of detection may also impact survival outcomes.
Clinical Relevance/Application: Advancements in adjuvant treatment have made regional recurrence of breast cancer very rare, with rates dropping to less than 2%. While it is well-known that interval breast cancers have worse prognoses, the clinical significance of interval regional recurrence in breast cancer, especially in women with a personal history of breast cancer (PHBC), is not well understood.

P1-12-11: Individualized Breast Cancer Treatment Strategies: Real-World Insights from the ROSE Study

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The ROSE study (NCT05377684) is an ongoing observational trial evaluating the Quality of Life (QoL) as the primary endpoint in premenopausal patients with Hormone Receptor (HR)+, HER2-negative early Breast Cancer (BC). The trial focuses on patients treated with a Luteinizing Hormone–Releasing Hormone (LHRH) analogue (triptorelin) combined with oral endocrine therapy, with or without chemotherapy depending on the risk of relapse, as determined by multidisciplinary meetings. Since April 2022 and up to October 2023 (18 months), the trial reached 50% enrollment of its target population. At this milestone, a predetermined interim analysis focused on the evaluation of baseline demographic characteristics, tumor features, and BC treatments. Other secondary objectives (e.g. hormonal levels) were not evaluated at this timepoint.

Out of the approximately 200 patients initially enrolled, 195 were deemed eligible for the study. All patients had estrogen receptor-positive early BC and, at baseline, were planned to receive adjuvant endocrine therapy with tamoxifen or aromatase inhibitors combined with triptorelin as ovarian function suppression therapy. The median age at BC diagnosis was 45.5 years, ranging from 30 to 56. Tumor characteristics showed that 97.4% of enrolled patients (n=189) had invasive carcinoma, while 2.6% (n=5) had carcinoma in situ. The grading distribution included 10.8% grade 1 lesions, 69.9% grade 2, and 19.4% grade 3. Staging data according to AJCC 2017 criteria were available for 187/195 patients and revealed the following distribution: 44.9% had stage IA BC (stage IB: 5.3%), 27.7% had stage IIA (stage IIB: 16%), and 5.7% had stage IIIA. The Ki-67 proliferation index was available for 193 patients, with a median value of 17% and a mean value of 22.3%.

Regarding therapeutic plans, the monthly triptorelin formulation was prescribed to 92.3% of patients (n=180), while the trimestral was prescribed to 7.7% of patients (n=15). The choice between tamoxifen or aromatase inhibitors depended on the relative risk of recurrence based on the aforementioned prognostic factors, with patients receiving the latter in higher-risk cases. Among the 195 patients, 27.2% (n=53) received adjuvant or neoadjuvant chemotherapy, classifying them as high risk for recurrence. Of these patients, nearly all received aromatase inhibitors and triptorelin (n=48). The remaining 72.8% of patients (n=142) were not considered high-risk enough to warrant chemotherapy, placing them in the intermediate-risk category. These patients were planned to receive triptorelin and either tamoxifen (n=28) or aromatase inhibitors (n=114) in chemotherapy-free treatment plans. The majority of intermediate-risk patients received aromatase inhibitors, indicating a higher risk profile, closer to the high-risk category.

The ROSE study highlights an Italian best practice approach that tailors therapeutic plans to the individual needs of each patient, allowing for treatments based on personalized risk assessments. This approach aligns with current national oncology guidelines by minimizing

chemotherapy use when possible and instead adopting individualized treatment plans based on specific prognostic factors. However, the observational nature of the ROSE trial provides a realistic snapshot of clinical practices, which may differ from the highly-selected populations typical of randomized controlled trials. This observational study of Italian best practices suggests the potential to redefine relapse risk cutoffs based on prognostic factors, providing more accurate and realistic patient-specific therapeutic approaches based on individual risk. The findings of this study, combined with results from ongoing post hoc analyses which aim to identify these new cutoffs, could potentially support the redefinition of relapse risk to better align with clinical practice, thereby fostering patient-specific therapeutic approaches.

P1-12-12: Leptomeningeal Metastases in Breast Cancer: Uni-institutional Experience

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Background: Breast cancer (BC) is the tumor most associated with leptomeningeal metastases (LM), a challenging complication of solid tumors with a poor prognosis and limited treatment options. This study explores the interplay between clinical presentation, treatment and survival outcomes in LM patients.
Methods: this is a retrospective cohort of patients with BC and a cerebrospinal fluid (CSF) positive for neoplastic cells between 2015-2021. Data on CSF cytology, neuroimaging, presenting symptoms, treatments and overall survival (OS) were collected. Symptoms were classified via hierarchical clustering into three neurological syndromes: intracranial hypertension, focal neurological deficit and diffuse encephalopathy. The Kaplan-Meier method was used for survival analyses. Hazard ratio (HR) and 95% confidence interval (95%CI) were calculated using Cox regression.
Results: Of 181 patients diagnosed with LM, 92 (50.8%) had BC as primary site and were included in this analysis. All patients were female with median age of 50.1 years at diagnosis of LM (younger for triple-negative breast cancer (TNBC): 46 years; older for RH+/HER2-: 52.2 years; p=0.09). 48 (52.2%) patients had ECOG-PS 3-4. The most common histology was invasive breast carcinoma of no special type (74 [80.4%]), followed by 9 (4.9%) lobular carcinoma. The most common BC profile was RH+/HER2- (34 [37.0%] patients; 7 [7.6%] luminal A and 27 [29.4%] luminal B), followed by TNBC (32 [34.8%]) and HER2+ (26 [28.2%]). The median time from cancer diagnosis to LM diagnosis was: 35.1 months (m) for HER2+; 20.8m for TNBC; 42.5m for RH+/HER2-; p=0.007. At the time of LM diagnosis, 84 (91.3%) patients had systemic metastatic disease, with 57 (62.0%) presenting brain parenchymal metastasis. The HER2+ profile was more associated with concomitant presence of brain metastasis (25 [96.1%] patients) when compared to RH+/HER2- (16 [47.1%]) and TNBC (16 [50.0%]); p<0.001. Patients were previously treated, on average, with 1-2 lines of chemotherapy in all profiles (p=0.2). RH+/HER2- patients also received 1-2 previous lines of endocrine therapy (ET). Most diagnoses were made during hospitalization

(78 [84.8%]), with 23 (25.0%) deaths before discharge. 82 (89.1%) patients had an MRI ≤ 1 month apart from the CSF. Of those, suggestive report findings of LM were found in 50 (54.3%), which was not prognostic for OS (HR=1.11, p=0.70). The median OS was 1.1m (0.7-4.7) for HER2+; 0.9m (0.6-1.6) for TNBC; 2.8m for RH+/HER2- (1.8-4.8); p=0.17. No differences in OS were found by neurological syndrome (intracranial hypertension, focal neurological deficit or diffuse encephalopathy). Post-LM diagnosis, only 47 (51.1%) patients received additional systemic therapies: 20 (21.7%) received chemotherapy (p=0.75); 10 (10.9%) RH+/HER2- patients received ET, 7 (7.6%) received intrathecal chemotherapy with methotrexate and 24 (26.0%) were submitted to radiotherapy (p=0.63). Patients who received intrathecal therapy had longer overall survival (4.0m [3.8-NA]; HR=0.38 [95%CI 0.15-0.96], p=0.04) when compared to other treatment options (3.1m [1.8-5-5]) or to no treatment (0.6m [0.4-1.12]).
Conclusion: In this cohort, patients who received intrathecal therapy had longer survival. Still, only 7 patients were prescribed this treatment. 25% of patients were not discharged after LM diagnosis and only half of the patients were able to receive any treatment strategy. Therefore, our cohort highlights the need for novel therapeutic approaches to improve outcomes.

P1-12-13: Characteristics and Prognosis of Inflammatory Breast Cancer according to Race: A population base study using SEER database

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Background: Inflammatory breast cancer (IBC) is classified as T4d in the TNM staging system and is notably aggressive and rare. We aim to understand the clinicopathological characteristics of IBC according to race and analyze both the features of IBC influencing prognosis and socioeconomic factors.
Method: Using the National cancer institute's Surveillance, Epidemiology, and End Results (SEER) Research data from 17 registries, we analyzed data from 7,075 women aged 20 and older diagnosed with IBC from 2010 to 2020. Results
Of 7,075 female patients with IBC, there were 48 (0.7%) American Indian/Alaska Native (AI), 468 (6.6%) Asian or Pacific Islander (API), 1238 (17.6%) Black, and 5285 (75.1%) White patients. The incidence rate of IBC was approximately 50% higher among those aged 50-69 and white patients had a higher incidence rate after the age of 70 compared to patients of other races. Stage IIIB was more common among White patients, while Black patients showed a higher proportion in stage IV. Triple-negative breast cancer (TNBC) was more prevalent among Black patients.
There was no significant difference in the rates of surgery and chemotherapy. However, complete response (CR) rate after neoadjuvant chemotherapy was lowest among Black patients. API and White patients had higher rates of being married, whereas 43.4% of Black patients were single. Also, Black patients had lower incomes.
Black patients had the shortest average overall survival (OS) at 29.6 months. The 5-year OS rates were 43.6% for API patients, and 42.2% for White patients. In contrast, the Black

patients was 28.3%. ($p < 0.001$). Economic income and marital status influenced OS rates, but residence was no significant difference in the race group. ($p = 0.107$).
Black patients tended to have an unfavorable prognosis due to higher rates TNBC subtype, advanced stage, lower CR rate. White patients had a lower stage and histological grade.
Conclusion: Black patients with IBC exhibited unfavorable prognoses compared to patients of other races. These results suggest that racial disparities in IBC prognosis are significantly influenced by the clinicopathological characteristics of the tumor, with socioeconomic factors also playing a role.

P1-12-14: Characteristics and outcomes of patients with triple negative breast cancer (TNBC) treated with neoadjuvant chemotherapy (NACT) according to race

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Introduction: Previous literature, originated mainly from the United States, describes differences in outcomes between races in patients (pts) with TNBC, with worse survival results in African-American women compared to the white population. Both inequalities in access to healthcare and different biological tumor behavior have been sought as possible explanations. This study tried to evaluate the differences in characteristics and outcomes of pts with TNBC treated with NACT according to race in a Brazilian cohort.
Methods: We retrospectively reviewed our institutional database to identify pts who underwent NACT for TNBC from 2012 and 2024 and collected data from medical records. Data regarding ethnicity, age, marital status, clinical stage, tumor characteristics and type of treatment were collected. Pts were classified by ethnicity into categories, mostly by heteroidentification registers at hospital admission: “White” (W), “Black” (B), (“Mixed or Browns” were categorized as Black) or “Others or unknown”. The last group represented less than 5%, and was excluded from analysis. Event-free survival (EFS) events were defined as death, disease recurrence or disease progression that precluded surgery. EFS and overall survival (OS) were analyzed from the first cycle of NACT until an event occurred. Survival was estimated with the Kaplan-Meier method and Cox regression model was used to calculate Hazard Ratio (HR). Chi-squared test was used to compare categorical variables.
Results: Of 737 pts, 41.4% were black. There was no significant difference between groups regarding median age (B: 48 years vs W: 49 years), marital status (married - B: 43.0% vs W: 42.6%, $p = 0.19$), as well as clinical stage III (B: 61.3% vs W: 63.7%, $p = 0.57$) and histological type (invasive ductal carcinoma - B: 88.2% vs W: 88.7%, $p = 0.48$). However, there was a difference in histological grade, with a higher proportion of black pts with grade 2 disease (39.0% vs 30.8%, $p =0.03$). A statistically significant difference was found in relation to the time of starting treatment, being greater in the black population (B: 2.3 months vs W: 2.0 months, $p = 0.03$). There were no significant differences in chemotherapy regimen, mostly AC-T (B: 87.5% vs W: 87.7%, $p = 0.35$) and type of surgery performed

(mastectomy - B: 47.9% vs W: 56.0%, p = 0.12), as well as pathological complete response rate (B: 28.5% vs W: 29.4%, p = 0.86). With a median follow-up of 61 months, white population had worse 5-year OS (72.9% vs 63.2%, HR 1.3, 95% Confidence Interval [95CI] 0.99 - 1.76, p =0.05) and 5 year EFS (65.5% vs 56.6%,HR 1.34, 95CI 1.04 - 1.72, p =0.02) than black population. When stratified according to histological grade, no differences were found between races for OS (p=0.20) and EFS (p=0.16) in grade 2 and for OS (p=0.28) and EFS (p=0.12) in grade 3 disease.
Conclusion: Black women had better EFS and OS than white women with early TNBC in our cohort. While reasons for this finding are not clear, it may have been influenced by a higher proportion of grade 2 disease in black pts. Furthermore, results may be influenced by heteroidentification bias, which may not reflect personal racial identification. This study highlights the importance of evaluating populations of different ethnicities to better understand racial disparities in TNBC.

P1-12-15: Real-World Assessment of biosimilar Trastuzumab MYL-1401O vs. reference Trastuzumab in HER2-Positive Breast Cancer: Utilization, Efficacy, and Safety in Asian patients

Ming-Chang Tsai

Background: MYL-1401O, a biosimilar of trastuzumab, has demonstrated efficacy and safety equivalent to reference trastuzumab (RTZ) in clinical trials for treating human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer (MBC). This study presents a real-world analysis comparing MYL-1401O with RTZ, focusing on single and dual anti-HER2 blockade therapies. The analysis encompasses neoadjuvant and first-line palliative treatments for HER2-positive early breast cancer (EBC) and MBC patients within a single hospital in Taiwan.

Methods: A retrospective analysis was conducted using medical records from Kaohsiung Veterans General Hospital. Patients treated with RTZ from January 2010 to December 2022 and MYL-1401O from January 2020 to December 2022 were included. The study focused on HER2-positive EBC patients who received neoadjuvant chemotherapy with RTZ or MYL-1401O ± pertuzumab (n = 140) and untreated stage IV MBC patients who received first-line palliative treatment with RTZ or MYL-1401O ± pertuzumab (n = 150). Primary endpoints included achieving pathological complete response (pCR) in the EBC group and progression-free survival (PFS) in the MBC group. Secondary endpoints were objective response rate (ORR), disease control rate (DCR), and cardiac safety. Subgroup analyses included patients receiving dual anti-HER2 blockade.

Results: Comparable pCR rates were observed between MYL-1401O and RTZ in the neoadjuvant chemotherapy group: 42.2% (19/45) for MYL-1401O and 51.6% (49/95) for RTZ (p = 0.301). Median PFS in MBC patients was similar between groups without statistically significant difference: not reached for MYL-1401O and 14.1 months for RTZ

(95% CI, 9.0-19.1, p = 0.061). ORR, DCR, and cardiac safety profiles did not significantly differ between MYL-1401O and RTZ.

Conclusions: This real-world study demonstrates that MYL-1401O is as effective and safe as RTZ in Asian patients with HER2-positive EBC or MBC, supporting its clinical utility in these populations.

P1-12-16: A Rare Case of Moderate Grade Intraductal Carcinoma in a Young Patient with known Neurofibromatosis 1

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Background: Neurofibromatosis type 1 (NF1) is a genetic disorder characterized by the development of tumors along nerves in the skin, brain, and other organs. It arises from mutations in the NF1 gene, which encodes neurofibromin, a tumor suppressor protein involved in regulating cell growth and division. NF1 patients have an increased susceptibility to various cancers, including breast cancer, though the precise mechanisms linking NF1 to breast cancer are still being investigated. Research indicates that the NF1 gene may play a role in modulating pathways involved in breast cancer development, such as the Ras signaling pathway. The risk of developing breast cancer before age 50 is estimated to increase fivefold in NF1 patients, with poor disease specific survival. Current NCCN screening guidelines suggest starting annual mammograms at age 30, with consideration of additional MRI screening. However, screening remains challenging in this patient population due to cutaneous neurofibromas, cafe-au-lait spots, and dense breast tissue. Enhancing our understanding of these connections could facilitate improved screening and management strategies for breast cancer in individuals with NF1.

Case Presentation: We present a case of a 17 year old female with a confirmed pathogenic NF1 mutation diagnosed in childhood, who presented with early-onset invasive ductal carcinoma (IDC). The patient presented with new-onset symptoms of right breast bloody nipple discharge and nipple retraction. Initial work-up with breast ultrasound noted only benign simple cysts. Further diagnostic work-up with MRI breast, however, demonstrated a 2 cm mass at the 4 o'clock axis of the right breast with associated 5 cm of segmental non-mass enhancement. Diagnostic mammogram revealed correlating right breast 5.8 cm segmental pleomorphic calcifications. Stereotactic core needle biopsy of these calcifications confirmed a diagnosis of IDC, grade 2, ER/PR +, HER2+ with extensive intermediate grade ductal carcinoma in-situ (DCIS). She underwent treatment with neoadjuvant TCHP followed by right nipple sparing total mastectomy, right sentinel lymph node biopsy (SLNB), and contralateral risk reducing nipple sparing mastectomy with direct to implant reconstruction. Pathologic examination of the right breast demonstrated extensive DCIS with no residual IDC, negative margins, pathologically negative sentinel lymph nodes (ypTis N0). Multidisciplinary adjuvant treatment included adjuvant Tamoxifen and Phesgo along with recommendations for post mastectomy radiation therapy.

Conclusion: Neurofibromatosis 1 and its heightened risk of early-onset breast cancer are well documented. However, diagnosing breast cancer in NF1 patients remains challenging due to prevalent cutaneous neurofibromas and dense breast tissue in young patients, which may obscure imaging and physical exam findings. While current guidelines recommend annual screening to begin at age 30, earlier initiation of high-risk screening with annual MRIs should be considered, potentially starting as early as age 18. Given the increased risk for breast cancer, it is crucial to implement age-appropriate diagnostic breast imaging in patients with known pathogenic NF1 mutations experiencing breast-specific symptoms.

P1-12-17: A case of sequential alpelisib and capivasertib in a patient with metastatic breast cancer harboring both a PIK3CA and AKT mutations

Sanjna Rajput, Karthik V. Giridhar

Introduction: Somatic mutations in the PIK3CA/AKT/mTOR pathway are associated with resistance to first-line endocrine therapy in hormone-receptor positive (HR+) breast cancer (BC). PIK3CA and AKT1 mutations are reported in 35-40% and 4-7% of HR+ BC respectively, with less published about the rates of co-occurrence (PMID: 32983983). Therapies targeting mutations in this pathway approved in conjunction with endocrine therapy include alpelisib, everolimus and capivasertib. Prior cohort data showed limited benefit of alpelisib after everolimus (PMID:36292649). Recent data notes that AKT emergent mutations may be a resistance mechanism to PIK3CA inhibition (PMID: 37916958).
As Capitello-291, BYLieve, and SOLAR-1 excluded patients with prior PIK3CA/AKT/mTOR inhibitors, minimal data exists on how to optimally select and sequence these therapies. Here we present a case of a patient with co-occurring PIK3CA and AKT1 mutations who gained clinical benefit from alpelisib and subsequently capivasertib.

Case presentation: A 64-year-old female presented with a left-sided parasternal mass. She had a prior estrogen receptor positive (ER+), human epidermal growth factor negative (HER2-) left mixed ductal and lobular carcinoma (pT2pN1a) 17 years ago, treated with lumpectomy, chemotherapy, radiation and 10 years of adjuvant endocrine therapy. 7 years later, PET-CT revealed presence of metastatic disease in the sternum and supraclavicular lymph nodes. Biopsy of the parasternal mass noted invasive ductal carcinoma, grade II, ER low+ (11-20%), progesterone receptor negative (PR-), HER2-. A second site biopsy was ER-, PR-, and HER2-. Tempus tumor sequencing of this sample noted both AKT1 E17K [variant allele fraction (VAF) 21.4%] as well as PIK3CA E545K mutation (VAF 8.1%).
The patient progressed on several lines of therapy due to adverse effects and disease progression. She received nab-paclitaxel/atezolizumab for 4 months, letrozole and abemaciclib for 2 months, letrozole alone for 6 months, and letrozole and ribociclib for 6 weeks. Given presence of PIK3CA mutation, 4th line treatment with fulvestrant and alpelisib was planned. However, the patient developed transaminitis after the first fulvestrant

treatment, prior to initiation of alpelisib, which resolved with steroids. After progressing on capecitabine after 2 months, alpelisib with exemestane was initiated. The patient had a partial metabolic response on PET/CT imaging and eventually progressed on alpelisib and exemestane after 15 months. Due to a low ejection fraction despite work with cardio-oncology, she was not a candidate for trastuzumab deruxtecan. She progressed on oral cyclophosphamide and methotrexate after 2 months and Sacituzumab govitecan after 3 months.
Guardant 360 liquid biopsy testing revealed the same prior AKT1 E17K (VAF 2%) and PIK3CA E454K alteration (VAF 1.7%). She started 9th line therapy with the pan-AKT inhibitor capivasertib with fulvestrant. Guardant Response assessment after 4 weeks noted clearing of the AKT1 and PIK3CA mutations with no cfDNA detectable. 2-month follow-up PET-CT revealed significant favorable response to treatment. At 6-month follow-up, the patient continues on this treatment.
We interrogated publicly available datasets in TCGA, MSK-IMPACT, METABRIC, and AURORA and note that across 7204 samples, mutation prevalence for PIK3CA and AKT1 was 39% and 5%, with significant mutual exclusivity and co-occurrence observed in only 41/7204 samples (0.6%) (PMID: 22588877).

Conclusion: This case report highlights a response of AKT1 targeted therapy with capivasertib in a patient who previously progressed on PIK3CA targeted therapy with alpelisib. Comprehensive biomarker assessment is needed to identify patients who may benefit from sequential therapies. There is a need for trials comparing therapies that target the PI3K pathway at distinct points, potential sequencing of these therapies, and the optimal sequence strategy.

P1-12-18: A case report of paraneoplastic rapidly progressive cerebellar syndrome in a patient with locally recurrent hormone receptor positive (HR+)/HER2- breast cancer

Kelsey Natsuhara, Michael D. Alvarado, Prashanth Ramachandran, Laura A. Huppert, Jessica Knapp, Sarah Donahue, Hope S. Rugo

Introduction: Paraneoplastic syndromes (PNS) are rare neurologic complications of breast cancer that can be life threatening. However, little is known about clinical risk factors for PNS. Furthermore, diagnosis can be challenging requiring a high index of suspicion to appropriately treat patients' PNS and their primary cancer.
Clinical Case: A 40 yr old woman with a history of locally recurrent right breast cancer presented with progressive neurologic symptoms. She was first diagnosed with stage IA (pT1bN0) multifocal grade 3 invasive ductal carcinoma with lymphovascular invasion; ER 90%, PR 10%, HER2-, recurrence score 31. After mastectomy, she received docetaxel + cyclophosphamide x 4 cycles and tamoxifen for 2 yrs, until she presented with a multifocal local recurrence with extension to skin (grade 3, ER>95%, PR-). She underwent surgical resection then received doxorubicin x 4 with ovarian function suppression (OFS). She then

developed a 2nd extensive local recurrence invading skin and muscle and started letrozole. Within 1 wk, she developed headaches, ataxia, dizziness, diplopia, and dysarthria that rapidly became disabling and was admitted to the hospital. MRI brain and spine were benign, except for subtle T2 hyperintensities in the cerebellum. Extensive infectious workup was negative. Cerebrospinal fluid (CSF) showed 72 WBCs with 97% lymphocytes. She was treated with broad spectrum antibiotics and antivirals for possible occult infection, but serial CSF sampling showed persistent lymphocytic pleocytosis. IgG index, oligoclonal bands, autoimmune encephalitis and paraneoplastic testing were negative. In consultation with neurology and oncology, she was diagnosed with probable paraneoplastic rapidly progressive cerebellar syndrome (RPCS). She was treated with 1g IV methylprednisone x 2 doses and intravenous immunoglobulin (IVIG). She was discharged and continued a slow steroid taper and monthly IVIG x 8 mos. Her symptoms began to improve after 1 mo of treatment and were largely resolved by 6 mos. For her breast cancer, she continued OFS + letrozole and, after significant neurologic improvement, underwent resection of the local tumor with 2 residual sub-cm foci followed by radiation and abemaciclib initiation. She remains stable on OFS, letrozole and abemaciclib 3.5 yrs after diagnosis of RPCS without worsening neurologic symptoms or breast cancer recurrence.
Discussion: Breast cancer is one of the most common solid tumors associated with PNS. The presentation of PNS varies widely, as any part of the nervous system can be affected. RPCS is one of the most frequent PNS in breast cancer. Patients typically presents with a severe pancerebellar syndrome that develops in <12 wks, including dizziness, nausea, gait instability, diplopia, ataxia, dysarthria and dysphagia. Symptoms typically stabilize after initial worsening, but if untreated, can leave patients with severe sequelae. Diagnosis requires ruling out alternative causes of cerebellar ataxia (e.g. metabolic causes), MRI brain, and CSF analysis. Though, MRI is not sensitive in the early stage of disease and CSF findings are often non-specific (e.g. elevated proteins and lymphocytes). RPCS is mediated by antibodies against onconeural antigens, with anti-Yo being most common in breast cancer. However, antibodies are identified in <50% of cases, making diagnosis difficult. Treatment typically includes high dose steroids +/- additional immunosuppressive therapies such as IVIG or plasmapheresis in severe cases. In addition, the underlying cancer must be treated for neurologic stabilization, but neurologic improvement is not always seen. Fortunately, our patient had significant neurologic improvement after treatment of her RPCS and breast cancer, with no signs of cancer recurrence after 3+ yrs.

P1-12-19: A case report of successful hair preservation using scalp cooling with doxorubicin and cyclophosphamide during pregnancy

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Introduction: The management of breast cancer during pregnancy requires complex decision making to optimize the health of the patient and minimize risks to the baby. Some systemic therapy agents, such as HER2-targeted agents, are contraindicated in pregnancy,

while for other agents there are less data. Doxorubicin + cyclophosphamide (AC) is a regimen that has been shown to be safe during pregnancy. However, hair loss is near universal without scalp cooling. There are no published reports of scalp cooling during pregnancy and the safety and activity of scalp cooling in pregnancy are unknown, though short term exposure to cold is not thought to be harmful.

Clinical Case: A 38 year old woman palpated a mass in her right breast. Breast ultrasound showed 1.5 cm and 1.3 cm masses with benign-appearing axillary lymph nodes. US-guided core biopsy revealed a grade 2 invasive ductal carcinoma (IDC) ER+, PR+, HER2+. At the time of diagnosis, she was found to be 10 weeks pregnant and desired to continue the pregnancy. Since HER2-targeted therapy is contraindicated during pregnancy, the patient started neoadjuvant systemic therapy with AC and used scalp cooling for hair preservation starting cycle 1. She received C1D1 at 14 weeks gestation and completed 4 cycles without complication. She had nearly 100% of her hair after 4 cycles of AC. At 30 weeks gestation, she underwent right partial mastectomy and sentinel lymph node biopsy which showed 2.5 cm of grade 2 IDC with 10% cellularity, ER+/PR+/HER2+. She had 6.5 cm of grade 2-3 DCIS, 1/1 lymph nodes with isolated tumor cells, and negative margins. At 34 weeks gestation, the patient was induced and delivered a healthy baby girl. At 2 weeks postpartum she started weekly paclitaxel, trastuzumab and pertuzumab (THP). She continued to use scalp cooling with minimal hair loss (<5%) after 12 weeks of THP. She has completed adjuvant T-DM1 and experienced no further hair loss.

Discussion: The diagnosis of breast cancer during pregnancy poses unique challenges both for treatment and symptom management. Data are limited in this space and largely from retrospective series, highlighting the need for careful monitoring and reporting of pregnant patients' experiences with breast cancer treatment. Hair loss is one of the most visible and psychologically distressing side effects of chemotherapy. Multiple randomized trials have demonstrated the efficacy of scalp cooling in reducing chemotherapy related hair loss, with more than half of women who use scalp cooling retaining more than 50% of their hair. However, rates of hair preservation are significantly lower with anthracyclines (16% in the SCALP trial). To our knowledge, there are no published reports on scalp cooling during pregnancy. This case is notable given the remarkable success of hair preservation with AC during pregnancy. In addition, scalp cooling was well tolerated for this patient and she went on to deliver safely. We hypothesize her notable hair preservation may be due in part to pregnancy related hair changes. Many women report thicker hair during pregnancy, due to slowing of hair follicle cycling with more follicles staying in growth phase (anagen) and fewer hairs entering resting phase (telogen), which results in less shedding and hair loss. After pregnancy, more hair follicles enter telogen phase resulting in postpartum hair loss (telogen effluvium). During her post-partum taxane treatment, she experienced ~5% hair loss, which may have represented telogen effluvium. This case demonstrates that scalp cooling can be safe and remarkably effective during pregnancy and should be considered in pregnant patients to mitigate the distress associated with alopecia.

P1-12-20: A rare case of metastatic atypical adenomyoepithelioma of the breast: challenges in systemic treatment.

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Introduction: Adenomyoepithelioma is a rare histologic type of primary breast tumor characterized by a biphasic presentation with cells of the epithelial and myoepithelial lineage that rarely become malignant and may metastasize. We present a case of metastatic atypical adenomyoepithelioma and the challenges associated with its systemic treatment.

Case Report: A 61-year-old woman with a history of grade 2 obesity, prediabetes and well-controlled hypertension presented with a lesion in her left breast. She had been under observation for about 7 years and had undergone several biopsies, all of which showed benign results and were treated as chronic granulomatous mastitis.

In 2022, she noticed a significant enlargement of the left breast lesion and sought our treatment. MRI of the breast showed an oval, exophytic lump in the anterior third of the left breast measuring 10.5x8.9x5.2 cm. Ultrasound-guided core biopsy revealed a ductal carcinoma in situ (DCIS) measuring 2.0 mm within a complex sclerosing lesion. A left mastectomy with sentinel lymph node biopsy was then performed. Pathologic analysis revealed an atypical adenomyoepithelioma with a size of 14.5 cm, mild to moderate atypia, clear margins and no involvement of the sentinel lymph nodes.

Staging with PET-CT revealed a hypermetabolic pulmonary nodule in the left lower lobe measuring 1.2 cm, which was confirmed by biopsy to be a metastasis of a primary atypical adenomyoepithelioma of the breast. The case was discussed in a tumor board to decide between SBRT and metastasectomy for the apparently solitary lung lesion. However, a PET-CT scan two months later revealed multiple lung nodules and multiple bone metastases.

First-line chemotherapy with weekly carboplatin and paclitaxel was initiated. After 12 weeks, a new PET-CT scan showed progression of the disease in both the bones and the lungs. The patient received second-line chemotherapy with liposomal doxorubicin as monotherapy, which also proved ineffective after 10 weeks, as the disease progressed in several bone segments and lung function deteriorated.

Genome profiling using NGS revealed mutations in AKT1, HRAS, IKZF1 and MPL, for which there are no targeted therapies in the Brazilian healthcare system. In addition, the TMB was 5 mutations/Mb without microsatellite instability. Other chemotherapy lines, including FOLFIRI and cisplatin with gemcitabine, were administered without satisfactory results. Although the patient had a good performance status (ECOG 1) and remained motivated for

new treatments, a sudden deterioration in consciousness occurred 16 months after diagnosis. She was taken to the nearest emergency room where a large ischemic stroke associated with a CNS tumor lesion was diagnosed, leading to her demise.

Discussion: The pathologic diagnosis of malignant adenomyoepithelioma is challenging because it is difficult to differentiate it from benign conditions such as intraductal papillomas, tubular adenomas, and sclerosing adenoses. In our patient, previous biopsies suggested granulomatous mastitis and the last biopsy revealed a large sclerosing adenomatous area with a small DCIS focus. Only analysis of the entire tumor sample led to the diagnosis of atypical adenomyoepithelioma. Reports from the literature emphasize the difficulty in characterizing morphological features indicative of malignancy.

The largest case series from the late 1980s and early 1990s reported 17 and 31 cases, respectively. More recent series are much smaller, emphasizing the rarity of the disease. Most cases are localized in the breast and can be well controlled by complete excision. However, local recurrences and metastases occur in up to 30% of patients and have a poor prognosis. The most common metastases include the thyroid, lung, CNS, bone and liver, suggesting hematogenous spread.

Treatment of malignant adenomyoepithelioma is not well established beyond complete removal at an early stage. The need for sentinel lymph node biopsy remains uncertain, and the efficacy of adjuvant chemotherapy has not yet been established. Chemotherapy for metastatic disease has generally shown limited efficacy. Therapies with anthracyclines, 5-fluorouracil and even eribulin have been reported, all of which have no proven efficacy.

Genomic profiling often identifies mutations in the AKT pathway, indicating a potential beneficial effect of targeted therapies in future trials. In this case, the patient had only received cytotoxic chemotherapy and had not responded to any of the therapies administered.

Conclusion: We await further studies to evaluate the role of molecular targeted therapies and immunotherapy in such a rare and aggressive malignancy. The case presented emphasises the need to continue research and development of effective treatment strategies for metastatic atypical adenomyoepithelioma.

P1-12-21: Angiosarcoma of the breast

Alexander V. Petrovsky, Danila A. Denchik, Yelena A. Kim, Elizaveta A. Golovina

Angiosarcoma of the breast is a rare malignant tumor of mesenchymal origin, rich in atypical cells of either endothelial or periocytic nature. It is characterized by rapid growth with a recurrent course, early metastasis of a hematogenic nature and a poor prognosis. Angiosarcomas account for less than 1% of all malignant breast tumors and less than 5-8% of soft tissue sarcomas. We report the case of a 28–year-old woman with a tumor in her

right breast that appeared during lactation. After performing a core biopsy of the formation, according to histological and immunohistochemical evaluations, primary angiosarcoma G1 (FNCLCC) was diagnosed with the presence of anti–CD31, anti-Fli-1 and anti-PanCK. From November 2021 to February 2022, 8 courses of neoadjuvant chemotherapy according to the HD AI scheme (doxorubicin + ifosfamide) were performed. Then, in March 2022, an oncoplastic sectoral resection of the right breast was performed. Postoperative histological examination: angiosarcoma G1 (according to the FNCLCC system) therapeutic pathomorphosis IIA according to Huvos. From 04/14/2022 to 07/05/2022, a course of adjuvant radiation therapy was performed only on the right breast without regional zones (3D CRT), photon energy 6/18MeV, single focal dose (SFD) 2 Gy, average focal dose 45.39 Gy. Currently, the patient is alive and has had a relapse-free course of the disease for two years. This clinical case shows the importance of continuity of treatment for angiosarcoma, which is a very aggressive disease.

P1-12-22: Breast Implant Associated Squamous Cell Carcinoma (BIA-SCC): A Mayo Clinic Multidisciplinary Approach to Treatment:

Dhauna Karam Prasad, Kimberly S. Corbin, Tina J Hieken, Samir Mardini, Malvika Solanki, Ciara C. O'Sullivan, Ciara O'Sullivan

Introduction: Breast implant associated squamous cell carcinoma (BIA-SCC) is a rare occurrence with approximately 400 cases reported to the FDA as of March 2023, however, the incidence is rising. Optimal management of this aggressive malignancy is not well defined, and is unfortunately associated with high morbidity and mortality. Herein, we describe the case of a woman who was diagnosed with and treated for BIA-SCC in a multidisciplinary fashion at the Mayo Clinic in Minnesota, USA.
Case Presentation: A 53-year-old post-menopausal woman presented in March 2024 with a 4-week history of pain, erythema and skin nodules concerning for an abscess of the right(R) breast implant. Her oncology history was significant for lobular carcinoma in situ of the left breast, and a right breast fibroadenoma (both diagnosed in 2009). She was treated with bilateral nipple-sparing mastectomies, which confirmed the aforementioned diagnoses. She subsequently underwent a bilateral breast reconstruction with gel implant placement in 2010. In 2014, she developed R breast pain secondary to implant capsular contraction, necessitating replacement of the R breast implant. She proceeded with a right-sided partial capsulectomy and bilateral silicone gel implant placement with fat injections. In March 2024, she presented with erythema, nodules and purulent drainage from the R breast which required hospitalization for IV antibiotics (minimally effective). Imaging (US and MRI of R breast) revealed complex intracapsular and subcutaneous fluid collections, enlarged right axillary and internal mammary chain lymph nodes, and 3 complex foci (the largest 4.3 cm) in the R breast. Core biopsy of a R breast lesion showed invasive carcinoma with squamous differentiation (triple negative, p63, p40-positive, PD-L1-positive [CPS >10]); right axillary lymph node FNA was negative for malignancy. Differential diagnoses were triple negative breast cancer vs. implant-associated squamous cell carcinoma vs. metaplastic breast cancer.

The diagnosis of BIA-SCC was made following multidisciplinary discussion, including a comprehensive review of the clinical presentation, case history, radiology and pathology reports.
She ultimately underwent surgical removal of the R breast implant with chest wall resection, capsulectomy and R axillary LN biopsy. Pathology revealed moderate to poorly differentiated invasive carcinoma with squamous differentiation, forming a 13.5 cm mass; 2 of 8 lymph nodes were positive for metastatic carcinoma. The patient was readmitted shortly after surgery complaining of dyspnea and chest pain. CT chest revealed a R pleural effusion. Diagnostic thoracentesis was negative for infection or malignancy. Despite broad-spectrum IV antibiotics, her symptoms worsened. A PET-CT revealed an FDG-avid infiltrative chest wall lesion (5.4 cm x 13 cm x 14.5 cm) worrisome for residual tumor, with a recurrent R pleural effusion. We commenced concurrent chemoradiation with weekly carboplatin (AUC 2), weekly paclitaxel (50 mg/m2) (1) and proton beam radiation (200cGy over 33 fractions). The patient responded clinically and radiologically to this approach . Next steps are to add pembrolizumab, and continue chemotherapy based on the KEYNOTE-522 regimen, continuing weekly carboplatin (AUC 1.5) and paclitaxel (80mg/m2) for a total of 12 weeks.. She will then complete 4 cycles of q 3 weekly adriamycin. cyclophosphamide and pembrolizumab. She is being monitored closely by medical and radiation oncology teams.

Conclusion: Awareness of BIA-SCC is key to early diagnosis and appropriate management of this rare and aggressive malignancy, as well as to ensure prompt reporting to regulatory authorities. Oncology and primary care providers should be vigilant when conducting long-term follow-up visits with patients who have had breast implants placed. Giventhe rarity of BIA-SCC , information sharing is critical to raise awareness and guide treatment recommendations. In-depth characterization of tumor genetics may aid development of personalized therapeutic strategies moving forward.
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P1-12-23: CASE SERIES OF PATIENTS WITH BREAST CANCER WHO USED HORMONAL IMPLANTS

Rafaella Britto Toledo, Antonio Carlos Buzaid, Carolina Cavalcanti Gonçalves Ferreira, Sara Batista Micheletti

Scenario: Symptoms attributed to menopause are the cause of many dissatisfactions and searches for Hormone Replacement Therapy. The numerous advantages associated with this replacement therapy have led to the widespread and unrestricted prescription of

different hormones. However, breast cancer remains the most common malignancy in Western countries in menopausal women and the estrogen receptor positive (ER+) subtype is the most prevalent. In patients with hormone receptor positive tumors, adjuvant endocrine therapy has a great impact on overall survival and the choice of endocrine therapy to be used will be determined by menopausal status. Objective: The primary objective of our study was to review the clinical characteristics and clinical course of patients that had hormonal implants and were diagnosed with breast cancer. Method: Case series of patients with hormone receptor-positive breast cancer who had undergone hormonal implants prior to the diagnosis of breast cancer. We retrospective reviewed the medical records of women with a histopathological diagnosis of invasive breast carcinoma aged over 18 years who reported having subcutaneous hormone replacement implants and were treated at Hospital Beneficência Portuguesa in São Paulo from November 2018 until November 2023. Results: Data were reported from 5 patients with hormone receptor-positive invasive breast carcinoma who had previously undergone SC implants as hormone replacement therapy. Among them, one patient whose last implant was more than 3 years old still had estradiol measured para mass spectometry (E2) of 23. Only after surgical removal of all her implants the E2 levels reduced to menopausal levels. Another had implants of about one year old and her E2 levels was 233. Her implants could not be surgically removed and the only adjuvant endocrine therapy possible was tamoxifen. Conclusion: Hormonal implants are a real problem in patients diagnosed with breast. E2 levels may remain elevated even many years after implant placement and some implants cannot be surgically removed resulting in chronic elevation of E2 levels. In these cases, only tamoxifen can be used as part of the adjuvant endocrine therapy.
Keywords: Hormone Replacement Therapy; Breast cancer; hormonal implant.

P1-12-24: Case Report: Activity of Pembrolizumab in Metastatic Merkel Cell Carcinoma and TNBC

Jessica Liu, Jane Kanowitz, Maryam Lustberg, Kelly Olino

The anti-PD-1 immune checkpoint inhibitor (ICI) pembrolizumab blocks the T-cell-inhibitory PD-1 pathway and has gained tumor agnostic approval for treatment of high mutational burden and PD-L1-expressing tumors [1,2; References Available].
Triple negative metastatic breast cancer (TNBC) is a highly aggressive subtype of breast cancer, accounting for 15-20% of incidence [3]. In combination with chemotherapy, pembrolizumab has improved overall survival in patients with metastatic TNBC [4,5,6]. Similarly, pembrolizumab has demonstrated tumor control against the neuroendocrine Merkel cell carcinoma (MCC), a rare and aggressive skin cancer [7,8,9]. Yet, for both MBC and MCC, study of ICIs' tolerability and efficacy in older patients (> 75 y.o.) is limited, despite higher incidence of cancer and reduced T-cell reserves [15].
Therefore, we present a case of a 98-year-old patient with metastatic MCC and locally advanced TNBC who achieved complete remission of both diseases on anti-PD-1 ICI pembrolizumab alone.

Case Presentation: A 98-year-old woman presented with a skin mass in the lower extremity in May 2022. The patient has a history of hypertension, hypercholesterolemia, aortic stenosis, and bradycardia with syncope, for which she had a cardiac pacemaker just prior to diagnosis. She is clinically frail and ambulates with a walker. Pathology reported the lesion as CD-20 negative, TTF-1 negative, and INSM1 positive, supporting diagnosis of neuroendocrine carcinoma. Given the small tumor size (1cm) and location on the medial aspect of the left ankle, she opted for one dose of 8Gy in August 2022 which led to complete regression of the lesion. In September 2023, biopsy showed in-transit Merkel cell carcinoma. Given good and improving performance status, she underwent PET CT for staging. PET CT imaging revealed a 3cm mass in the right lobe of the liver and a 3cm left breast mass. Laboratory data noted stage II renal insufficiency. Biopsy of the liver mass revealed Merkel cell carcinoma. Breast biopsy identified a poorly differentiated invasive ductal carcinoma mass, stage IIB TNBC with PDL-1 combined positive score (CPS) > 10 by pathology.
CARG (Cancer and Aging Research Group Chemotherapy Toxicity Calculator), a validated geriatric chemotherapy toxicity assessment tool, was utilized. Her CARG score of 15 indicated a 92% risk of grade 3 or higher toxicity, making chemotherapy an unfavorable palliative option.
As such, pembrolizumab (anti-PD-1 ICI) was recommended. Beginning November 2023, the patient received four treatments (200mg every 3 weeks) of pembrolizumab without toxicity. After the first cycle, her breast mass and skin lesions were no longer palpable. PET and clinical examination in March 2024 demonstrated complete radiographic and clinical remission of all sites of disease. Presently, there has been no clinical evidence of recurrence and most recent imaging has been negative.
Discussion: Both advanced MCC and TNBC are rare and aggressive carcinomas that have been found to respond to PD-1/PD-L1 targeted immunotherapy, with comparable ICI efficacy in patients aged younger or older than 85 years [6,12]. While recent multicenter, prospective studies did not find a correlation between age and immune related adverse event (irAE) occurrence, older patients were more likely to discontinue treatment due to irAEs [12,13,14,15]. Thus, assessment of functional status, comorbidities, and psychosocial factors is increasingly important for selecting geriatric patients for immunotherapy.
Our 98-year-old patient was diagnosed with MCC metastatic to the liver and PD-L1 positive TNBC. The complete remission of all sites of disease with pembrolizumab alone presents a remarkable case of PD-1 ICI-induced regression of advanced MCC and TNBC. Thus, this case suggests PD1-inhibitor immunotherapy can be effective, tolerated, and may be considered in older adults who are otherwise fit.

P1-12-25: Circulating Tumor DNA (ctDNA) Detection of Local Recurrence in a Patient with Early-Stage Triple Negative Breast Cancer

Samer Alkassis, Marla Lipsyc-Sharf, Shiliang Zhang, Caterina Gianni, Arielle Medford, Aditya Bardia, Shahryar Ashouri, Nimmi Kapoor

Introduction: Triple negative breast cancer (TNBC) accounts for 15-20% of invasive breast cancer cases. While most patients with early-stage TNBC are treated with curative intent, approximately 50% of patients experience recurrence in the first 5 years. There are no current recommended assays for early signs of recurrent disease, though novel diagnostic tests are currently in development. Circulating tumor DNA (ctDNA) assessment has been investigated as a way to evaluate response to treatment, determine prognosis, and detect minimal residual disease (MRD) after curative-intent treatment. However, while ctDNA detection is known to be strongly associated with poor prognosis, the incorporation of ctDNA evaluation in early breast cancer care has been challenging due to lack of evidence of clinical actionability. No data are currently available regarding the predictive utility of ctDNA detection on local recurrence. Herein, we present a case of a patient with early-stage TNBC who had positive ctDNA testing in the adjuvant setting that identified local recurrence.

Case: A 53-year-old thin woman palpated a mass in the right breast. A mammogram and ultrasound revealed an 8 mm lesion, and biopsy showed a grade 3 TNBC. CT scan of the chest, abdomen, and pelvis (CT CAP) revealed no evidence of metastatic disease. MRI of the right breast showed conglomerate area spanning up to 5.2 cm, with possible nipple involvement. She received 4 cycles of neoadjuvant carboplatin and Taxol, due to patient preference, followed by repeat MRI showing the same size of the mass with decreased enhancement. She then had bilateral skin-sparing mastectomy, including nipple removal, with implant reconstruction. Pathology revealed an 8 mm TNBC and involvement of one lymph node. She then completed 4 cycles of adjuvant cyclophosphamide and doxorubicin. One month after completing adjuvant chemotherapy, she had positive plasma ctDNA testing. A subsequent PET/ CT scan showed no evidence of metastatic disease. She had three additional plasma ctDNA tests at 2, 3, and 6 months after initial testing which were positive with increasing variant allele frequency. After additional PET/CT and CT CAP at 3 and 6 months from initial PET/CT scan respectively, an MRI breast was performed showing multiple masses in the skin flap of the reconstructed right breast. She underwent complete right mastectomy and implant removal which confirmed multifocal TNBC in the skin flap. She subsequently completed a course of radiation to the right breast, with negative plasma ctDNA afterwards.

Discussion: The detection of ctDNA post-treatment in early stage TNBC is a strong predictor of recurrence risk. During neoadjuvant chemotherapy, improvement in ctDNA detection has been associated with improved distant recurrence-free survival, even in patients with extensive residual cancer. The c-TRAK TN prospective trial in adjuvant TNBC found that ctDNA detection was associated with poor prognosis, with median time from ctDNA detection to recurrence of 4.1 months. However, to our knowledge, MRD testing has not yet been systematically utilized for detecting local recurrence. Fortunately, our patient did not have evidence of distant metastatic disease and dedicated breast imaging was obtained to identify local recurrence.

Conclusion: This case highlights the potential of MRD assessment in the adjuvant setting in TNBC to identify local recurrence and, in cases of adjuvant ctDNA detection, guide thorough evaluation with both systemic scans and dedicated primary breast imaging. As

demonstrated here, this may impact treatment and surveillance. Further prospective trials are needed to determine the clinical utility of MRD assessment for prognosis and early detection of local and distant recurrences in this setting.

P1-12-26: Clinical Case Vignette - Molecular PET Imaging to Guide Selection for Endocrine Therapy in Estrogen-Receptor Positive Metastatic Breast Cancer

Wenhui Zhou, Benjamin Franc, Eric Rosen

Introduction: Approximately 80% of all breast cancers are estrogen receptor (ER) positive, and determining ER status is vital for selecting appropriate treatment. 16α-[18F]-fluoro-17β-estradiol (18F-FES) is an FDA-approved radiopharmaceutical that enables non-invasive, in vivo assessment of ER status in patients with recurrent or metastatic breast cancer.
Clinical case: A 60-year-old woman diagnosed with right breast cancer (invasive ductal carcinoma, ER+/PR+/HER2-) underwent bilateral nipple-sparing mastectomy with clear margins and negative sentinel lymph nodes. The patient opted against adjuvant chemotherapy or radiation. About 6 months after surgery, biopsy confirmed metastatic breast cancer of the ER+/PR+/HER2- subtype. Due to the ER positivity of her metastatic breast cancer, she underwent an 18F-FES PET scan which revealed ER+ metastatic lesions in the right chest wall, right axillary and mediastinal lymph nodes, as well as in the axial and appendicular skeleton. This patient was deemed a candidate for and received first-line endocrine therapy (Anastrozole).
A 67-year-old woman diagnosed with left breast cancer (invasive ductal carcinoma, ER+/PR+/HER2-) underwent breast conservation surgery followed by adjuvant radiation. She remained on anastrozole for approximately six years until a re-staging 18F-FDG PET scan revealed hypermetabolic thoracic lymphadenopathy, pulmonary, hepatic, and osseous lesions. Considering the ER positivity of her initial cancer, an 18F-FES PET scan was performed, showing no uptake in any of the metastatic lesions. Liver biopsy confirmed metastatic breast cancer of ER-/PR-/HER2- subtype. The combination of disease progression on endocrine therapy and negative ER expression in both the liver biopsy and 18F-FES PET scan indicated acquired resistance and likely transformation to triple-negative disease. Consequently, endocrine therapy was discontinued, and she was switched to alternative cytotoxic chemotherapy (Abraxane).
Conclusion: These two complementary clinical vignettes illustrate the value of 18F-FES PET in patient selection for endocrine therapy. Together, these examples advocate for wider implementation of the recent NCCN guidelines recommending the use of 18F-FES PET in patients with metastatic or recurrent ER+ breast cancer.

P1-12-27: Clinical Case Vignette: Estrogen Receptor Targeting PET Imaging to Monitor Treatment Response to Endocrine Therapy in Lobular Breast Cancer

Wenhui Zhou, Benjamin Franc, Eric Rosen

Introduction: Estrogen receptor (ER)-targeting therapeutics play a large role in breast cancer treatment, given that 80% of breast cancers are ER positive. Molecular-based imaging strategies targeting ER are evolving to become integral to breast cancer management. 16α-[18F]-fluoro-17β-estradiol (18F-FES) is an FDA-approved PET agent that enables non-invasive imaging of ER status in patients with recurrent or metastatic breast cancer.

Clinical case: A 57-year-old woman with metastatic breast cancer (invasive lobular cancer, ER+/PR+/HER2-) who was initially diagnosed after bilateral salpingectomy for a left adnexal mass that showed metastatic ILC, and subsequent workup showed biopsy-confirmed primary ILC in the right breast. Her initial staging 18F-FDG PET scan showed no evidence of metabolically active malignancy. She underwent lumpectomy and axillary lymph node dissection, revealing 4/4 positive nodes. Due to the ER positivity of her metastatic breast cancer, a re-staging 18F-FES PET scan was performed, revealing 18F-FES uptake in subcentimeter right axillary and subpectoral lymph nodes. She was deemed eligible for and received first-line endocrine therapy (Letrozole) in combination with a CDK4/6 inhibitor (Ribociclib). At the 3-month follow-up 18F-FES PET scan, decreased FES avidity was noted in the right axillary and subpectoral lymph nodes, which completely resolved by the 1-year follow-up 18F-FES PET scan.

Conclusion: This case vignette illustrates the value of 18F-FES PET not only for patient selection for endocrine therapy but also for predicting the likelihood of treatment response. Thus, 18F-FES PET offers a promising opportunity for molecularly targeted PET radiopharmaceuticals to guide and monitor treatment in patients with metastatic or recurrent ER+ breast cancer.

P1-12-28: De Novo Metastatic Breast Cancer to the Orbit: A Case Study

Haley Westervelt Mabry, Katherine Ansley, Christina Cramer

Among new hormone receptor positive and HER2-negative breast cancer cases, less than 4% of patients aged 41-60 are diagnosed with metastatic disease at presentation. Of this small percentage, very few cases of orbital metastasis as the first presenting finding have been identified. We present the case of a 44 year old female who initially presented with redness and discharge of the left eye and was diagnosed with conjunctivitis. Despite treatment with courses of moxifloxacin eye drops, fluoromethalone eye drops, and oral amoxicillin, her symptoms persisted after 2 months prompting an ED visit. A CT scan demonstrated an osseous mass of the left sphenoid bone with a soft tissue mass involving

the left superolateral orbit, anterior and middle cranial fossa with associated proptosis. MRI scan of the orbits revealed an aggressive mass of the left frontal bone and left sphenoid wing with extraosseous soft tissue components. Further full-body imaging showed 2 small masses measuring 1.9 and 1.8 centimeters in the lower outer quadrant of the left breast and an enhancing lesion of the left parietal bone concerning for metastatic lesion but otherwise no convincing evidence of metastasis within the intradural parenchyma of the brain, abdomen, pelvis, or spine. She underwent left orbitofrontal craniotomy for resection and biopsy without complications; pathology revealed metastatic adenocarcinoma of breast primary. Tumor markers were found to be estrogen receptor and progesterone receptor positive and HER2 receptor negative. Subsequent breast biopsy confirmed consistent tumor markers within the breast masses. She received gamma knife radiation to the left periorbital region. At the time of gamma knife, 17 Gy was prescribed to the 59% isodose line to cover the high-risk post-operative peri-orbital dura. MRI 8 weeks later showed no evidence of local recurrence or new intracranial metastases. Radiation was followed by first line medical therapy for metastatic breast cancer with abemaciclib, an aromatase inhibitor and ovarian function suppression with goserelin. In conclusion, we present a rare case of metastatic breast cancer with the presenting feature of orbital mass; this case will contribute to earlier diagnosis and treatment in subsequent cases as well as add to the small body of known cases to further characterize long term prognosis of this specific type of organ involvement of metastatic breast cancer.

P1-12-29: Extreme response to sacituzumab-govitecan in a patient with metastatic triple-negative breast cancer

Allison Poles, Melody Cobleigh, Gene Solmos

Background:In 2020, Sacituzumab-govitecan was granted accelerated approval by the US Food and Drug Administration (FDA) for locally advanced or metastatic triple-negative breast cancer in patients who had received two or more chemotherapy regimens. Since then, very few case reports have demonstrated exceptional response. This presentation describes the remarkable clinical course of a patient with metastatic breast cancer who had an extreme response to sacituzumab-govitecan and has remained disease-free years after stopping treatment.

Findings: A 64-year-old female underwent a screening mammogram in the fall of 2018 which showed faint macrocalcifications in the lower outer quadrant of the right breast, warranting biopsy. One year later, she returned for further work-up after palpating a mass in the lower outer quadrant of the right breast. Diagnostic mammogram at that time demonstrated a 2.8 cm spiculated mass with suspicious microcalcifications in the lower outer quadrant of the right breast, and subsequent biopsy showed grade 3 triple-negative invasive ductal carcinoma, Ki-67 88%. Staging work-up revealed lung nodules, with subsequent excision revealing metastatic adenocarcinoma, consistent with breast primary. The patient started nab-taxel and atezolizumab, but experienced disease

progression[MC1] and was subsequently started on sacituzumab govitecan-hziy. After only a single cycle of sacituzumab govitecan, the breast mass decreased from 9 cm to 5 cm on physical examination and axillary lymphadenopathy resolved. After 3 cycles, the mass had shrunk to 2 cm. She underwent 22 cycles (ultimately stopped due to toxicity) with subsequent lumpectomy and sentinel node biopsy revealing pathologic complete response (pCR) in both breast and lymph nodes. Over the last two years since stopping sacitizumab govitecan, the patient has had serial exams, computerized tomography (CT) scans and mammograms every 3 months (nearly five years after her initial biopsy-confirmed diagnosis and nearly six years after her initial mammogram) and has no clinical or radiographic evidence of disease.

Conclusions: We are documenting an exceptional response to sacitizumab govitecan in a patient who presented with metastatic triple-negative breast cancer (TNBC). Despite notable disease progression on prior regimens, sacitizumab-govitecan showed dramatic and rapid response, with complete pathologic response (pCR) in breast and lymph nodes. Almost five years after this patient's initial diagnostic mammogram, and two years after stopping treatment with sacituzumab-govitecan, she remains without evidence of disease. Such findings are important to share in hopes that more research can focus on extreme responders. Results of next generation sequencing of her tumor will be presented.
Source:
1. Bardia A, Hurvitz SA, Tolaney SM, Loirat D, Punie K, Oliveira M, et al. Sacituzumab Govitecan in Metastatic Triple-Negative Breast Cancer. New England Journal of Medicine. 2021 Apr 22;384(16):1529–41.

P1-12-30: Genetic Predisposition to Cancer Associated with a Germline Pathogenic BRCA2 Variant: A Clinical Case Report

Zaida Morante, Henry Gomez

BACKGROUNDBRCA1 and BRCA2 are tumor suppressor genes involved in DNA repair and maintaining genomic stability. The presence of pathogenic variants (PV) in BRCA2 is associated with an increased risk of cancer in males, mainly for breast, prostate, and pancreatic cancer.Breast cancer in males is a rare disease. In the United States, it accounts for 1% of all breast cancer cases and 1% of all malignant neoplasms in males. In Lima, Peru, the cancer registry reported 31 new male cases during the years 2013-2015, out of a total of 7068 cases in both sexes.A lifetime risk of 1.8% to 7.1% at 70 years of age has been estimated for breast cancer in males carrying PV in BRCA2. They are characterized by presenting more advanced clinical stages (CS) at diagnosis compared to their female counterparts, with a high expression of estrogen receptors (ER), greater proliferative activity, and more aggressive behavior.Regarding prostate cancer, those with PV in BRCA2 have an 8.6-fold increased risk of developing prostate cancer by the age of 65 compared to the general population, with a higher likelihood of lymph node involvement and distant metastases at onset.Although the risk of cancer in women with PV in BRCA1 and BRCA2 has

been extensively investigated, the characteristics of cancer in male carriers of these mutations have not been adequately studied. This case report follows the CARE Guidelines. Case Presentation: 67-year-old male with a family history of prostate, breast, and pancreatic cancer on the paternal side (table 1), carrying a PV in BRCA2, was diagnosed in 2006. A routine screening in March 2022 found a PSA level of 8.58 ng/dL. On digital rectal examination (DRE), a 60 x 50 mm, non-tumorous, soft prostate was observed. Considering the history of carrying a PV in BCRA2, a prostate MRI was included in the screening, which reported findings consistent with a PI-RADS 4 score. Prostate core biopsy guided by ultrasound confirmed the presence of an acinar adenocarcinoma, Gleason score 7 (4+3) in the left lobe, corresponding to a stage IIA prostate cancer (T2bN0M0), ISUP 3, of unfavorable intermediate risk.The patient was treated with high-dose-rate brachytherapy (HDR-BT) to the prostate, followed by external beam radiation therapy (EBRT) using VMAT (volumetric modulated arc therapy) technique. Subsequently, he received androgen deprivation therapy (ADT) for 6 months with triptorelin and bicalutamide. He was placed under observation and follow-up with a PSA value of 0.2 ng/mL.In May 2023, a 1 cm retroareolar nodule was detected in the right breast, with no palpable axillary lymph nodes. Mammography revealed a high-density nodule measuring 10.8 mm, located retroareolar towards the upper-outer quadrant of the right breast. Ultrasound confirmed an irregular solid nodule measuring 10.3 x 9.2 mm with no axillary lymphadenopathy (figure 1.). Fine-needle biopsy confirmed an infiltrating carcinoma, ductal variety, NST/NOS subtype, intermediate grade. Immunohistochemistry (IHC) reported positive estrogen receptors (ER) (+++/100%), positive progesterone receptors (PR) (+++/60%), negative Her2/Neu, and KI67 15%. Tomographic studies showed no evidence of metastatic lesions.Bilateral total mastectomy was performed, with pathology reporting infiltrating breast carcinoma, NST/NOS subtype, intermediate grade, 20% TILS, associated ductal carcinoma in situ of 10%, negative lymphovascular invasion, and positive perineural infiltration. The tumor size was 14 mm, with nipple and terminal duct involvement due to direct extension of in situ and invasive carcinoma. No tumor infiltration of the skin was reported, and surgical margins were clear. Micrometastasis of 0.2 mm was found in 1 of 3 examined lymph nodes. The pathological stage was pT1c pN1mi.The Oncotype DX study had a Recurrence Score (RS) of 22 points, leading to a recommendation for adjuvant chemotherapy with Docetaxel-Cyclophosphamide for 4 cycles, followed by tamoxifen.

Discussion: Identifying the BRCA1 and BRCA2 genes has revolutionized how we use genomic information in individuals at high risk of developing cancer. However, most studies have focused on women, and the information available for men is limited.Contrary to what was reported in the clinical case, the typical presentation in carriers of PV in BRCA2 is of an aggressive disease, with a Gleason score ≥ 8, more advanced local extension (T3/T4), lymph node involvement, and the presence of metastasis at diagnosis.For male carriers of PV in BRCA2, prostate cancer screening is recommended from the age of 40 through PSA testing and DRE. With PSA levels ≤3 ng/mL and a normal DRE, repeating screening tests every 1 to 2 years is suggested. However, if the PSA level is >3 ng/mL or an abnormality is found in the DRE, a multiparametric prostate MRI is recommended.Although conservative management with prostate radiation therapy was chosen in this case, a widely accepted approach for

patients with unfavorable intermediate-risk is radical prostatectomy (RP). A meta-analysis by Luo et al. evaluated the benefit of RP versus observation in patients with localized disease. A 9% reduction in mortality risk and a 43% reduction in disease progression were observed.Regarding breast cancer in men, the age at diagnosis in patients with PV in BRCA2 precedes that of non-carriers by approximately a decade. The ductal histological subtype is the most prevalent, representing approximately 90% of cases. The Luminal A immunophenotype corresponds to 42% of reported cases. It is characterized by larger tumors, a higher frequency of lymph node metastasis, and distant metastases, resulting in a less favorable prognosis and lower survival rates compared to breast cancer in women. Xingyu Chen et al. reported a 10-year estimated survival rate of 53.9% versus 68.5% in men and women, respectively.For carriers of PV in BRCA2, education and training in breast self-examination and clinical breast examination every 12 months, starting at age 35, are recommended. Additionally, annual mammography should be considered from the age of 50 or 10 years before the first known case of breast cancer in men in the family, especially for those with PV in BRCA2 or for whom the lifetime risk of breast cancer is up to 7%.Regarding breast surgery, observational studies describe that breast-conserving surgery in men is associated with similar survival rates compared to total mastectomy. Like in the female population with clinically negative breast cancer and axilla, sentinel lymph node biopsy is the standard approach.The performance of contralateral risk-reducing mastectomy (CRRM) in patients carrying PV in BRCA2 is controversial. There is no high-quality evidence to support CRRM to reduce the risk of contralateral breast cancer in men. Whether the benefits of overall survival (OS) and disease-free survival (DFS) outweigh the associated costs and disadvantages has not been conclusively determined.S. Shak et al., in the analysis of 347 men and 82,434 women using Oncotype DX, obtained a similar distribution for both sexes based on RS, with an average RS of 18.1 (± 11.2) for men and 19.1 (± 10.2) for women. Similarly, Tal Grenader et al. found similar data regarding RS in the Oncotype DX analysis of 65 male patients and 2,455 women. These results support, to some extent, the use of Oncotype DX to guide breast cancer treatment in men.Although initially designed for patients without lymph node involvement, Oncotype DX was included in premenopausal women with 1 to 3 positive axillary lymph nodes in the RxPONDER study. In this study, the benefit of adjuvant chemotherapy in premenopausal women with an RS ≤ 25 was demonstrated.Wang et al., in the analysis of 848 men and 110,898 women with breast cancer evaluated using Oncotype DX, found that the distribution and RS patterns associated with mortality differ between men and women. Intermediate and high-risk RS values, defined by the TAILORx study parameters (11-25 and ≥26), were associated with a higher mortality risk in men and had a lower risk threshold than the female population. Additionally, there was little benefit of chemotherapy in men with intermediate risk.Currently, there are no clinical practice guidelines supporting the use of Oncotype DX to predict the benefit of adjuvant chemotherapy in men with breast cancer. However, the recommendation made in this patient is based on the inference of data from studies conducted in the female population.

Conclusion: Men carrying PV in BRCA2 have a higher relative risk of breast and prostate cancer, both occurring at younger ages than the general population and exhibiting

characteristics that confer greater aggressiveness and a less favorable prognosis. Oncotype DX is a genomic test that provides prognostic and predictive information on early breast cancer in women; however, information regarding its use in men is still limited. Further research is essential to establish precise guidelines for the Recurrence Score (RS) in male patients with breast cancer. Tumor suppressor genes BRCA1 and BRCA2 are involved in DNA repair and are crucial in maintaining genomic stability. Early detection through screening and appropriate medical follow-up improves the prognosis in patients carrying PV in BRCA2. Therefore, risk control monitoring and adequate genetic counseling are necessary.

P2-01-01: Lifestyle Linked Biomarker is Associated with Poor Prognosis in Estrogen Receptor Negative Breast Cancer

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Background: Advanced glycation end-products (AGEs), reactive metabolites produced as a by-product of sugar metabolism, have been linked to higher breast cancer (BC) risk and increased mortality after BC diagnosis. The total body AGE pool is composed of endogenous AGEs and exogenous AGEs (consumed mainly through processed and fried foods common in Westernized diets). Diets high in AGEs have been linked to BC risk and outcomes. In our prior work, we reported that higher serum AGE (sAGE) levels, a reflection of total body AGE, are associated with reduced overall and BC-specific survival. sAGE was positively associated with BMI ($p<.0001$, $rs = 0.10$) and negatively associated with physical activity ($p< .001$, $rs = -0.06$). Comparing the highest quintile to the lowest quintile, sAGE was negatively associated with all survival outcomes in the entire cohort (overall survival (OS) HR=1.66 p=.014, recurrence free survival (RFS)=1.66 P=.004, BC specific survival (BCSS) HR=1.78 p=.013, distant metastasis free survival (DMFS) HR=1.81 p=.004). Here we explore the relationship between sAGE and BC outcomes by hormone receptor status in the Women's Healthy Eating and Living (WHEL) study.
Methods: The WHEL randomized 3088 BC patients stage I-III who completed their primary therapy to a high-vegetable, low-fat diet or control and followed for a median of 7.3 years. Main outcomes were invasive BC events (recurrence or new primary N=518), death due to BC (N=262) and deaths from any cause (N=315). Fasting blood was collected at study entry. sAGE was measured as the AGE metabolite carboxymethyllysine (ug/ml). sAGE was log transformed and corrected for plate batch effect via linear regression, and analyzed in continuous scale and in quintiles. The relationship between sAGE and survival endpoints was calculated using Kaplan-Meier and Cox regression models. In multivariable Cox models, we evaluated the interaction between sAGE (in quintiles) and ER or ER/PR status, adjusting for potential confounding covariates (age, race, BMI, smoking, alcohol use, physical activity, tumor characteristics).
Results: 2564 participants had sAGE available. After excluding samples for excessive variabilities, 2315 samples were analyzed. Patient characteristics: median age 52, 85%

white, 79% menopausal, 70% received chemotherapy, 43% node positive, 71% stage II or III. 1727 patients were ER+ and 556 were ER-. Raw corrected sAGE ranged from 0.0-48.15 ug/ml (median 7.39); logged and corrected ranged from -5.04-1.67. In the multivariable Cox model, the main effects of sAGE were significant for all the survival endpoints (OS p=0.023, RFS p=0.042, DMFS p=0.012, BCSS p=0.028), as well as the sAGE and ER interaction effects (OS p=0.017, RFS p=0.011, DMFS p=0.015, BCSS p=0.008). The strongest associations between sAGE and BC outcomes were seen within ER- cases: ER- patients of the highest sAGE quintile showed significantly higher risk compared to those with the lowest quintile (OS HR=2.37 p=.02, RFS HR=2.02 p=.02; BCSS HR=2.74 p=.01, DMFS HR=2.62 p=.01). Among the patients with the highest quintile sAGE, ER- cases showed consistently higher risk than ER+ (OS HR=1.83 p=.03, RFS HR=1.78 p=.02; BCSS HR 2.39 p=.004, DMFS HR=1.58 p=.11). Similar but slightly less significant results were seen with combined ER/PR status, likely due to the reduced sample size from missing values.
Conclusions: In a cohort of women with early BC, higher sAGEs were associated with worse survival outcomes in BC, with a stronger association in ER- BC compared to ER+ BC. AGEs may represent a novel, lifestyle-linked, modifiable prognostic biomarker in ER- BC. Interventions aimed at lowering sAGE levels should be tested in this high-risk subgroup for their impact on prognostic and metabolic biomarkers as well as clinical outcomes.

P2-01-02: Patient's Preference for Shared Decision Making-Based Distant Metastasis Surveillance and Its Effect on the Quality of Life: A Prospective Pragmatic Trial

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Background: Major guidelines recommend against the routine use of surveillance imaging tests to detect distant metastasis in patients with early-stage breast cancer, as such tests in asymptomatic patients do not significantly impact survival rates and often lead to unnecessary follow-up procedures and increased patient anxiety. Despite these guidelines, a significant number of imaging studies are still performed, highlighting a gap between recommendations and clinical practice. This study aims to assess the patient's preference for the use of surveillance imaging tests for distant metastasis detection and to evaluate degree of quality of life associated with different surveillance strategies in early breast cancer patients.

Methods: A prospective pragmatic clinical trial was conducted from July 2021 to December 2023 at three breast cancer centers in Seoul, South Korea. The study initially aimed to enroll 264 patients, but enrollment was stopped after enrolling 238 patients. Patients with early-stage breast cancer who had completed treatment within the past two years were enrolled and randomized into a shared-decision making (SDM) group or a control care group. Patients in SDM group were educated with videos and leaflets about the lack of clinical

evidence for performing imaging tests to detect asymptomatic distant metastasis. The control care group underwent surveillance imaging studies of institutional practice during the follow-up period, while the SDM group was followed according to their preference for distant metastasis surveillance. Primary endpoints were quality of life (QoL), anxiety, and depression at enrollment, and at 6-month, 1-year, and 2-year visits. The Functional Assessment of Cancer Therapy-Breast (FACT-B) and the Hospital Anxiety and Depression Scale (HADS) were used for assessments.

Results: A total of 787 questionnaires were collected from 238 patients. The FACT-B total score of the control group was significantly higher at enrollment (107.6 ± 21.1 vs. 102.0 ± 20.8, p=0.040), but converged over time with no significant differences at subsequent visits. The overall anxiety score (6.1 ± 3.3 vs. 5.5 ± 3.7, p=0.017) was significantly higher in the SDM group, but the difference was insignificant when compared at each visit. There were no significant differences in depression score at any time point. In the SDM group, 117 patients were asked for their preferences for distant metastasis surveillance and 33 patients (29.1%) preferred not to receive unnecessary imaging. At enrollment, this guideline-based surveillance group had significantly higher FACT-B total scores compared to those who preferred imaging for distant metastasis (113.9 ± 15.6 vs. 97.4 ± 20.5, p<0.001). The difference remained significant at 6-month visit (107.7 ± 15.4 vs. 98.6 ± 20.8, p=0.026), but not at 1-year or 2- year. Similarly, both anxiety and depression scores were significantly lower in those who preferred guideline-based surveillance at enrollment, but not at subsequent time points.

Conclusion: This pragmatic trial demonstrates that with adequate patient education and shared decision making, guideline-based surveillance imaging does not negatively impact patients' quality of life, anxiety, or depression level. These findings support an evidence-based strategy for the surveillance of patients with early-stage breast cancer.

P2-01-03: Is trastuzumab associated with new-onset hypertension in adolescents and young adults with breast cancer?

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Background: Studies have documented new-onset hypertension (hereafter referred to as "hypertension") and heart failure during and after breast cancer therapy. Trastuzumab, a monoclonal antibody used to improve survival in women with human epidermal growth factor receptor 2 (HER-2) positive breast cancer, carries a risk of cardiovascular complications. While most congestive heart failure events are reversible after trastuzumab is paused or stopped, the reversibility of trastuzumab-induced hypertension is uncertain. To date, no study has investigated hypertension as a potential adverse effect of trastuzumab therapy in adolescent and young adult (AYA) cancer survivors. Methods

Using data from Kaiser Permanente Northern and Southern California, we included all female AYAs ages 15–39 years, diagnosed with a first primary invasive breast cancer between 2006 and 2020, who survived at least 2 years after diagnosis. Patients were categorized into two groups: those who received chemotherapy plus trastuzumab (exposed group) and those who received chemotherapy without trastuzumab (non-exposed group). We looked at their outcomes 2 years post-diagnosis. We compared the cumulative incidence of hypertension between exposed and non-exposed groups and evaluated factors associated with hypertension. Hypertension was defined according to criteria described in the Pathways Heart Study, using the International Classification of Diseases codes and anti-hypertensive medications.

Results: We identified 2,371 AYA women with breast cancer; 33.4% of patients received trastuzumab. During a median follow-up of 5.8 years, the 5-year cumulative incidence of hypertension did not differ between the exposed (6.1%, 95% Confidence Interval (CI) 4.5–8.0%) and non-exposed (6.0%, CI 4.9–7.3%) groups. In the multivariable model, trastuzumab administration was not associated with hypertension (hazard ratio (HR)=1.06, CI 0.78–1.45). Factors associated with higher risk of hypertension included older age at diagnosis (35–39 vs. 15–34, HR=1.56, CI 1.15–2.11), non-Hispanic Black/African American (HR=2.55, CI 1.64–3.95) or non-Hispanic Asian/Pacific Islander (HR=1.99, CI 1.34–2.97 vs. non-Hispanic White) race/ethnicity, overweight/obesity (body mass index >25kg/m2, HR=2.57, CI 1.87–3.53 vs. normal weight), and ever smokers (HR=1.53, CI 1.10–2.12 vs. non-smokers).

Conclusion: Trastuzumab was not associated with an increased risk of hypertension in AYA chemotherapy recipients, but there was a higher risk of hypertension among non-Hispanic Black/African American and non-Hispanic Asian/Pacific Islander women, older age at diagnosis, overweight/obese patients, and smokers. Strategies promoting healthy diet, exercise, and smoking cessation should be employed for AYA patients with breast cancer who are at higher risk of hypertension.

P2-01-04: Impact of Mobile Healthcare Apps on Patient-Reported Quality of Life after Breast Cancer Surgery: A Randomized Controlled Trial

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Background: While numerous mobile healthcare applications (apps) have been developed, there remains a paucity of evidence regarding their efficacy in enhancing the quality of life for cancer patients. To bridge this gap, we conducted a randomized trial involving 320 breast cancer patients to evaluate the impact of utilizing apps postoperatively for six months on their overall well-being.
Methods:Between November 2020 and September 2021, we enrolled stage 0-III breast

cancer patients aged 20-60 who underwent surgery at Asan Medical Center. These participants were randomly assigned in a 1:1:1:1 ratio to three commercial apps (A for general health management, B for walking encouragement, and C for cancer-specific support) or a control group receiving conventional care. Comprehensive assessments were conducted at baseline, 6 months, and 12 months using the five-dimension EuroQol five-level version (EQ-5D-5L) questionnaires. Quality of life was measured using both the index score and visual analog scale (VAS) of EQ-5D-5L. Additionally, we investigated the proportions of patients who reported problems in the five dimensions.
Result: While the overall cohort showed no significant improvement in index score or VAS with the apps, a subgroup analysis revealed notable trends. High-compliance users of app B reported a lower proportion of problems in usual activities compared to the control group (0% vs. 27.4%, P < .001). Additionally, among those undergoing adjuvant chemotherapy—a population typically experiencing decreased quality of life—app C users demonstrated higher index scores than the control group (0.908 vs. 0.761, P = 0.004).
Conclusion: Our study did not demonstrate the effectiveness of mobile apps in improving the quality of life for postoperative breast cancer patients. However, these healthcare apps showed the potential to enhance the quality of life not only among patients with high app adherence but also among those whose quality of life was initially compromised.

P2-01-05: Quality-adjusted Time Without Symptoms of disease progression or Toxicity of treatment (Q-TWiST) Analysis of Sacituzumab Govitecan vs Chemotherapy in Previously Treated Patients with HR+/HER2– Metastatic Breast Cancer

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Background: The randomized, open-label phase 3 TROPiCS-02 study demonstrated that sacituzumab govitecan (SG) was associated with significantly improved overall survival (OS) and progression-free survival (PFS) vs chemotherapy for patients with previously treated hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2– [HER2 immunohistochemistry 0, 1+, or 2+/in situ hybridization-negative]) metastatic breast cancer (mBC). SG also had a manageable safety profile consistent with prior clinical studies in mBC. We present the results of a quality-adjusted time without symptoms of disease progression or toxicity of treatment (Q-TWiST) analysis to better assess the benefit and risk of SG treatment vs chemotherapy while accounting for patient quality of life.
Methods: Survival time was partitioned into 3 health states as follows: A) TOX: with toxicity (treatment-emergent adverse event [TEAE] of grade ≥ 3) before disease progression until TEAE resolving, disease progression, death, or end of follow-up, whichever was first; B) TWiST: without toxicity and before disease progression; and C) REL: disease progression until death or end of follow-up, whichever was earlier. Health state utilities were determined using published literature. Q-TWiST was calculated as the utility-weighted sum

of health state durations, and differences in Q-TWiST and relative Q-TWiST gains between the treatment groups were assessed. The established threshold for clinically important relative Q-TWiST gain is ≥10%. The utility weighting for the health states was: u-TWiST- = 0.715, u-TOX- = 0.605, and u-REL-- = 0.443. A threshold analysis was conducted in which u-TOX- and u-REL- were varied between 0 and 1 to assess robustness of treatment benefit in relation to utility values. This was performed with u-TWiST- = 0.715 (base case, stable disease with no toxicity) and u-TWiST- = 1 (best overall health state). Another sensitivity analysis was conducted to show restricted mean Q-TWiST difference at various follow-up times up to approximately 39 months.
Results: SG demonstrated significantly longer Q-TWiST (9.7 months; 95% CI, 8.9-10.5) vs chemotherapy (8.1 months; 95% CI, 7.3-9.0) in patients with HR+/HER2– mBC with a difference of 1.6 months (95% CI, 0.4-2.7; P = .0067) between treatment groups. The relative Q-TWiST gain was 10.8%, passing the threshold for clinical meaningfulness. SG had a longer TWiST by 2.0 months (95% CI, 0.1-3.9) compared with chemotherapy. In the threshold sensitivity analysis, adjusted weighted utilities of --u-TOX- and u-REL- had minimal impact on relative Q-TWiST gain with SG vs chemotherapy. With u-TWiST = 0.715, Q-TWiST gain depended primarily on u-TOX- value, and Q-TWiST gain was ≥ 10% when u-TOX- was > 0.07. Q-TWiST gain ranged from 9.9% to 11.4%. When u-TWiST- was set to 1, Q-TWiST gain also depended mainly on u-TOX- value, but Q-TWiST gain was ≥ 10% for all values of u-TOX- and u-REL-. In this analysis, Q-TWiST gains ranged from 13.9% to 15.3%. In both cases, Q-TWiST gains were statistically significant across the entire range of u-TOX- and u-REL- values. In the sensitivity analysis, Q-TWiST gain increased or was maintained with longer follow-up time.
Conclusions: In patients with HR+/HER2– mBC, SG showed a clinically meaningful and statistically significant improvement in Q-TWiST time compared to chemotherapy, and this improvement was enhanced with longer follow-up. The results from this analysis further strengthen the benefit-risk for SG as a treatment for patients with HR+/HER2– mBC.

P2-01-06: Supportive care concerns of young women living with metastatic breast cancer from an ongoing prospective virtual intervention

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Background: Adolescents and young adults (AYAs) living with metastatic breast cancer (MBC) experience physical and psychosocial difficulties often amplified by a disrupted life trajectory. Data regarding age-specific needs and concerns of AYAs within the context of the MBC disease experience are lacking. We describe psychosocial and supportive care concerns among AYAs with MBC to better understand and address their survivorship needs over time.
Methods: AYAs (18-39 years) diagnosed with MBC participating in an ongoing prospective intervention study (Young, Empowered and Strong [YES], NCT04379414) at Dana-Farber Cancer Institute complete an electronic REDCap survey at baseline (BL) and every 6 months

(mos) post-enrollment for 3 years, then annually for 2 years. Through the YES web-based portal, participants respond to electronic patient-reported outcomes and receive information on ways to manage symptoms and concerns they endorse in addition to other resources. Here we describe REDCap survey-reported psychosocial and supportive care concerns at BL, 6-, and 12-mos. Clinical information was abstracted from medical records. Concerns were assessed with items adapted from the AYA Health Outcomes and Patient Experience survey and dichotomized (not at all/a little, somewhat/very concerned). Financial burden was assessed using National Health Interview Survey items such as burden from diagnosis (a little/none, a lot/some). We used generalized estimating equations to model proportional changes in concerns from BL to 6- and 12-mos.
Results: As of 5/1/2024, 100 of 103 (97.1%) women sent the BL survey completed it. Of those eligible (N=91), 53 (58.2%) completed the 6 mo survey; 23 (25.3%) did not respond, 11 (12.1%) died, and 4 (4.3%) had withdrawn. At 12-mo, 40 of the 75 eligible participants responded (53.3%); 24 (32%) did not respond, 9 (12%) died, and 2 (2.7%) had withdrawn. Median age at enrollment was 37 years (range=26-45), 33 years at MBC diagnosis (range=22-39), and time from metastatic diagnosis to enrollment was 15 mos (range=0.2-96.3). Time from primary to metastatic diagnosis varied: 34.0% de novo, 17.0%, 3 mos-2 years and 49.0% ≥2 years. Most participants were white (85%), non-Hispanic (92%), and had a college degree or higher (80%).
Concerns were pervasive at BL, but many concerns lessened over time including the possibility of the cancer worsening (BL: 92.0% v. 6 mo: 86.8% v. 12 mo: 71.8%, p=0.02), potential long-term side effects of treatment (71% v. 56.6% v. 47.4%, p= 0.01), potential long-term effects of cancer on health (70.0% v. 60.4% v. 46.2%, p=0.03), and a family member's risk of getting cancer (39% v. 41.5% v. 20.5%, p=0.01). Though other concerns followed a similar pattern over time, differences were not statistically significant: how to check for signs of cancer worsening (61.6% v. 52.8% v. 41.0%, p=0.06), physical fitness/exercise (54.6% v. 50.9% v. 43.6%, p=0.48), nutrition/healthy diet (51.5% v. 45.3% v. 35.9%, p=0.19), and having financial support for medical care (40.0% v. 45.3% v. 33.3%, p=0.31). BL financial burden difficulties were endorsed least (a lot/some: 41.2%) followed by those at 12- (47.4%) and 6-mo (55.8%) but were not significantly different (p=0.14).
Conclusion: Among AYAs with MBC enrolled in the YES study, selected cancer-related concerns improved over time, still a substantial proportion continue to experience concerns over time. These preliminary results suggest an intervention to support young survivors with MBC may improve AYA concerns while others may need additional attention; alternatively, some concerns may wane as patients adapt to diagnosis and treatment. Addressing concerns such as financial toxicity, physical and mental health, disease progression, and treatment side effects, may optimize disease expectations and wellbeing.

P2-01-07: The Co-Occurrence of Obesity and Cancer-Related Fatigue and Their Combined Impact on Physical Function in a Nationwide RCT of 456 Breast Cancer Survivors (a URCC NCORP Study).

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Background: Cancer-related fatigue (CRF) is one of the most prevalent and debilitating toxicities - experienced by nearly half of breast cancer survivors, with over 60% reporting their fatigue as moderate to severe. Up to 75% of breast cancer (BC) survivors also report being overweight or obese. Both CRF and obesity are independent risk factors for impaired physical function which ultimately impacts cancer survivors' ability to perform essential daily activities, such as personal hygiene, meal preparation, engaging in physical activity, working, and engaging in meaningful relationships. Despite their high prevalence, the co-occurrence of CRF and obesity is not well characterized and how these conditions may synergistically impact physical function is not fully understood.
Methods: We conducted a secondary analysis of baseline data from 456 BC survivors who participated in a multicenter, nationwide phase III RCT comparing the effects of Yoga for Cancer Survivors (YOCAS©®), CBT-I, and a behavioral placebo on insomnia. The Brief Fatigue Inventory (BFI) was used to assess CRF (0-10 scale; mild = 1-3; moderate = 4-6; severe = 7-10). Anthropometric measures, height and weight, were utilized to calculate body mass index (BMI; kg/m2). Cardiorespiratory function was assessed via 6-minute walk test (6MWT Distance, 6MWTD [in feet]). Muscular strength was assessed via hand grip dynamometry strength test (kg). Age-adjusted marginal mean BFI scores (total, severity, and interference with activities of daily living) were calculated according to BMI category (normal weight = 18.5 - 24.9 kg/m2; overweight = 25 - 29.9 kg/m2; obese >30 kg/m2) via the Least Squares Means method. We also conducted an age-adjusted logistic regression for the odds of moderate-severe CRF according to BMI category. Finally, we cross-classified participants into four groups 1) normal weight with mild CRF (N=53), 2) normal weight with moderate-severe CRF (N=121), 3) overweight/obese with mild CRF (N=54), and 4) overweight/obese with moderate-severe CRF (N=228). We calculated age-adjusted marginal mean physical function measures according to these categories via the Least Squares Means method.
Results: 456 BC survivors (avg. age 56 years, 1.9 years post primary treatment, 86% White, 81% receiving hormonal therapy) were recruited from University of Rochester Cancer Center NCI Community Oncology Research Program (URCC NCORP) Research Base-affiliated community oncology practices across the nation. BC survivors who are obese (mean: 4.9, SE: 0.1) or overweight (4.4 ± 0.2) experienced moderate CRF while BC survivors who are normal weight experience mild CRF (3.8 ± 0.2; both $p<0.05$). We report similar trends in the CRF severity (normal weight = 5.0 ± 0.2, overweight = 5.6 ± 0.2, obese = 6.1 ± 0.1; both $p<0.05$) and CRF interference subscales (normal weight = 3.3 ± 0.2, overweight = 3.7 ± 0.2, obese = 4.3 ± 0.2; both $p<0.05$). Logistic regression revealed the odds of moderate-severe CRF were over 2x higher (OR [95%CI] = 2.24 [1.38-3.66]) among obese survivors when compared to normal weight survivors. Overall, overweight/obese breast cancer

survivors with moderate-severe CRF had significantly worse cardiorespiratory function (6MWTD = 1,432.4' ± 19.4) compared to other groups (overweight/obese and mild CRF = 1549.3' ± 26.5, normal weight and moderate-severe CRF = 1613.5' ± 39.9, or normal weight and mild CRF = 1706.2' ± 40.2; all p<0.001). No significant difference in hand grip strength was observed between any of the groups (p-trend=0.365).
Conclusion: Obese BC survivors are twice as likely to have moderate-severe CRF compared to their normal weight counterparts. Further, BC survivors with co-occurring overweight/obesity and moderate-severe CRF have significantly worse cardiorespiratory function than those with just one or neither of these morbidities.
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P2-01-08: Follow-up in Early and Locally Advanced Breast Cancer Patients: An EORTC QLG-BCG- ROG study.

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Background: Breast cancer (BC) is the most frequent type of cancer in women worldwide. Evidence-based follow-up strategies regarding risk, patients' needs and quality of life are lacking. The main objectives of this study are to determine the range and prevalence of physical, psychological and social problems following breast cancer treatment, and to identify patterns of physical, psychological and social problems based on demographic and clinical factors.
Methods: The EORTC 1617-QLG-BCG-ROG is an international cross-sectional non-interventional follow-up study in patients who are disease-free at least 1 but no more than 3 years since completion of primary treatment (except hormonal treatment) for early BC (EBC) and locally advanced BC (LABC). Institutional, demographic, tumour and treatment history data were collected. Patients completed a PRO questionnaire set once including the EORTC QLQ-C30, QLQ-BR45, EORTC QLQ-SHQ-22, EORTC QLQ-OUT-PATSAT-7, one question regarding follow up strategy from the patient´s point of view, and the Distress Thermometer, 139 items in total. A total of 830 patients would allow adequate estimation of prevalence rates and 90% power to detect a 10% difference between two cohorts. Patients were stratified by age, NPI risk score and treatment to ensure adequate representation within each stratum.
Results: Between November 2020 and September 2022, a total of 833 patients were enrolled in 25 institutions (of which 17 had a dedicated breast unit) across 10 countries. Of these 833 patients, 686 (82%) had EBC and 144 (17%) LABC. A total of 805 (97%) questionnaires set were completed, either in whole or partially, by eligible patients. More than 60% reported maximal score on the role and social functioning scale, as well as 50% reported no symptoms on nausea/vomiting, diarrhoea, insomnia, appetite loss and

constipation. Regarding stage of the disease, EBC patients showed statistically significant better (at 1% level of significance) scores in the physical functioning, role functioning, and cognitive functioning scales of EORTC C30 compared to the patients with LABC. Regarding age, young patients (< 50y) showed statistically significant worse scores in emotional functioning, cognitive functioning, social functioning, fatigue, and insomnia and reported more financial difficulties compared to the older population. Regarding type of therapy, patients receiving chemotherapy showed statistically significant worse scores in the physical functioning, role functioning, cognitive functioning, social functioning, fatigue, nausea/vomiting, pain, and financial difficulty scales, compared to the patients without chemotherapy even though the therapy had ended 1 to 3 years before.
Further analyses of EORTC QLQ-BR45, EORTC QLQ-SHQ 22, and EORTC QLQ-OUT-PATSAT7 including comparison with normative data, are currently being performed and will be presented at SABCS 2024.
Conclusions: Patients with EBC and LABC showed a distinct increase in physical functioning, role functioning, and cognitive functioning scales during follow-up after completion of primary treatment. Results showed that especially young patients and patients received chemotherapy are vulnerable groups, even after the end of therapy. These problems were associated with cognitive functioning, social functioning, fatigue, and financial difficulty. Information gained from this study will be useful for refining follow-up programs in EBC and LABC.

P2-01-09: Patient-reported outcomes in premenopausal breast cancer patients with or without ovarian suppression therapy – a subgroup analysis from a Brazilian prospective cohort

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Background: Young women with breast cancer (BC) treated with chemotherapy or endocrine therapy (ET) often experience physical and psychosocial changes during treatment, partly due to non-physiological menopausal symptoms. These symptoms tend to be worse with ovarian function suppression (OFS), and it can significantly affect the quality of life (QoL), leading to nonadherence to treatment. Our study aims to assess patient-reported outcomes (PRO) in premenopausal breast cancer patients receiving or not OFS.
Methods: This report is a sub-analysis of our institutional multicenter, prospective, observational study involving female patients with BC who received treatment in private healthcare facilities in the Brazilian states of Rio de Janeiro and São Paulo. For this analysis, we focused on patients aged 50 years or younger with stage I to III invasive breast cancer who underwent adjuvant ET between 01/01/2013 and 01/31/2023. Patients were divided based on whether they received OFS. PRO was evaluated through EORTC-QLQ-BR23 functional scales at baseline, 3, 6, 9, 12, and 24 months. Patients were included before any systemic treatment, including chemotherapy. We used linear mixed models with

adjustments for the cancer stage to compare the scales between the two groups. Results were reported relative to the baseline category, using 95% confidence intervals and p-values. The R software, version 4.1.2, was used for the analyses.
Results: In total, 363 patients met the inclusion criteria. Of these, 290 received ET alone, and 73 were assigned to receive OFS+ ET. Patients who received OFS were more likely to be younger (64.4% vs. 25% <40 years), had a more advanced cancer stage (31% vs. 13% stage III), and were more likely to receive chemotherapy (90.4% vs. 73.4%). The number of patients who completed the questionnaires was 363, 152, 272, 250, 252, and 171 at baseline, 3, 6, 9, 12, and 24 months, respectively. Regarding the functional scales "sexual functioning" and "sexual enjoyment," despite the absence of statistical significance between groups, the OFS group experienced a more pronounced clinically significant (>10 points) decrease in the first 6 months of treatment and an inability to return to baseline after 24 months. At months 6 and 12, a higher number of sexually active patients reported not having sexual desire or satisfactory sex. Furthermore, after 24 months of treatment, patients in the OFS group were more likely to report hot flashes, headaches, being physically less attractive, less feminine, less sexual interest, and less sexual activity.
Conclusion: Despite OFS contributing to improved disease-free survival and overall survival in adjuvant treatment, its side effects significantly affect the patient's long-term QoL. Discussing these side effects with patients before starting systemic therapy and developing treatment plans is crucial to enhance the QoL of patients dealing with OFS-related concerns. Our study emphasizes the need for a personalized, empathetic approach to patient care and regular sexual health assessments by oncology healthcare professionals.

P2-01-10: A real-world evidence study of an online Mindfulness-Based Stress Reduction intervention: Effects on the symptomatic burden of patients with breast cancer

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Introduction: Patients with breast cancer (BC) experience significant psychological distress, fatigue, insomnia, and menopausal symptoms throughout their treatment and survivorship. Mindfulness-Based Stress Reduction (MBSR) has been implemented to alleviate these symptoms. Our group previously demonstrated in a randomized study with BC survivors that an online MBSR program is beneficial. This study now aims to evaluate the effectiveness of online MBSR in reducing the symptomatic burden of patients with BC in a real-world, uncontrolled setting.
Methods: Women aged ≥18 years, diagnosed with BC, were invited to participate. The 8-week online MBSR intervention was provided by a certified instructor. Data were collected at 3 timepoints: baseline, 1 week after program completion, and 1 month thereafter. Intervention effectiveness was assessed using the following outcome measures: GAD-7 (anxiety), PHQ-9 (depression), FACIT-F (fatigue), MAAS (mindfulness state), ISI (insomnia), CWS (worry of cancer recurrence), and MEN-QoL (menopausal symptoms). Data on

satisfaction and attendance barriers were also collected. Linear mixed models including evaluation timepoint, antidepressant use, menopausal status, clinical BC stage, BC treatment modalities, and recurrence as fixed effects, and individual participants as a random effect were used to assess outcome changes.
Results: A total of 37 patients were included. Their median age was 53 years (IQR: 49-62). The majority were married (56%), college-educated (53%), most frequently stay-at-home spouses (39%), and publicly insured (50%). Most were diagnosed with stage II (33%) or III (33%) BC. A considerable percentage of participants were undergoing active treatment, mainly with endocrine therapy (53%), chemotherapy (14%), or anti-HER2 therapy (14%). Notably, antidepressants were used by 22% of participants and 14% were already practicing mindfulness.
Participants were able to attend a median of 6 MBSR sessions (IQR: 5-8). After taking part in the program, most participants were very satisfied (78%) or satisfied (11%), and rated the program as very useful (84%). The most frequently reported barriers for attendance were lack of time/inability to conform to time schedule (19%), personal/family commitments (16%), and forgetfulness (14%). At the first follow-up, a significant mean difference was observed in GAD-7 (-2.79 points, $p<0.01$), PHQ-9 (-3.15 points, $p<0.01$), FACIT-F (-2.54 points, $p=0.03$), MAAS (+0.65 points, $p=0.01$), CWS (-3.54 points, $p<0.01$), but not in ISI (-2.02 points, $p=0.08$) or MEN-QoL (+0.41 points, $p=0.44$). One month after program completion, significant improvements persisted in GAD-7 (-2.82 points, $p<0.01$), PHQ-9 (-3.29 points, $p<0.01$), FACIT-F (-3.61 points, $p<0.01$), MAAS (+0.92 points, $p<0.01$), and CWS (-2.69 points, $p=0.01$). At this timepoint, a significant mean change in the ISI scale was also observed (-3.64 points, $p<0.01$), but no significant changes were found in MENQoL (+0.32 points, $p=0.86$).
Conclusion: An online MBSR program is an effective intervention to reduce the symptomatic burden of patients with BC in a real-world setting. Participants are highly satisfied with the online modality and find this resource useful. The significant improvements observed in GAD-7, PHQ-9, FACIT-F, MAAS, and CWS scores suggest a substantial impact on patients' quality of life. Given the confirmed efficacy of online programs, it is crucial to incorporate these feasible and accessible interventions into routine care to benefit a larger number of patients.

P2-01-11: Supportive Care Service Utilization Among Long Term Metastatic Breast Cancer Survivors

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Background: Patients living with metastatic breast cancer (MBC) have unique psychosocial and medical needs, which often go unrecognized. Many MBC patients are now living years on chronic therapy. Understanding current referral patterns and utilization of supportive care services is a necessary step to improving patient access and aligning services with patient needs. We examined supportive care service utilization among long term MBC survivors to determine sociodemographic, psychosocial, and clinical predictors of

utilization.
Methods: 224 patients from the Ohio State University Stefanie Spielman Comprehensive Breast Center, who had been diagnosed with MBC for ≥1 year, were asked to complete a one-time, online survey to assess their quality of life, self-rated health, symptoms, supportive care needs, and reflections on their cancer diagnosis and treatment. Multivariate logistic regression was used to determine sociodemographic (age, race, education, income, rural/urban residency), psychosocial/quality of life (PROMIS physical component score, PROMIS mental component score, social support), and clinical factors (time since diagnosis, current treatment, metastasis site, fatigue, pain, and sleep) associated with utilization of supportive care services.
Results: The mean age of the participants was 60.0 years old (±12.8 years) and the average time since stage IV diagnosis was 5.9 years (±4.9 years). Most participants were female (n=221), white (92%), non-Hispanic (98%), and about half had a total household income of < $75,000. Approximately 50% had a bachelor's degree or higher, and 71% resided in urban areas and 29% in rural areas in the state. Most participants were currently receiving bone health support (47.8%), endocrine therapy (43.4%), or CDK4 inhibitor therapy (33.3%). About two-thirds of participants had metastases to the bone (66%) and 17% had metastases to the brain. Participants with the highest rate of referral to supportive services were those with metastases to the liver (49%), followed by bone (46.6%), brain (44.7%), lymph nodes (43.6%), and other sites (33.3%). Over 25% of participants used 3 or more services, however, the majority of participants reported not using any supportive care services (55.3%). The three most common referrals among the MBC survivors were for nutrition/dietitian services (19.3%), physical therapy (18.4%), and counseling services (18.0%). However, only 15% of participants actually reported receiving either nutrition/dietitian care (15.4%), physical therapy (14.9%), and/or counseling services (10.5%). Patients receiving services were more likely to be non-white (p=0.008), more highly educated (p=0.02), receiving chemotherapy (p=0.03), and with lower (worse) PROMIS physical and PROMIS mental scores (ps<0.01), higher levels of fatigue and pain (ps<0.02), and lower self-rated quality of life (p=0.005). In multivariate logistic regression models, those with better PROMIS mental health scores also had lower odds of using supportive care services (OR: 0.93, 95% CI: 0.86, 0.99, p=0.04).
Conclusions: Survivors living with MBC in this study reported lower rates of referral and use of supportive care services than expected. Those who engaged with supportive care services often used multiple services compared to only one. Given this dichotomy, future studies are needed to determine if low rates of engagement are due to patient wishes or variability in screening and referral practices. More research is needed to determine processes that equitably and efficiently screen, refer, and engage eligible MBC patients with guideline-based supportive care services.

P2-01-12: Association of Employment Characteristics and Food Insecurity Risks among Women with Breast Cancer

Michael Halpern, David Gimeno Ruiz de Porras

Introduction: Food insecurity (i.e., inconsistent access to nutritionally adequate and safe food) is estimated to occur among 17%-55% of individuals diagnosed with cancer. Little is known about how employment and its characteristics after a cancer diagnosis may mitigate food insecurity risks. We examined associations between food insecurity, employment, and availability of paid sick leave among women diagnosed with breast cancer in the U.S.
Methods: The study sample (n=604) was women aged 18-65 with non-missing employment status and a self-reported history of breast cancer (diagnosed age 18 or older) from the 2020, 2021, and 2022 National Health Interview Survey (NHIS), a nationally representative household survey of the civilian noninstitutionalized U.S. population. Bivariate (Chi-Square) analyses of NHIS food insecurity items by employment status and, among currently employed women, availability of employer-provided sick leave were conducted using PROC SURVEYFREQ in SAS 9.4 adjusting for the complex survey design of the NHIS.
Results: Compared with not currently employed women (n=234), currently employed women (n=370) had higher levels of education and were younger. There were no differences in years since breast cancer diagnosis, marital status, household race/ethnicity composition, or household urban/rural status between the two groups. Currently employed women were less likely ($p<0.05$) to report food insecurity in the past 30 days across multiple domains (less likely that food runs out; food didn't last and didn't have money for more; couldn't afford balanced meals; cut the size of meals, skipped meals, ate less, or went hungry due to money; and lost weight or didn't eat for a whole day because there wasn't enough money for food) and less likely to report overall food insecurity. Among currently employed women, those with paid sick leave were less likely ($p<0.05$) than those without paid sick leave to report that their food didn't last and that they ate less due to money. Those with paid sick leave also reported greater food security overall and had less worry about paying medical bills if they got sick or had an accident.
Conclusions: Continued employment after a breast cancer diagnosis is associated with a significantly decreased risk of food insecurity. Available paid sick leave decreased food insecurity among women continuing employment. Support to facilitate continued employment and return-to-work and workplace accommodations, including paid sick leave can have a positive impact on the well-being of women with a history of breast cancer.

P2-01-13: Palliative Care Use and End-of-Life Care Quality in HR+/HER2- Metastatic Breast Cancer

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Background: Metastatic breast cancer (MBC) is incurable, but therapeutic advances have improved clinical outcomes for patients with hormone receptor positive (HR+), human epidermal growth factor-2 negative (HER2-) disease. Palliative care (PC) is recommended to alleviate distress of patients with advanced cancer and reduce administration of aggressive care at the end of life. Intensive end-of-life (EoL) care is associated with greater physical and emotional distress of patients and caregivers. Despite current guidelines, there is limited data in prior literature on PC use and EoL care of patients with MBC benefitting

from therapies such as cyclin-dependent kinase 4/6 inhibitors (CDK4/6i). The aim of this study is to describe referral to PC, hospice utilization, and EoL outcomes, including rate of aggressive EoL care, in patients with HR+/HER2- MBC.
Methods: A secondary analysis of data from a retrospective review of patients with HR+/HER2- MBC treated with endocrine therapy +/- CDK4/6i for first-line MBC at the Duke Cancer Institute between 1/2012 and 12/2017 was performed. Data extracted from the electronic medical record included demographic and clinical characteristics at MBC diagnosis. Variables added for this analysis and abstracted through 3/15/24 included PC involvement, hospice use and duration of care, and EoL care outcomes, including place of death, healthcare utilization in last 30 days of life, and chemotherapy in last 30 and 14 days of life. PC and hospice utilization and EoL care data were analyzed with descriptive statistics.
Results: Of 102 patients in the cohort, 85 died during the study period, 77 of whom had complete EoL care data. Mean age at metastatic diagnosis was 61.9 (SD 12.1; range 27-84 yrs) and mean age at death was 66.0 (11.9; 33-86 yrs). Over half (n=42/77, 55%) received aggressive EoL care. Half of the cohort received some form of PC (51/102, 50%). Among the deceased, rates of aggressive EoL care were comparable between those who engaged with PC (24/45, 53%) and those who did not (18/32, 56%). Of those who received PC, in-hospital referrals were more common (31/51, 61%) than ambulatory referrals (19/51, 37%). Similar proportions engaged with PC in outpatient clinics (28/51, 55%) as those with exclusively inpatient PC encounters (23/51, 45%). Median number of outpatient PC visits was 2 (range 1 – 16 visits among deceased). Among the indicators of aggressive EoL care, multiple emergency department (ED) visits (22/79, 28%) and hospital admissions (18/79, 23%) in the last 30 days of life as well as in-hospital location of death (20/85, 24%) were the most common. Chemotherapy was administered within the last 30 days for 16 patients (16/80, 20%) and within the last 14 days of life for 9 patients (9/80, 11%). Among the 72% (61/85) who enrolled in hospice, 9% (7/82) were on hospice care for ≤ 3 days. Of the 15 patients who received hospice care for more than 3 days and received aggressive EoL care, most had multiple ED visits (n=11) and/or hospital admissions (n=7) in the last 30 days of life.
Conclusion: This real-world study demonstrates that patients with HR+/HER2- MBC receive aggressive care at EoL despite some engaging with PC and many enrolling in hospice care. PC involvement did not appear to impact receipt of aggressive EoL care. This may be a result of the low median number of outpatient PC visits, representing insufficient PC "dosage" to facilitate complex symptom management and advance care planning required to reduce aggressive EoL care. Interventions to enhance PC engagement and decrease aggressive EoL care are needed to reduce in-hospital deaths, frequency of ED visits, hospitalizations, and intensive care unit admissions in the last month of life, and to ensure patients with MBC experience hospice services for a sufficient duration to derive meaningful benefit.

P2-01-14: Impact of Psychological Support on Quality of Life, Coping Strategies, and Anxiety and Depression in Breast Cancer Patients: A Comparative Randomized Study

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Recent advancements in breast cancer treatments have improved survival rates, yet the focus remains primarily on survival outcomes rather than quality of life and psychological factors. This study investigates the impact of psychological support on anxiety, depression, coping strategies, and quality of life in early invasive breast cancer (BC) patients. Conducted at ARNAS Civico hospital in Palermo, Italy, this randomized comparative study assigned patients to either standard care (control group) or standard care supplemented with psychological support (intervention group).
Assessments were conducted pre-surgery and at 8 months post-treatment, coinciding with the completion of a 9-session support program for the intervention group. The study utilized QLQ-C30, QLQ-BR23, HADS, and Brief COPE questionnaires. Data analysis employed two-way repeated measures ANOVA and mixed-effects models to explore temporal trends and group differences over time. Statistical significance was set at p-values below 0.05 and t-values exceeding twice the standard deviation in mixed-effects models. A total of 65 patients participated, with 34 in the intervention group and 31 in the control group. Median age differed significantly between groups (p=0.001). At baseline, significant differences were observed in self-blame (p=0.02) and financial difficulties (p=0.02). Anxiety and depression (HADS-T) increased over time in patients without psychological support (HADS-T = 3.42, t = 3.46), while those receiving psychological support showed reduced levels of anxiety and depression (HADS-T = -6.94, t = -4.87). Emotional functioning (int: 13.69, t=2.72; ctrl: -6.09, t=-1.74), social functioning (int: 14.48, t=2.82; ctrl: -13.72, t=-3.11), cognitive functioning (int: 14.79, t=2.50; ctrl: -17.45, t=-4.25), and insomnia (int=-19.39, t=-2.72; ctrl=11.78, t=2.39) significantly improved over time in the intervention group, while declining in the control group. Global quality of life significantly improved from baseline for patients receiving psychological support (7.33, t=2.1) but declined for those in the standard care group (-7.78, t=-3.22). Patients in the intervention group showed improvements in body image (17.57, t = 3.56) and future perspective (19.65, t = 3.56), and reductions in hair loss (-28.44, t = 2.70) and systemic therapy side effects (-11.51, t = 2.64). In contrast, the control group experienced significant deterioration in these aspects over the study period. Regarding coping strategies, the support group showed significant improvements in problem-focused coping (int: 5.47, t=6.23; ctrl: -4.26, t=-7.03) and emotion-focused coping (int: 3.97, t=4.19; ctrl: -2.57, t=-3.94) at the second assessment. Avoidance behaviors decreased in both groups (int: -0.95, t=-1.56; ctrl: -0.87, t=-2.074). The intervention group showed positive outcomes in informational support (1, t=2.81), planning (1.53, t=5.37), positive reframing (1.75, t=4.89), active coping (1.24, t=4.13), behavioral disengagement (-1.074, t=-3.46), self-distraction (1.37, t=4.51), denial (-1.14, t=-2.94), acceptance (1.61, t=5.64), and emotional support (1.23, t=3.44).

The findings highlight that psychological support significantly enhances various aspects of patient well-being, contributing positively to improving overall quality of life.

P2-01-15: Survivorship attributes in women with hormone receptor positive breast cancer at long term follow-up in the CLEAR Study of Late Recurrence.

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Background: Limited data are available on long-term breast cancer (BC) survivorship. We examined this issue in women with hormone receptor positive BC recruited at least 4 years post-diagnosis onto a prospective cohort study that investigates survivorship as well as host and circulating factors associated with risks of late recurrence.
Methods: At study entry (2019-2022), 423 women provided information regarding overall health, marital status, current living situation, vitamin/supplement/alternative therapy use, participation in supportive activities. They also completed the Attachment subscale of the Social Provisions Scale and the Holmes and Rahe Stress Scale.
Results: Mean age was 61.4 ± 9.8 years, 93.6% were post-menopausal. 322 (76.1%) identified as white, 16 (3.8%) black, 70 (16.5%) Asian, and Latin America/Hispanic 9 (2.1%). Mean time from surgery to enrolment 7.5 ± 2.8 years, mean CTS5 score 4.2 ± 0.6 % per year. 100 (23.6%) tumors were HER2+, 368 (87%) received chemotherapy. 274 (64.8%) were still on endocrine therapy (ET): 194 (45.9%) on AIs and 80 (18.9%) on tamoxifen at the time of enrolment. Mean duration of ET 6.5 ± 2.4 years. Mean BMI was 27.3 ± 5.9 kg/m2: 25.8% obese, 34.3% overweight, 38.5% normal weight. 213 (50.4%) consumed alcohol in the last year, mean 5 ± 4.3 drinks per week. 377 (89.1%) used dietary supplements: 328 (77.5%) vitamin D, 93 (22.0%) multivitamins, 80 (18.9%) vitamin C and 172 (40.7%) calcium, 66 (15.6%) reported using alternative supplements, notably cranberry and Coenzyme Q10. 276 (65.2%) were married, 65 (15.4%) separated/divorced, 35 (8.3%) widowed, 47(11.1%) never married. Most lived with a partner 279 (66%); 59 (13.9%) with other family members or friends and 82 (19.4%) lived alone. Overall health during the past year was considered stable or better by 306 (84.2%) subjects. 266 (62.9%) reported engaging in at least 1 of the following supportive activities therapy, meditation, yoga, and group exercise. Mean score on the Attachment subscale of the Social Provisions Scale was 15 + 1.7 (range 0 to 16); 254 (60%) reported the maximum score, indicating excellent close attachment and support. In contrast, 154 (36.4%) scored in the highest risk category (>300) on the Stress Scale. The most frequently endorsed items were changes in family get togethers, social activities, vacations, health of family members and work conditions, likely reflecting the COVID pandemic.
Conclusions: In the CLEAR study, with a mean of 7.5 years post-diagnosis, BC survivors reported strong support, with strong emotional ties and stable overall health. However, they experienced high stress and high alcohol consumption, possibly related to the COVID

pandemic. Further analysis addressing diet, physical activity and medical status, and change over time are ongoing.

P2-01-16: Pharmacogenomic variants and risk of adverse events in breast cancer patients treated with Trastuzumab-deruxtecan: results from the PROCURE project

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Background: Trastuzumab-deruxtecan (T-DXd) has shown an unprecedented clinical benefit in advanced breast cancer. Despite its meaningful anticancer outcomes, around 15-20% of patients have to discontinue T-DXd due to related toxicity (mainly pneumonitis/interstitial lung disease). To date, there is no evidence of the potential impact of pharmacogenomic variants and the risk of treatment-related adverse events (TRAE) in breast cancer patients treated with T-DXd.
Methods: The PROCURE project is a translational research study comprising 26 Spanish institutions that aims to analyze pharmacogenomic variants and risk of toxicity in patients with breast cancer treated with T-DXd. Eligible patients had to receive at least one dose of T-DXd for its current approved indications in advanced HER2+/HER2-low breast cancer, with a minimum follow-up of 3 weeks to collect data on early-onset toxicity. Patients who had discontinued the treatment were also eligible. Patients' demographics and clinical variables were gathered from medical records in an electronic database. Emerging adverse events were registered following CTCAE V.5 guidelines. One blood sample was collected for DNA extraction. Pharmacogenomic analyses included the determination of UGT1A1*28 allele and a massive SNP genotyping array with over 1.9 million genetic markers and enriched in pharmacogenomics variants (Infinium Global Diversity Array with Enhanced PGx, Illumina).
Results: A total of 329 female patients were enrolled in the study from July 2022 to March 2024 with available genetic information. At data cut-off (1 July 2024), clinical data was available for 315 (95.7%). The median age was 56 years and the median duration of T-DXd treatment was 12.0 months (95%CI 10.4–14.2). Regarding UGT1A1 genotypes, 36 patients (11.4%) were *28/*28 homozygous (poor metabolizers), 143 (45.3%) were *1/*28 heterozygous (intermediate metabolizers), 1 (0.3%) was *1/*37 and 135 (42.7%) were *1/*1 (wild type). According to UGT1A1 genotypes (in this order: *1/*1, *1/*28, *28/*28) we observed the following TRAE of any grade: neutropenia (14.0%, 15.3%, 8.3%), anemia (13.2%, 7.6%, 11.1%), thrombocytopenia (4.4%, 4.2%, 11.1%), diarrhea (19.9%, 16.7%, 16.7%), vomiting (16.2%, 14.6%, 13.9%), pneumonitis/ILD (10.3%, 8.3%, 8.3%), dose

reductions (28.9%, 27.8%, 34.8%), and treatment discontinuation (28.9%, 26.7%, 17.4%). We observed a higher incidence of grade 3 gastrointestinal toxicity in poor metabolizers (*28/*28) compared to the other two genotypes: diarrhea (0.7%, 0.7%, 2.8%), and vomiting (0.7%, 0.0%, 5.6%). The incidence of drug-induced pneumonitis/ILD in our study was 9.2% (29/315). We did not find an association between the UGT1A1 variants and the incidence of pneumonitis. In the SNP array, 314 of 315 samples and >95% of SNVs passed quality control. A logistic regression analysis of pneumonitis/ILD revealed more than 25 markers with unadjusted p-value <5x10-5 (additive model). Detailed pharmacogenomic TRAE analyses will be presented during the conference.
Conclusions: Descriptive analyses from the PROCURE project show a similar incidence of all grade TRAE irrespective of UGT1A1 allelic variants. We observed a higher incidence of grade 3 gastrointestinal toxicity in UGT1A1 *28/*28 carriers. Potential pharmacogenomic markers of ILD were identified by a massive SNP genotyping array in unadjusted analysis that warrants further research.

P2-01-17: Genomic predictors of response among patients with hormone receptor-positive (HR+)/HER2- metastatic breast cancer (MBC) receiving the AKT inhibitor (AKTi) ipatasertib combined w/ endocrine therapy & a CDK4/6 inhibitor (CDK4/6i) in TAKTIC trial

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Background: Treatment of HR+/HER2- MBC often involves an antiestrogen agent and CDK4/6i, and following disease progression, multiple therapies are approved in the second line. Management is increasingly guided by a precision-based approach, including the use of AKTi in tumors harboring an AKT1 or PIK3CA mutation or PTEN loss. However, little is known regarding molecular factors that mediate resistance to AKTi. Results from the TAKTIC trial demonstrated antitumor activity and tolerability of the AKTi ipatasertib with endocrine therapy (ET) +/- palbociclib post-CDK4/6i (Wander et al., 2023). We hypothesize that next-generation sequencing (NGS) of tumors among patients (pts) receiving ipatasertib could inform genomic predictors of response to AKTi.
Methods: TAKTIC was a phase Ib open-label trial evaluating ipatasertib in combination with fulvestrant, an aromatase inhibitor, or fulvestrant + palbociclib, in participants with HR+/HER2- MBC who received ≥1 line of prior therapy for MBC and had exposure to CDK4/6i (NCT03959891). An exploratory objective of TAKTIC was to identify genomic biomarkers that correlate with response to an AKTi-based combination regimen. Blood samples for circulating tumor DNA analysis were drawn at routine timepoints and archival tumor tissue was obtained. Mutational profiling was performed using commercially available NGS-based assays (frequently via Guardant360). Progression free survival (PFS) was estimated using the Kaplan-Meier method, and survival analysis was implemented with

the Breslow approximation for ties. Univariable and multivariable hazard ratio (HR) and 95% confidence interval (CI) analyses were estimated using a cox proportional hazards model.
Results: TAKTIC accrued 77 pts (6/2019 – 2/2022), enrolling 35 on doublet therapy (AKTi + antiestrogen) and 42 on triplet therapy (AKTi, fulvestrant, and palbociclib). Baseline NGS results were available in 58 of 77 pts, and alterations in PI3K/AKT pathway genes were found in PIK3CA (43%), AKT1 (7%), and PTEN (10%). Mutations in ESR1 (29%) were seen at rates consistent with prior studies in MBC post-ET. A subgroup of 20 pts who received the triplet ipatasertib regimen and had baseline NGS data within 60 days of drug start were analyzed. Mutations in PIK3CA (n=7, 35%; n=5 polyclonal) and PTEN (n=4, 20%; n=1 polyclonal) were detected, as were alterations in ESR1 (n=5, 25%), FGFR1 (n=4, 20%), KRAS (n=4, 20%), and ERBB2 (n=3, 15%); no baseline AKT1 mutations were seen in this subgroup. Univariate gene analysis demonstrated that FGFR1 amplification was associated with shorter PFS (HR 5.42, 95% CI 1.3 – 22.1, P=.019), and ERBB2 alteration trended toward worse outcomes (HR 3.38, 95% CI 0.8 – 13.6, P=.086); no significant difference was seen between ESR1 mutant vs. ESR1 wild-type tumors (HR 1.6, 95% CI 0.6 – 4.6, P=.384). Multivariate gene analysis demonstrated that PIK3CA/AKT1/PTEN altered tumors (n=9) had greater response to the AKTi triplet combination compared to tumors without mutations in this pathway (n=11) (median PFS 505 vs. 114 days, HR 0.2, 95% CI 0.1 – 0.7, P=.015). Breast cancers harboring alterations that upregulate RAS pathway signaling (KRAS/NRAS, BRAF, FGFR1/2, ERBB2, EGFR; n=9) showed a trend toward inferior outcomes compared to non-altered disease (n=11) (median PFS 114 vs. 253 days, HR 2.0, 95% CI 0.8 – 5.3, P=.160). Additional analyses at the individual gene and pathway level will be presented at the meeting.
Conclusions: Genomic insights using NGS suggest that MBC post-CDK4/6i is more susceptible to an AKTi-based treatment with ipatasertib in the presence of a PI3K/AKT/PTEN pathway mutation, whereas alterations in FGFR1 are associated with worse outcomes. This effort is one of very few studies prospectively evaluating mediators of AKTi response, an area of active interest given changes in the therapeutic landscape. The results presented here are hypothesis-generating; future work is underway to further expand upon these data.

P2-01-19: Measurement of ADC Targets HER2 and TROP2 in Breast Cancer for Accuracy and Selection

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Introduction: With over 700 antibody drug conjugates (ADCs) in clinical trials, there are new challenges for protein diagnostics. ADCs have a measurable target and likelihood of response increases with increased target expression. But as multiple ADCs are approved for the same indication, measurement of target also could play a role in sequencing of therapy.

To accurately compare competing ADCs, the targets must be quantitatively measured, not read, as there is broad variability in reading/scoring target expression by conventional semi-quantitative IHC tests. We have previously described a quantitative immunofluorescent (QIF) method (called HS-HER2) to measure HER2 protein in attomoles per square millimeter (amol/mm2). Our new duplex QIF (TROPLEX™) assay measures both HER2 and TROP2 protein targets. We show validation of the TROPLEX assay and a prospective assessment to compare relative levels of target expression. We envision use of this assay to help sequence patients for Trastuzumab deruxtecan (T-DXd) and Sacituzumab govitecan (SG).

Methods: We constructed cell lines microarrays (CMAs) with 9 cell lines expressing different levels of HER2 and TROP2 proteins, then created HER2 and TROP2 standard curves of QIF scores based on targeted mass spectrometry measurements. This provided a conversion from QIF signal to (amol/mm2) for each target. We then tested the TROPLEX assay on a 40 case CLIA lab validation set and a prospectively collected cohort of 93 breast cancer cases. Both validated HS-HER2 and TROPLEX assays were performed using the Leica BOND RX autostainer and imaged with the CyteFinder II HT multiplex fluorescent imaging platform from RareCyte, and molecular compartmental analysis was performed in QuPath with the Qymia extension.

Results: Validation of the TROPLEX assay showed inter-operator reproducibility (R2 = 0.99 for HER2 and R2 = 0.94 for TROP2), inter-assay CVs of 10% for HER2 and 9.8% for TROP2, and intra-assay CVs of 6.4% for HER2 and 6.2% for TROP2. Since the threshold of ADC response is not known for either target, the assays were made as sensitive as possible. The limit of detection (LOD) for HER2 was 360 amol/mm2 and for TROP2 it was 325 amol/mm2. In the TROPLEX prospective trial 2% of the cases were below the LOD for HER2 and 0% for TROP2. Other analytic parameters are in process. Measurement of HER2 protein in 40 cases selected for CLIA lab validation we found a very high correlation (R2 = 0.9) between HER2 measured by the validated HS-HER2 assay compared to the TROPLEX assay. In prospective trials, we found that HER2 expression ranged from about 100 to nearly 7200 amol/mm2 and TROP2 showed a range from about 900 to 22000 amol/mm2. The TROPLEX assay showed no significant correlation between HER2 and TROP2 expression but many cases that are high for one target are low for the other (see figure 1). While TROP2 IHC is not standard of care, we could compare measured levels of HER2 to IHC scores. We found that the first quartile of quantitative HER2 shows 57% cases scored as IHC=0 and 30% cases scored as IHC=1. In the second quartile of quantitative HER2 we found 29% cases scored as IHC=0 and 42% cases scored as IHC=1. This distribution shows the inaccuracy of conventional HER2 IHC compared to quantitative assessment.

Conclusion: Based on our validation and prospective studies, the multiplex QIF TROPLEX assay is highly sensitive for both HER2 and TROP2. The assay was sufficiently accurate and reproducible for validation in a CLIA lab. Studies are underway to determine the threshold for ADC response and to assess the value of this quantitative ranking assay to select between ADCs with the same indication.

P2-01-20: Impact of Exercise on Immune Biomarkers in Benign Breast Tissue from Women with High Mammographic Breast Density (MBD)

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Background: Increased physical activity (PA) is associated with lower risk of all subtypes of breast cancer in both pre- and post-menopausal women, but the biological mechanisms through which PA impacts breast carcinogenesis are not well understood.

Methods: We conducted a single-arm pilot study to evaluate the impact of an aerobic and strength training PA intervention on tissue biomarkers in inactive women at increased risk of breast cancer due to the presence of increased mammographic breast density (MBD). Eligibility criteria included having at least 50% MBD (BIRADS C or D); engaging in <90 minutes per week of moderate or vigorous PA (MVPA); age <60 years; no concurrent use of hormone replacement therapy, oral/implantable birth control agents, or chemoprevention for breast cancer; and no prior history of breast cancer. Participants (pts) took part in a 12-week aerobic and strength-training intervention including 2 supervised exercise sessions and 1+ session of home-based aerobic exercise. Pts underwent biopsy of benign breast tissue at baseline and 12-weeks of mirror-image sites in opposite breasts. Biopsies were timed to coincide with the luteal phase of the menstrual cycle in pre-menopausal participants. Areas of interest (AOIs) were marked from terminal ductal lobular units (TDLU's), ductal epithelium, and stroma by a breast pathologist and by staining for pan-keratin and CD45. RNA-sequencing of the AOIs was performed using GeoMx platform. Changes over time between pre- and post-intervention biopsies were evaluated using paired t-tests on average (per patient) immune cell type abundances that were estimated from gene expressions using the GeomxTools R package. All p-values were adjusted for multiple testing using the Benjamini-Hochberg procedure. Single cell spatial analysis targeting 20 immune and proliferative markers was performed using Co-detection by indexing (CODEX), a multiplexed imaging method, in 11 paired biopsies with sufficient tissue remaining after gene expression analysis. Analysis included cell segmentation and clustering with deconvolution of cellular populations followed by comparisons between matched samples pre and post exercise. Comparisons differences in number and density of cellular populations, cell-cell interactions and cell neighborhoods.

Results: Thirty pts were enrolled between 10/2018 and 11/2019. Due to the COVID-19 pandemic, 3 participants did not undergo a week-12 biopsy and 1 additional patient was lost to follow up for non-COVID related reasons, leaving 26 participants with paired pre- and post-intervention tissue. Average age of study pts was 47.6 (±5.7) years, average BMI was 29.2 (±6.3) kg/m2, and median minutes of MVPA at baseline was 72.5 (range 0-360). Pts attended an average of 20.4 of 24 exercise training sessions (85% adherence) and increased exercise by a median of 92.5 minutes/week (range -140 to 700). Cell abundance based on gene expression data showed increased CD8+ memory T cells (mean difference (MD)=1.34, standard error (SE)=0.85), neutrophils (MD=2.25, SE=0.83), and NK cells

(MD=1.18,SE=0.57) in the epithelium (N=11 patients) and increased neutrophils (MD=1.00, SE=0.33) in the stroma (N=10 patients) after the 12-week exercise intervention at a 25% FDR threshold. Details of the CODEX analysis will also be presented.

Conclusions: In this single-arm pilot study exploring the impact of an exercise intervention on biomarkers in benign breast tissue in women at increased risk of breast cancer due to elevated MBD, there was evidence of immune changes in post-exercise tissue samples, providing some of the first evidence that exercise could impact gene expression in benign breast tissue. This work provides a foundation for further work exploring the potential pathways through which exercise could prevent breast cancer in at-risk populations.

P2-01-21: Baseline circulating tumor cells (CTCs) predict for progression-free survival (PFS) after ablation on NRG-BR002, a randomized phase II/III study of ablation vs. standard systemic therapy care (SOC) for oligometastatic breast cancer (OMBC).

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Background: NRG-BR002 randomized patients with OMBC to SOC with vs. without ablation (surgery or radiation) of all visible metastases. The phase II PFS go signal for improvement with ablation was not met. Blood for CTCs was collected from consenting patients and sent for the FDA-approved CellSearch CTC assay and High-Definition Single Cell CTC Analysis (HDSCA). The primary CTC objective was that pretreatment CTCs (PreRX CTCs) would be prognostic for PFS. Secondary objectives included baseline CTCs being predictive for the effect of RX on PFS and CTC post-treatment clearance rate correlating with PFS.
Statistical Design and Methods: A secondary endpoint analysis of the prognostic effect of PreRX CTCs on PFS and the interaction between study arm and the PreRX CTCs on PFS were evaluated for both assays using multivariable Cox proportional hazards models (MVA). CellSearch CTCs were dichotomized 0 vs. ≥ 1 and HDSCA CTCs were assessed by < vs. ≥ lowest quartile (Q1). Due to small numbers of CTC+ cases, CTC clearance correlation with PFS was not able to be tested. Descriptive results are presented.
Results: Of 125 phase II eligible patients, 108 (86%) consented to blood collection. Among the cohort with CTC data (n=62 for CellSearch, n=60 for HDSCA), demographic and treatment variables were balanced between study arms and similar to the trial population. Thirty-six (58%) and 26 (42%) had CellSearch CTC = 0 and ≥1, respectively. Fifteen (25%) and 45 (75%) had HDSCA CTCs < Q1 and ≥ Q1, respectively. Median follow up at the time of analysis was 48 months. PreRX CTCs (CellSearch and HDSCA) were not prognostic for PFS. In each of the analyses for PreRX CTC (0 or < Q1) being predictive for PFS, after adjusting for number of metastases (1 v >1), the interaction between study arm and CTCs had a HR >

3, reaching statistical significance for HDSCA (p=0.024; HR 5.58, 95% CI: 1.26, 24.77) and trending towards statistical significance for CellSearch (p=0.063; HR 3.39, 95% CI: 0.94, 12.25). Patients in the ablation arm, with HDSCA PreRX CTCs < Q1, experienced better PFS than those with PreRX CTCs ≥ Q1; 78% vs. 33% at 2 years, respectively, as compared to patients on the control arm (17% vs. 55%). Patients in the ablation arm, with CellSearch PreRX CTCs = 0, also experienced better PFS than those with PreRX CTCs ≥ 1; 54% vs. 27% at 2 years, respectively, as compared to patients on the control arm (35% vs. 61%). For CellSearch only, 19/62 patients (9 SOC and 10 ablation) had positive PreRX and PostRX CTC values. In the SOC arm, clearance of CTCs was seen in 6 patients (all progression-free) and no clearance in 3 (2 progressed/1 died). In the ablation arm, clearance (n=5)/decrease (n=2) of CTCs was seen in 7 patients; however, only 3 remained progression-free; and an increase was seen in 3 patients whose disease progressed. For the 9 patients across both arms with PreRX and PostRX CTCs that progressed, all 9 developed new metastatic sites. Conclusion: Lower PreRX CTCs predicted for improved PFS after ablation using the HDSCA assay. The pre-specified CTC analyses provide consistent results using two independent assays highlighting the robustness of the finding. These hypothesis-generating findings suggest that PFS is improved in CTC negative OMBC patients who are treated with ablation.

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P2-01-22: NSABP B-31 confirms the prognostic value of the NeoALTTO-developed S5 genomic classifier

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Background: S5 and S18 gene classifiers were generated in the NeoALTTO study and were associated with a poor prognosis in patients treated with trastuzumab + chemotherapy (ESMO 2022). The associations of S5 and S18 with prognosis were confirmed in the Italian GHEA (Group HEreceptin in Adjuvant treatment) expanded access study. Now we report on a pre-planned, retrospective study (NRG Ancillary project AP2220) testing the association of S5 and S18 with the prognosis of patients from NRG Oncology/NSABP B-31 to confirm their prognostic value. NSABP B-31 was a phase III, randomized trial to assess the safety and efficacy of the addition of trastuzumab (H) to doxorubicin and cyclophosphamide followed by paclitaxel (ACT) in patients with operable, node-positive, HER2-positive breast cancer. This analysis benefits from a large patient sample, an extended follow-up duration, and detailed treatment information.

Materials and methods: Among the 2,130 patients who participated in B-31, tissues were available for 1,579. Available Affymetrix gene expression data from 731 patients were used

to associate S5 and S18 with outcomes. The S5 and S18 scores were analyzed as (i) continuous and (ii) dichotomized variables using a median predefined cut-off. Co-primary endpoints for this study were disease-free survival (DFS) and overall survival (OS) as estimated by the Kaplan-Meier method and Cox models.

Results: The patient populations of the original NSABP B-31 trial and the current gene expression study were very similar. The median follow-up time was 7 years. Main patient characteristics were: median age 50 (range 26-78), distribution of positive nodes (58% with 1 to 3, 28% with 4 to 9, and 14% with ≥10), tumor sizes in cm (39% ≤2, 52% >2 to ≤5, and 9% >5), and estrogen receptor (ER) status (53% positive and 47% negative). Of 731 patients, 370 were randomized to ACT and 361 to ACTH. No statistically significant associations with outcomes were observed with the S18 score, but the S5 score was significantly associated with DFS after adjustment for clinical variables (age, tumor size, node and ER status) and treatment, both as continuous and dichotomized variables (C-indexes were 0.65 [95% CI: 0.61-0.69] and 0.66 [95% CI: 0.61-0.70], respectively). The hazard ratio (HR) for dichotomized (high v low) S5 score was 1.50 (95% CI: 1.14-1.99, p=0.004). Notably, patients classified as S5-high also had a higher risk of death with a C-index of 0.69 (95% CI: 0.64-0.75) and HR of 1.42 (95% CI: 0.95-2.12). No significant interaction between the S5 score and the treatment arm or ER status was observed.

Conclusions: In patients with early-stage HER2-positive, node-positive breast cancer, the S5 score was confirmed as an independent prognostic factor, whereas the S18 score was not confirmed. The S5 score might be useful to identify patients with a favorable prognosis who would be suitable for treatment with trastuzumab + chemotherapy alone in the adjuvant setting.

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P2-01-23: A 140-gene Machine Learning Classifier Predicts Survival and Response to Chemotherapy and Immunotherapy in 2500 TNBC

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Background: TNBC is molecularly heterogeneous, showing varied responses to therapeutic agents (Txt). We developed a 140-gene classifier (TNBC-ICR) based on random-forest (RF) to cateogorize TNBC into four biological subgroups. It was trained and tested on 551 TNBC (Zhu ESMO Congress 2023), and had better precision in predicting time to recurrence with anthracycline-based (A) txt compared to TNBC-Baylor subtypes (Burstein Clin Cancer Res, 2015) in TACT2 trial (NCT00301925).

Here, we assessed the genomic characteristics and clinical value of TNBC-ICR classifier to predict response for standard of care and emerging Txt.
Methods: TNBC-ICR, consisting of 1000 decision trees, assigned each case to immune-enriched (IM), luminal-AR (LAR), mesenchymal-like (MES) or highly proliferative basal-like (BL-Prolif) based on RF probabilities linked to these subgroup features.
Association with genomic characteristics and prognosis were tested in The Cancer Genome Atlas (n=155, TCGA Nature, 2012), METABRIC (n=269, Curtis Nature, 2012), and SCAN-B (n=571, Saal Genome Med, 2015).
Association with pathological complete response (pCR) was tested across 4 neoadjuvant (NAT) clinical studies: 1) a combined cohort of MDACC, ISPY-1, LBJ/IN/GEI and USO (MDACC, n=188, Hatzis JAMA 2011), 2) CALGB 40603 (n=389, NCT00861705), 3) BrighTNess (n=482, NCT02032277) and 4) ISPY-2 (n=364, NCT01042379). These studies, all including Paclitaxel (P), assessed the additional benefits of Bevacizumab (Bev), Carboplatin (Carbo), PARP inhibitor (V) or Pembrolizumab (Pem), followed by A and Cyclophosphamide (C).
Gene expression data (GE) was available for all studies, with batch correction applied to reduce platform effects before using the TNBC-ICR classifier.
Statisical analyses included multivariable Cox regression models to assess the hazard ratio (HR), chi-squared tests to compare genomic characteristics and pCR rates among the subgroups and logistic regression models to assess the significance and odds ratio (OR) of tumor probabilities to IM, LAR, MES and BL-Prolif related to pCR.
Results: Using whole exome sequencing data from TCGA and METATRIC (n = 306), TP53 was the most frequently mutated gene, observed in IM (88%), MES (76%), BL-Prolif (85%) and LAR (68%, $p = 0.008$), with PIK3CA mutations in LAR (41%) and the others (7%, $p < 0.001$).
The pCR rates for IM, LAR, MES and BL-Prolif were as follows:

MDACC (P+AC), 33% (18/54), 10% (3/30), 25% (9/36) and 48% (29/60), $p = 0.002$;
CALGB 40603 (P+Carbo+AC+Bev), 67% (76/103), 56% (30/54), 36% (31/86) and 54% (73/136), $p < 0.001$;
BrighTNess P+Carbo+V arm: 69% (51/74), 29% (8/28), 62% (34/55) and 43% (34/80), $p < 0.001$; P+Carbo arm: 73% (29/40), 37% (7/19), 62% (13/21) and 48% (20/42), $p = 0.03$; in P arm: 43% (16/37), 17% (3/18), 27% (8/30) and 34% (13/38), $p = 0.21$;
ISPY-2 P arm: 22% (6/27), 50% (5/10), 12% (3/26) and 14% (3/22), $p = 0.06$; P+Pem arm: 100% (8/8), 60% (3/5), 40% (2/5) and 55% (6/11), $p = 0.098$;

In P arms, pCR rate was generally low but addition of either Carbo or V increased pCR rates in IM and MES subtypes and addition of Pem over P increased pCR rates for IM and BL. There was no consistent association of TNBC-Baylor subtypes to pCR in these studies.
Statistical modelling of TNBC-ICR classifier on txt response showed that probability to IM-subgroup predicted pCR (OR = 3.52, $p < 0.001$) in NAT and better recurrence-free survival in adjuvant chemotherapy-treated subgroups of SCAN-B (HR = 0.38, $p = 0.04$) and METABRIC (HR = 0.3, $p = 0.04$).

Conclusion: Tumors with higher IM-features had increased pCR rates after NAT. We validated the reproducibility and clinical validity of TNBC-ICR classifier in predicting differential response to therapies, including taxane and immunotherapy, demonstrating its potential as a practical clinically relevant integrative biology-driven machine learning algorithm.

P2-01-24: Immune activation of tumor cells and microenvironment as assessed by PD-L1 expression and interferon gamma signaling predict long term disease-free and overall survival: Results of the prospective randomized neoadjuvant ABCSG 34 trial

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Background: Novel neoadjuvant treatment options including combinations of chemotherapy with immune-checkpoint inhibitors have improved pathologic complete remission (pCR) rates and long-term outcomes in early-stage breast cancer. To better understand individual risk of relapse and stratify patients for post-neoadjuvant risk-adapted therapeutic interventions beyond the crude dichotomization of pCR, novel predictive factors serving as surrogate for immune-activation and long-term outcome are warranted. The interferon gamma signaling pathway is a key modulator of antitumor immune effects. In the present study, we assessed the role of PD-L1 and the interferon gamma related proteins STAT1 and IRF1 in different tissue compartments as prognostic and predictive biomarkers in the prospective randomized ABCSG 34 trial.
Patients and Methods: In total, 289 tumor samples of 400 patients with early HR+/HER2- or TN breast cancer (BC) or triple-negative BC within the ABCSG 34 trial were available: 210 HR+/HER2 BC and 79 TN patients received neoadjuvant chemotherapy (anthracycline and taxane-based) and endocrine therapy, respectively. Patients were randomized 1:1 to receive either Standard of Care (SoC) or SoC plus the MUC1-based antitumor vaccine tecemotide. Expression of PD-L1 (Immune Score, IC: SP142 clone, Ventana and Combined Positivity Score, CPS: SP263 clone, Ventana), as well as STAT1 and IRF1 (both Cell Signaling Technology) expression at baseline, surgery (where available), and the change in expression during therapy was correlated with patient outcome. Also, STAT1 and IRF1 expression was determined in tumor cells, TILs and stromal fibroblasts. Expression levels were correlated with invasive disease-free (IDFS), distant relapse-free (DRFS) and overall survival (OS). Association with outcomes and use of tecemotide were estimated using univariable stratified (chemotherapy vs endocrine therapy cohort) Cox regression. For continuous markers the hazard ratios (HR) depict the change in hazards for an absolute 10%-point increase of the marker.
Results: Higher PD-L1 IC in biopsy samples was associated with a better DRFS (HR 0.92, 95% CI 0.84 – 1; p = 0.048). In surgical specimens, a positive compared to a negative CPS

was associated with improved DRFS (HR 0.31, 95% CI 0.10 – 0.99; p = 0.048). However, an increase in CPS during neoadjuvant therapy was associated with worse OS (HR 1.03, 95% CI 1 – 1.05; p = 0.032). Pre-therapeutic IRF1 expression in TILs was associated with a better IDFS (HR 0.64, 95% CI 0.42 – 0.98; p = 0.039) and DRFS (HR 0.55, 95% CI 0.34 – 0.88; p = 0.013). Post-therapeutic proportion of IRF1 expressing tumor cells was associated with a better DRFS (HR 0.62, 95% CI 0.42 – 0.92; p = 0.018). STAT1 expression in any of the compartments at any time did not show significant association with long-term outcome. These effects were independent of biological tumor subtype (HR+/HER2- or TNBC). No significant associations between the expression of the investigated biomarkers and any potential tecemotide benefit could be identified.
Conclusion: The results of this study indicate that in the ABCSG 34 trial, PD-L1 positivity as determined by IC and CPS was able to predict better DRFS and OS. However, in patients with residual tumor, an increase in CPS during treatment was associated with decreased OS - a result that needs to be interpreted with caution, since no “post treatment sample” can be accessed in cases of pCR. In addition, IRF1 expression both in tumor cells and TILs predicted an improved IDFS and DRFS. STAT1 expression in any of the tissue compartments did not demonstrate significant association with survival. Despite a limited sample size, these results suggest that immune activation status of tumors and TILs as well as the microenvironment at the beginning of neoadjuvant treatment predict prolonged long-term outcomes, even when RCB/PCR rates remain unchanged.

P2-01-25: Circulating tumor DNA clearance by neoadjuvant chemotherapy or breast surgery detected using an ultrasensitive ctDNA MRD assay in early breast cancer

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Introduction: Presence of circulating tumor DNA (ctDNA) after treatment for early-stage breast cancer (EBC) is associated with poor outcomes, yet accurate detection of ctDNA clearance after treatment requires ultrasensitive methods. Here, we describe the clearance of ctDNA in EBC by multiple treatment modalities, including surgery and neoadjuvant chemotherapy (NAC), using an ultrasensitive ctDNA minimum residual disease (MRD) assay.
Methods: The MSK-LINC study prospectively collected blood samples from EBC patients throughout their clinical care at MSK. MRD was monitored using Foresight CLARITY, an ultra-sensitive MRD research assay with LOD95 below 1 part-per-million (ppm). Whole genome sequencing (WGS) of primary tumor and WBC samples was used to identify tumor-derived phased variants. Personalized MRD assays were then designed to detect ctDNA. We assessed the relationship between ctDNA during distinct phases of treatment and clinical outcomes, including NAC, surgery, or adjuvant treatment.

Results: We analyzed 390 samples from 50 EBC patients (Stages: I=22%, II=56%, III=22%) treated with curative-intent surgery for diverse subtypes (62% HR+/HER2-, 24% HER2+, 14% TNBC). Pretreatment ctDNA was present in 72% of patients, with a median variant allele frequency (VAF) of 100 ppm (range 8 - 226,000 ppm). Following neoadjuvant (44%) or adjuvant (48%) chemotherapy, 14% distant relapses were observed.
Among 18 patients with detectable ctDNA before or during NAC, 56% cleared their ctDNA prior to surgery and remained disease free, with 60% of these achieving pathological complete response (pCR). In contrast, 44% of patients were ctDNA MRD positive (ctDNA+) at the last time-point prior to surgery (median 5 ppm, range: 3-12); 50% of these patients experienced disease recurrence and none achieved pCR. Clearance of ctDNA prior to surgery was associated with significantly improved DFS ($p = 0.012$).
When considering the effect of surgery on patients with residual ctDNA after NAC, 63% had undetectable ctDNA at the first post-operative time-point while 38% did not, with ctDNA levels ranging from 0.9 to 322 ppm. All ctDNA+ patients experienced relapse. Among patients who cleared ctDNA at any postoperative timepoint, only one (13%) experienced relapse; ctDNA was detected in the next blood sample, 15 months prior to relapse. All patients experiencing relapse had ctDNA detected prior to recurrence by serial monitoring.
Among evaluable patients not receiving NAC, 15 patients had ctDNA detected pre-surgery (median ctDNA level 40 ppm, range 8-913), with 87% achieving ctDNA clearance post-surgery. The two patients without ctDNA clearance post-surgery (VAF pre- vs post-surgery 124→3.8 ppm and 40→10 ppm) were HR+/HER2- and experienced no relapse. Interestingly, both patients received adjuvant chemotherapy and endocrine therapy, leading to ctDNA clearance at subsequent timepoints. Among 13 cases with ctDNA clearance post-surgery, 92% remain disease free. We observed a single late relapse at 54 mo in a patient with TNBC who cleared ctDNA at the post-operative landmark, with ctDNA detection re-emerging prior to relapse.
Conclusion: In EBC, ctDNA clearance by NAC, surgery, or adjuvant therapy is strongly associated with favorable outcomes. Ultrasensitive MRD analysis demonstrates that surgery also leads to a high rate of ctDNA clearance, suggesting the ability to measure and differentiate responses to both local and systemic therapies. Ultrasensitive ctDNA in EBC is likely to become an important biomarker of response to therapeutic interventions and warrants further study.

P2-01-26: Carboplatin added to neoadjuvant chemotherapy in a randomized phase II study: Complex DNA based biomarkers predict response in hormone receptor positive and triple negative breast cancer.

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Background: Carboplatin (Cb) improves treatment efficacy among patients with triple negative breast cancer (TNBC). However, not all patients need additional chemotherapy and the benefit for patients with hormone receptor positive breast cancer (HR+BC) has not been extensively investigated. Tumor-specific copy number alterations may predict sensitivity to DNA damaging agents such as Cb. The I-BCT-1 (Improved Breast Cancer Therapy – 1) study was designed to discover biomarkers associated with treatment response, in patients with high-risk HR+BC and TNBC.

Methods: Patients with HER2 negative high risk (by Ki67, ER/PgR and/or grade) cT2-4 tumors and no distant metastases were randomized 1:1 to paclitaxel (80 mg/m2, Q1W) alone or in combination with Cb (AUC6 Q3W), for 12 weeks, followed by four cycles of EC90 (epirubicin 90 mg/m2 and cyclophosphamide 600 mg/m2; Q3W). Tumor sampling was performed before treatment start, along the treatment, and at surgery, but only data related to initial sampling is reported here. Complex biomarkers based on somatic copy number alterations (the complex arm-wise aberration index (CAAI), loss of heterozygosity (LOH) and homologous recombination deficiency (HRD)) status were determined based on exome sequencing data. The influence of Cb on chemotherapy delivery and toxicities was analysed.
Results: In the 187 patients randomized, the median follow-up was 4.7 years (range 1.0 – 7.9 years). Among the TNBC patients (n=72), the pCR rate was 69% in the Cb group (n=35) vs. 43% in the control group (n=37; p = 0.036). For HR+BC patients (n=115), the pCR rate was 15% in the Cb group vs. 11% in the control group (n.s.), but the fraction of these patients with residual cancer burden (RCB) 0 or 1 increased from 16 to 35% by Cb treatment (p = 0.033). For TNBC patients, event-free survival (EFS) was significantly improved in the Cb group compared to the control group (p=0.017, HR 0.24, 95% CI 0.07-0.86). For HR+BC patients, no improvement in EFS was observed (p=0.684, HR 1.19, 95% CI 0.52-2.71). The proportion of patients with serious adverse events was 42.1% in the Cb group vs. 18.5% in the control group (p<0.001).
In TNBC patients receiving Cb, high CAAI was associated with an improved pCR rate (p=0.029). HRD has earlier been investigated as a marker for several DNA targeted therapies, including Cb, but no significant difference in pCR was found in the TNBC patients according to HRD status. In HR+BC patients receiving Cb, the pCR rate was significantly higher in patients with HRD tumors vs. patients with non HRD tumors (p=0.002; not associated with germline BRCA status).
In TNBC patients with a low genomic fraction of LOH, the treatment outcome was better in the Cb group than in the control group (p=0.005). In TNBC patients who did not receive Cb, high genomic fraction of LOH was associated with a good prognosis. This was validated in an independent dataset of TNBC patients (n=144, p=0.034), indicating a limited additional effect of Cb for patients with tumor harbouring a high fraction of LOH.

Conclusion: In TNBC patients, Cb improves the pCR rate and EFS. In HRBC patients, Cb improves the favorable outcome with RCB 0/1, but not EFS. The results suggest that different complex DNA based biomarkers predict response in hormone receptor positive

and triple negative breast cancer: HRD should be further investigated as a predictive marker for Cb benefit in hormone receptor positive patients, while genomic fraction of LOH may be an important marker for treatment selection in triple negative breast cancer patients.
Clinical trial identification: EudraCT number: 2013 – 004418 – 17 / ClinicalTrials.gov ID NCT02546232

P2-01-27: Utility of Plasma Circulating DNA Tumor Fraction in Bone-Only Metastatic Breast Cancer: A Real-world Outcomes Study

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Introduction: Among the >168,000 women living with metastatic breast cancer (MBC) in the U.S., bone metastases (mets) are common, developing in 50-70% of MBC patients (pts). Notably, approximately one-third of MBC pts develop bone mets without other visceral (V) mets, termed 'bone-only' (BO) MBC. Standard imaging is insufficient to reliably detect and track BO progression, resulting in many BO pts being excluded from clinical trials, and leaving providers lacking valuable knowledge to inform treatment decisions. Evidence suggests that the tumor-derived fraction of circulating DNA (TF) is highly prognostic in MBC. This study aimed to (1) evaluate differences in TF distribution between pts with BO and V MBC; (2) investigate the prognostic value of TF near the time of start of therapy; and (3) determine baseline lab and clinical factors associated with TF.

Methods: This study included MBC pts who underwent Foundation Medicine liquid comprehensive genomic profiling, with samples collected up to 60-days prior to start of therapy. Pts clinical data was obtained by the U.S.-wide de-identified Flatiron Health and Foundation Medicine real-world clinicogenomic breast database (CGDB), from ~280 U.S. cancer clinics (~800 sites of care) between 01/2011 and 12/2023. Real-world overall survival (rwOS) was compared between TF group (<1% vs. 1-10% vs. >10%) and stratified by BO and V (with or without bone) MBC. Multivariable analyses were adjusted for baseline lab and clinical factors, including age, ECOG score, race/ethnicity, hormone receptor statuses, histology, line of therapy, practice type, stage at diagnosis, adjuvant therapy, menopausal status, number of mets, albumin, alkaline phosphatase (ALK), serum creatinine, hemoglobin, lactate dehydrogenase (LDH), and neutrophil-to-lymphocyte ratio. The association between TF and baselined lab and clinical features was performed by linear regression.

Results: A total of 778 pts were included in the study (155 BO and 622 V MBC, 1 missing mets data). Of these, 299 had TF <1% (TF-low), 175 had TF 1-10% (TF-intermediate [int]) and 304 had TF>10% (TF-high). Prior to 1st line of therapy, there was no significant difference in proportion of pts with detectable ctDNA; comparing BO MBC pts to V MBC pts

(n=256, BO 39% TF-low, 18% TF-int, 43% TF-high; and V 38% TF-low, 18% TF-int, and 44% TF-high; p=1.0), a similar distribution was seen among all evaluable samples. Among patients with BO MBC, TF was prognostic: BO MBC pts with TF-low demonstrated improved rwOS relative to TF-int (median not reached vs. 31.1 months, hazard ratio (HR) 2.19, 95% confidence interval (CI) 1.1-4.35) and TF-high (median 23.5 months, HR 2.07, 95% CI 1.12-3.82; log-rank p=0.027). Multivariable analyses confirmed the independent and additive association of TF and less favorable rwOS, including BO vs. V status. In multivariable analyses evaluating clinicopathologic factors associated with TF, V mets were not associated with higher ctDNA TF and, conversely BO MBC was not significantly associated with TF values (p=0.106). Higher TF was independently associated with ECOG PS 1+ (p=0.023), 2+ sites of mets (p<0.001), albumin (p=0.043), ALK (p<0.001), and LDH (p=0.002); lower TF was independently associated with estrogen receptor status positive (p=0.017).

Conclusions: BO MBC Pts are as likely to have detectable circulating tumor DNA as MBC Pts with V mets, – particularly prior to 1st line of therapy when ctDNA levels would be expected to be lowest and in multivariate modeling controlling for clinicopathologic factors. There are no associations between V vs. BO mets and TF groups, and BO Pts with TF <1% have significantly better prognoses than those with TF ranging from 1-10% or >10%. TF is a useful biomarker in BO prognosis offering the same utility in BO as it does in V MBC.

P2-01-28: Expression of Antibody-Drug Conjugate targets in tumor and normal tissue from patients with metastatic breast cancer

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Background. The ideal Antibody-Drug Conjugate (ADC) target should have a high specificity for an antigen expressed in tumors, with low heterogeneity between tumor sites, and a low expression in normal tissue to avoid on-target toxicity. 72 targets for ADCs are currently being clinically studied in solid tumors with 2 targets (HER2, TROP2) already having an approved indication for metastatic breast cancer (mBC). Here, we investigated the mRNA expression levels of all 72 targets in 910 tumor and normal tissue samples from 30 patients enrolled in our rapid autopsy program UPTIDER (NCT04531696).
Methods. 910 samples (58 primary untreated (P), 753 metastatic (M), 99 normal (N)) from 30 UPTIDER patients underwent bulk mRNA sequencing with data available to study gene expression of 72 targets. 23 patients had HR+/HER2-, 6 triple-negative (TNBC), and 1 HER2 amplified primary BC. 18 patients had invasive breast cancer of no special type (NST), 7 invasive lobular carcinoma (ILC) and 5 mixed (3 NST/ILC, 1 NST/metaplastic, 1 both NST and ILC). Normal samples were selected based on the absence of oncological and non-oncological organ disease and on representation across available organs and patients. M

and N samples from 33 different organ sites were considered for the analysis. Normalized gene expression was reported as a continuous parameter. Weighted Spearman correlation on median gene expression was used to identify co-expressed/mutually exclusive targets. Associations between gene expression and independent co-variate (sample status: M vs P) were assessed by linear mixed quantile regression with random effect on patient ID to correct for inter-and intra-patient heterogeneity. No formal association test was performed between M and N.
Results. The 5 targets with the highest median expression in M are: FN1, MUC1, LAMP1, HER3 and SLC39A6 (median range: 8.46-10.34). TROP2 and HER2 had the 9th and 27th highest expression (median: 7.73 and 4.99). The 5 targets with the highest median expression in N are: FGFR2, SLC39A6, FN1, ALCAM and MUC1 (range: 9.43 and 10.45). TROP2 and HER2 had the 21st and 36th highest expression (median: 5.70 and 4.46). 15 targets had relatively consistent low expression across all normal tissues, which did not include the 5 highest expressed targets in M. Moderate levels of HER2 expression were found across several normal tissues, including bone marrow, heart and kidneys, but not in the lung. For TROP2, higher expression was seen in normal bladder, kidney, lung, pancreas and peritoneum, but not in bone marrow. Intra-patient inter-M heterogeneity, assessed by interquartile range (IQR), was high for MUC1, FN1 and VTCN1 (median IQR: 1.38–1.65) but relatively low for HER2 and HER3. Reduced and higher expression in M vs P was reported for 11 and 4 targets. Almost no differential expression was seen between HR+/HER2- vs TNBC, ILC vs NST and ER+ vs ER- metastases. A strong positive correlation (R>0.6) in M samples was observed for 20 pairs, including HER3-CEACAM5, and a strong negative correlation (R<-0.6) was observed for 6 pairs, including HER2-GPNMB. Organ specific expression was observed for a few targets in bones, brain, lung, muscle, pleura and subcutaneous M but not for M in breast contralateral, liver, lymph nodes, peritoneum and spleen. In N, CA9 was strongly expressed in bladder, CFC1B in bone marrow, DLL3 and ST8SIA1 in brain, CEACAM6 and CLDN18 in lung, DPEP3 in thoracic lymph nodes, CLDN6 in pancreas and CDH6 in peritoneum. No association was observed for age at death, post-mortem interval and changes in target expression in both M and N samples.
Conclusions. Our study brings novel data on the mRNA expression of ADC targets in M and N samples in mBC, both for targets with approved clinical indication (HER2 and TROP2) as for those being clinically investigated.

P2-01-29: Prognostic value of circulating tumor DNA in metastatic breast cancer

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Introduction: Circulating tumor DNA (ctDNA) is widely used in the management of metastatic breast cancer (MBC) for tumor genotyping and detection of actionable alterations. Whether detectable ctDNA and its levels are associated with worse prognosis

has not been well established yet in MBC. Available data are limited by small sample sizes and/or suboptimal ctDNA assays. In this study, we aim to evaluate the prognostic value of ctDNA detection in a large cohort of patients (pts) with MBC.
Methods: We integrated clinical and ctDNA sequencing data from MBC pts treated at our institution (MSK) who underwent at least one ctDNA assessment using MSK-ACCESS, a 129-cancer-related gene assay. The assay demonstrated high sensitivity and specificity to detect tumor-derived variants as it utilizes matched WBC sequencing to filter out biological noise originating from clonal hematopoiesis or germline variants. We selected the first ctDNA assessment performed in the metastatic disease course. ctDNA level was defined as the highest variant allele frequency (maxVAF) of the detected tumor-derived variants. In case of no detectable ctDNA mutation, ctDNA level was considered as zero. Association between ctDNA detection and its levels with overall survival (OS) was assessed using uni- and multivariate left-truncation corrected Cox proportional hazard models.
Results: Overall, 766 pts with at least one MSK-ACCESS between 8/2019 and 4/2024 were identified. Of those, 685 pts with complete clinical information were included in the analysis: 72% HR+/HER2-, 15% TNBC, and 13% HER2+. At the data lock, 42% of the pts had died. MSK-ACCESS was performed at or after the metastatic disease diagnosis with a median interval of 15 months (IQR: 1.4-46, 33% within 3 months). ctDNA was detectable in 73% of pts (at least one tumor-derived mutation), with a median maxVAF of 2.4% in ctDNA+ pts (IQR: 0.63-12.7%) and 34% of pts having >2 mutations. Median ctDNA levels were strongly associated with the number of metastatic sites (2.9% for ≥3 sites, 1.6% for 2, 0.7% for 1; p=0.00047), and presence of liver metastases (1.5% vs 0.76%; p=0.0013). ctDNA levels were lower in ctDNA+ pts with HR+/HER2- as compared to TNBC or HER2+ (median 1.9%, 4.2% and 4.6%, respectively, p=0.031). Detection of ctDNA was a strong prognostic factor for OS with a univariate HR of 3.9 (95% CI: 2.7-5.8) and multivariate HR of 3.7 (95% CI: 2.5-5.4). ctDNA levels were also strongly prognostic: ref: not detected, <1%: HR=2.2 (95% CI: 1.4-3.5), 1-10%: HR=3.9 (95% CI: 2.6-5.9), >10%: HR=7.3 (95% CI: 4.8-11.0), p for trend <2e-16. The number of mutations detected was associated with OS; HR=2.8 (95% CI: 1.9-4.2) for 1-2 mutations and HR=5.8 (95% CI: 3.9-8.6) for > 2 mutations, compared to none. In the analysis by receptor subtype, ctDNA levels were prognostic for both HR+/HER2- (p<2e-16) and TNBC (p=0.00045) but not for HER2+ (p=0.32). ctDNA level remained an independent prognostic factor for OS in a multivariable analysis adjusted for visceral metastasis, number of metastatic sites (at the time of metastatic relapse) and receptor subtype (HR=6.7, 95% CI: 4.4-10.2 for maxVAF >10% vs 0). Similar results were obtained using median, mean, and geometric mean VAF to estimate ctDNA levels. When restricting the analysis to HR+/HER2- MBC pts with a ctDNA collected within 3 months from metastatic diagnosis (n=152), ctDNA levels remained an independent prognostic factor for OS after adjusting for presence of visceral metastasis and number of metastatic sites (HR=4.5 95% CI: 1.6-13 for maxVAF >10% vs 0).
Conclusion: In this large cohort of MBC pts, ctDNA detection and levels emerged as strong prognostic indicators for OS, independently from disease burden. Our data suggest that ctDNA levels can provide valuable insight into tumor biology as well as disease burden and may be utilized for early prognostication in MBC.

P2-01-30: Gene Expression patterns in early ER+/HER2- breast cancer treated with neoadjuvant chemotherapy versus CDK4/6 inhibitor therapy: results from the Swedish PREDIX Luminal B trial

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Introduction: CDK4/6 inhibitors are approved in ER+/HER2- breast cancer at the adjuvant and metastatic settings, however their role as neoadjuvant therapy is still under investigation. The aim of this study was to identify gene expression-based predictive biomarkers for response to the combination of a CDK4/6 inhibitor and endocrine therapy versus chemotherapy in clinically defined high-risk, early luminal breast cancer.
Methods: The PREDIX Luminal B clinical trial (NCT02603679) enrolled ER+/HER2- breast cancer patients with tumor size at least 20mm and/or positive nodal status. Patients were randomized 1:1 to receive preoperatively either weekly paclitaxel for 12 weeks followed by palbociclib and endocrine therapy for 12 weeks (Arm A), or the reverse sequence (Arm B). The primary outcome was the objective radiologic response at 12 weeks (ORR12) while pathologic response to treatment was a secondary outcome. Core needle biopsies were obtained at baseline, after 12 weeks of treatment and at the time of surgery. Total RNA-sequencing was performed using bulk RNA extracted from fresh frozen biopsies from all timepoints. RNA libraries were constructed using the Illumina Stranded Total RNA library prep kit with Ribo-Zero Plus and sequencing was carried out on the Illumina NovaSeq X Plus System. The raw RNA-sequencing data were subjected to pre-processing and quantification using the nf-core/rnaseq bioinformatics pipeline (version 3.3). Differential expression analysis was performed, followed by gene set enrichment analysis (GSEA) using the Hallmark gene sets sourced from the MSigDB database. Finally, single-sample predictor (SSP) models were employed for both classifying patients into one of the five PAM50 subtypes (SSP-PAM50) and estimating the risk of recurrence (ROR) score (SSP-ROR).
Results: The study enrolled a total of 181 patients, with 179 forming the intention-to-treat population (39% premenopausal, 66% node positive). After quality control, 170 patients had evaluable RNA-sequencing data at baseline and 133 of those had matched data for week 12. Residual Cancer Burden (RCB) status was available for 132 patients, with 16 of them having RCB 0/1. In patients treated with palbociclib and endocrine therapy, the ORR12 was higher for the Luminal B intrinsic subtype compared to other subtypes (Odds Ratio (OR)= 2.81, 95% CI 1.03-8.08, p = 0.04) at baseline, but not in patients treated with paclitaxel (OR = 1.50, 95% CI 0.52-4.45, p = 0.43). ROR did not differ between responders and non-responders in either arm (p=0.76 in arm A, p=0.15 in arm B). GSEA showed that responders in chemotherapy arm had tumors with upregulation of immune-related genes and downregulation of the ER-pathway (adjusted $p < 0.05$) at baseline. In contrast, responders in arm B were characterized by upregulation of cell cycle related genes, MYC and E2F program (adjusted $p < 0.05$), and this was confirmed by interaction analysis for ORR12 and arm B for the reference arm A. Patients achieving RCB 0/1 were enriched for immune related pathways at baseline (adjusted $p < 0.05$). Longitudinal analysis between baseline

and week 12 for the Luminal B group revealed a change of intrinsic subtype under treatment and the direction of the change was similar in both arms, with most (93.4%) converting to either Luminal A or Normal-like.
Conclusion: Objective response of ER+/HER2- breast cancer to neoadjuvant palbociclib/endocrine therapy or paclitaxel is governed by different biologic processes. Further translational studies are ongoing to identify predictors of response and the best sequence of treatments.

P2-02-01: Enhancing chemotherapy sensitivity in triple-negative breast cancer through knockdown of the 'Dark' druggable gene SCYL3

Hannah Engebretson, Steven W. Wall, Audra Lane, Sara Savage, Bing Zhang, Gloria V. Echeverria

Triple Negative Breast Cancer (TNBC) is an aggressive subtype of breast cancer with limited targeted therapy options for patients lacking deleterious BRCA1/2 mutations. TNBC has a five-year survival rate of less than 11% once the disease has metastasized which is alarming given that 45% of patients have residual disease after undergoing neoadjuvant chemotherapy (NACT). Therefore, it is crucial to identify therapeutic regimens that increase chemotherapy sensitivity. We previously demonstrated that mitochondrial oxidative phosphorylation (OXPHOS) is elevated in TNBC cells that survive NACT after administration of DNA damaging chemotherapeutics (e.g. doxorubicin [DXR] and carboplatin [CRB]) but not taxane treatments (e.g. docetaxel [DTX] and paclitaxel [PTX]) (PMIDs 30996079 and 36813854). Other groups have provided evidence that induction of mitophagy, a process whereby damaged mitochondria are degraded, can improve taxane sensitivity in TNBC. Thus, identifying mechanisms regulating OXPHOS and mitophagy could lead us to promising therapeutic targets to overcome chemoresistance in TNBC. In 2014, the NIH launched the "Illuminating the Druggable Genome (IDG)" project that identified approximately 4,000 genes with therapeutic potential based on classification of a G-protein coupled receptor, kinase, nuclear receptor, or ion channel domain. Cross-referencing this list across public and proprietary databases to subclassify them based on current knowledge revealed 328 "dark" genes with unknown functions (PMID: 29472638). We determined co-expression of each dark gene in the BRCA proteomics data from the Clinical Proteomic Tumor Analysis Consortium (PMID: 37582339). We then used gene set enrichment analysis to identify positive enrichment of OXPHOS-related metabolic pathways from WikiPathways. Genes co-expressed with Saccharomyces cerevisiae yeast 1 (SCY1) Like Pseudokinase 3 (SCYL3) were enriched for in the electron transport chain and OXPHOS pathways. SCYL3 was previously shown to negatively regulate mitophagy in hepatocellular carcinoma (HCC) (PMID: 36440258). Based on our prior work demonstrating the crucial role of OXPHOS in TNBC and the potential mechanism and relationship between mitophagy and taxane sensitivity, we sought to ascertain the role of SCYL3 in TNBC cell survival, chemotherapeutic resistance, metabolic rewiring, and mitophagy. Transient siRNA-mediated knockdown of SCYL3 in human TNBC cell lines MDA-MB-468 and SUM159 caused no defect in cell survival as

evidenced by cell counting assays. To ascertain the role of SCYL3 in chemotherapeutic resistance, we treated TNBC cells with IC50 doses of conventional chemotherapies and monitored cell numbers following SCYL3 knockdown. Our data provide evidence in the aforementioned cell lines that SCYL3 knockdown increased sensitivity to taxanes but not DNA-damaging agents. Our preliminary findings also indicate that knockdown of SCYL3 in cultured human TNBC cells leads to decreased levels of the negative regulator of mitophagy, Presenilin-associated rhomboid-like (PARL) protein. These findings suggest inhibition of SCYL3 improves taxane sensitivity in TNBC cells, potentially through activation of mitophagy. Continued experimentation will focus on direct measurement of mitophagy rates and functional studies to ascertain the potential role of SCYL3 in mitochondrial phenotypes and chemoresistance.

P2-02-02: CHEMOTHERAPEUTIC TOPOISOMERASE 2 POISONS GENERATE TREX1 RESISTANT DNA FRAGMENTS THAT INDUCE A POTENT cGAS/STING RESPONSE

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Tumours with genomic instability display enhanced immunogenicity and potential for response to immune checkpoint blockade (ICB). Interestingly, chemotherapy mediated DNA damage can also stimulate the immune system through activation of the cGAS/STING innate immune pathway and therefore, improve clinical outcomes in combination with ICB. Here we set out to compare the ability of classically used chemotherapies to activate innate immunity, as well as characterise the mechanism(s) behind it. We identified topoisomerase 2 (TOP2) poisons, such as anthracyclines, as the most effective activators of cGAS/STING, leading to a potent type I interferon response. Mechanistically, we demonstrate that abortive TOP2cc repair intermediates (dsDNA fragments containing 5'-phosphotyrosyl residues) get encapsulated within micronuclei and activate cGAS, which can be blocked with antimitotic agents (e.g. taxanes). Crucially, we discovered that these 5'-phosphotyrosyl linked fragments are resistant to nuclease degradation by the cytoplasmic nuclease TREX1, which stabilises them, resulting in robust cGAS/STING mediated inflammation. Finally, using in vivo modelling, we confirmed that Top2 poisons enhance anti-tumour responses to ICB in comparison to taxanes. In line with this, using pre and on-treatment breast tumour biopsies, we have shown that anthracycline-based chemotherapy induces a robust type I interferon response in breast tumours, driving tumour lymphocytic infiltration. Moreover, following anthracycline treatment with taxane treatment results in supression of lymphocytic infiltration in these same tumours. Collectively, our findings reveal that TOP2 poison mediated DNA lesions enhance cGAS substrate availability by impairing cytoplasmic dsDNA degradation, highlighting their ability to drive anti-tumour immune responses and enhance the therapeutic efficacy of ICB.

P2-02-03: The RAR gamma nuclear receptor agonist IRX5010 has combination inhibitory effects with an anti-PDL-1 checkpoint inhibitor on the growth of EMT-6 triple negative breast cancer

Martin Sanders, Vidyasagar Vuligonda

Research Objectives and Rationale: We previously presented (2023 SABCS) that monotherapy with the RAR gamma agonist IRX5010 inhibits tumor growth in the triple negative breast cancer EMT-6 syngeneic mouse model; and in a human Her2+ JIMT-1 breast cancer xenograft mouse model. Growth inhibitory effects of IRX5010 in both models were accompanied by increased tumor infiltrating effector memory T-cells. We presented (2024 FASEB Retinoids Conference), effects of monotherapy with IRX5010 on inhibition of tumor growth accompanied by development of tumor infiltrating effector memory T-cells in mouse models of lung, (Lewis Lung Cancer); colorectal (MC38) and prostate cancers (MyC-CaP). We observed in these studies inhibition of tumor infiltration by myeloid derived suppressor cells, suggesting that combination treatment with IRX5010 and checkpoint inhibitors could increase inhibition of tumor growth. We now present data on IRX5010 treatments with anti-PD-1 or anti-PDL-1 monoclonal antibody checkpoint inhibitors in an EMT-6 triple negative breast cancer model.
Experimental Methods: EMT-6 cells were injected into Balb/c mice and allowed to grow to 50-150 mm3. Then treatments were started and maintained until tumors grew to 1500 mm3. Treatment was administered as daily oral doses of IRX5010 at 10 mg/kg/day, or vehicle. Murine monoclonal anti-PD-1 or anti-PDL-1 antibodies were injected ip every three days at 10 mg/kg. Tumor sizes were assessed by calipers every three days. Toxicity assessment was done by weighing the mice every three days. Flow cytometry of harvested tumors for tumor infiltrating T-cell subtypes, and myeloid derived suppressor cells was done at study end.
Results: Monotherapy with either IRX5010 or murine anti-PDL-1 monoclonal antibody had substantial inhibitory effects relative to vehicle on tumor growth in the syngeneic EMT-6 triple negative breast cancer model; -61% for IRX5010, and -75% for murine anti-PDL-1. Monotherapy with murine anti-PD-1 had modest inhibitory effect on tumor growth, -14%. Combination treatment with IRX5010 plus murine anti-PDL-1 had an additive effect resulting in 84% inhibition of tumor growth relative to vehicle, a 9% additional inhibition of tumor growth compared to monotherapy with anti-PDL-1 alone. Combination treatment with IRX5010 plus murine anti-PD-1 had a modest additive effect, resulting in -65% inhibition of tumor growth relative to vehicle, a 4% additional inhibition of tumor growth compared to monotherapy with IRX5010. Flow cytometry for T-cells subsets and myeloid derived suppressor cells was performed. These results are pending at the time of submission of this abstract. The data will be presented at the symposium.
Conclusions: Treatment of the EMT-6 murine model of triple negative breast cancer with the RAR gamma selective agonist compound IRX5010 plus the checkpoint inhibitor monoclonal murine anti-PDL-1 resulted in 84% inhibition of tumor growth, a 9% increase of inhibitory treatment effect over anti-PDL-1 alone. Combination treatment with IRX5010 with monoclonal anti-PD-1 had only modest additive effects on inhibition of EMT-6 tumor

growth. All treatment regimens were well tolerated as assessed by change in weight. These findings are the first demonstration of additive effects of combination treatment with an RAR gamma nuclear receptor agonist and an anti-PDL-1 monoclonal antibody checkpoint inhibitor in a triple negative breast cancer mouse model. They support future translation of combination treatment with IRX5010 plus an anti-PDL-1 monoclonal antibody checkpoint inhibitor into clinical trials in triple negative breast cancer patients.

P2-02-04: Investigating Semaphorin7a in Mammary Gland Involution and Postpartum Breast Tumorigenesis

Lauren Cozzens, Traci R Lyons

Women with postpartum breast cancer (PPBC), breast cancer diagnosed within 10 years post-childbirth, have an increased risk of metastasis and death compared to nulliparous women or women diagnosed during pregnancy. Unfortunately, what causes this increased risk is not well understood. Studies have shown that postpartum involution (INV), the process by which the lactating mammary gland re-models to a morphologically near prepregnant state, is a driving force of tumor progression. Our lab has identified Semaphorin 7a (SEMA7A), a signaling molecule, as upregulated in the mammary gland during INV and in PPBC tumors compared to nulliparous mammary glands or tumors, respectively. SEMA7A promotes both mammary epithelial cell (MEC) survival during INV, and tumor cell survival in models of PPBC. Studies have found that constitutive Sema7a knock-out (KO) mice exhibit a decrease in the proportion of mammary progenitor cells (MPCs) during INV as compared to wild-type (WT) mice. Notably, MPCs have been identified as the cells of origin for many breast cancer tumors. SEMA7A has also been shown to promote progenitor-like phenotypes in vitro in both normal MECs and breast tumor cells. Despite these findings, the mechanism by which SEMA7A promotes progenitor cell phenotypes during INV and how these phenotypes may contribute to PPBC tumorigenesis has yet to be explored. Therefore, we hypothesize that SEMA7A promotes progenitor-like and pro-survival phenotypes in MPCs during involution to contribute to PPBC tumor initiation. To model differentiation during lactation, and cell death programs during INV, we have utilized and characterized in vitro cell models of lactation and INV. Preliminary studies have shown that SEMA7A upregulates expression of the transcription factor SRY-box 2 (SOX2) in both normal MECs during INV and in breast cancer cells. Interestingly, SOX2 has been shown to maintain luminal progenitor cells and breast cancer stem cells and thus may mediate SEMA7A's promotion of progenitor cell phenotypes. SEMA7A is known to signal through integrins to mediate its downstream effects and preliminary work has suggested SEMA7A may facilitate upregulation of SOX2 through MAPK signaling. To test the impact of SEMA7A on SOX2 transcriptional activity during INV, we have begun to utilize a SOX2 reporter vector developed in the laboratory of Dr Lalage Wakefield. To model the impact of SEMA7A on PPBC tumorigenesis in vivo, we have also combined the whey acidic protein-transforming growth factor alpha (WAP-TGF) mouse model with our SEMA7A KO mice. The

goal of these studies is to inform improved prevention and treatment strategies for patients with PPBC.

P2-02-05: Mechanism of action of gedatolisib in combination with fulvestrant and/or palbociclib in estrogen receptor positive breast cancer models

Stefano Rossetti, Aaron Broege, Megan Seibel, Jhomary Molden, Igor Gorbatchevsky, Brian Sullivan, Lance Laing

Background: Therapies targeting PI3K, AKT, and mTOR (PAM) in combination with fulvestrant are approved to treat patients with HR+/HER2- advanced breast cancer. Due to the crosstalk between the PI3K/AKT/mTOR (PAM) pathway and the Estrogen Receptor (ER) and CDK pathways, resistance can arise through compensatory mechanisms when only one of these pathways is inhibited. In a phase 1b clinical trial in patients with HR+/HER2- advanced breast cancer, the combination of gedatolisib, a pan-PI3K/mTOR inhibitor, with endocrine therapy and palbociclib showed promising efficacy and safety compared with the published results for standard-of-care therapies. A Phase 3 trial (VIKTORIA-1) evaluating gedatolisib plus fulvestrant with and without palbociclib is underway in patients with advanced breast cancer. In the present study, we used multiple ER+ breast cancer cell lines to investigate the mechanism of action of gedatolisib and single node PAM inhibitors in combination with fulvestrant and/or palbociclib.
Methods: A panel of breast cancer cell lines with mutated or non-mutated PAM pathway genes (e.g. PIK3CA, PTEN) were evaluated for their responses to gedatolisib and single node PAM inhibitors (PI3K, AKT, mTORC1) alone or in combination with fulvestrant and/or palbociclib. Cell viability, growth rate, and cell death were evaluated by luciferase/fluorescence-based assays. Cell cycle and DNA replication were assessed by EdU incorporation and flow cytometry. ER activity was assessed by analysis of ER-target genes, while CDK pathway activity was assessed by analysis of phospho-RB and E2F-target genes. Breast cancer xenograft studies evaluating gedatolisib plus fulvestrant and/or palbociclib in vivo were also performed.
Results: Gedatolisib significantly reduced cell viability and growth rate in all cell lines tested, and the addition of fulvestrant and/or palbociclib significantly increased its growth-inhibitory effects. Mechanistically, the gedatolisib/palbociclib/fulvestrant triplet demonstrated greater inhibition of DNA replication and counteracted adaptive responses associated with single drug treatment, such as induction of ER activity by gedatolisib and reactivation of CDK signaling after palbociclib treatment. Previously, the in vivo efficacy of the gedatolisib/palbociclib/fulvestrant triplet was confirmed in a breast cancer xenograft model. In comparative studies, gedatolisib plus fulvestrant/palbociclib induced greater growth-inhibition relative to single-node PAM inhibitors plus fulvestrant/palbociclib.
Conclusions: The combination of gedatolisib, fulvestrant, and palbociclib exerted greater growth-inhibitory effects than each drug alone and showed superior efficacy relative to single node PAM inhibitors combined with fulvestrant and palbociclib. This study provides

a strong mechanistic rationale for combining gedatolisib, fulvestrant, and palbociclib for the treatment of advanced breast cancer.

P2-02-06: Molecular heterogeneity in adjacent normal tissue among Chinese breast cancer patients

Hela Koka, Bin Zhu, Priscilla Lee, Kevin Wang, Avraam Tapinos, Difei Wang, Gary M. Tse, Koon-ho Tsang, Cherry Wu, Chad A. Highfill, Kristine Jones, Belynda Hicks, Amy Hutchinson, Montserrat Garcia-Closas, Stephen Chanock, David C. Wedge, Lap Ah Tse, Xiaohong R. Yang

Background: Breast cancer (BC) stands as a prominent contributor to cancer-related fatalities on a global scale. Traditionally, the primary focus in assessing recurrence risk and guiding treatment decisions has revolved around scrutinizing the primary tumor's histopathological characteristics and molecular compositions. However, recent findings suggest that exploring normal tissue adjacent to the tumor (NAT) could unveil valuable insights into the intricate characteristics of BC advancement and patient prognoses. Specifically, in our recent work, we discovered three phylogenetic tree groups of NAT and tumor tissues that were associated with distinct tumor and tumor microenvironment (TME) features using the whole genome sequencing data collected from 43 Hong Kong BC patients (Zhu et al. under review). Here, we aimed to further characterize the molecular heterogeneity of NAT by integrating methylation and gene expression profiling data in an expanded sample set, which may deepen our understanding of tumor evolution.
Methods: Genome-wide DNA was extracted from fresh frozen paired tumor/NAT tissues in 188 patients diagnosed with breast cancer and treated in Hong Kong, China. DNA was then profiled using the Infinium Methylation 850K BeadChip array. Horvath clock was utilized to estimate epigenetic age, and MethylCIBERSORT was used to estimate cellular composition using the methylation data. For a subset of the patients (N= 76), we also analyzed RNA sequencing (RNA-Seq) data and used CIBERSORTx to estimate the proportions of immune cell subpopulations in NAT samples. Risk factor and clinical information was available for most patients.
Results: The uniform manifold approximation and projection (UMAP) identified two distinct clusters of NAT samples (cluster 1, N= 139; cluster 2, N= 49) based on the top 10K most variable methylation probes. The two clusters were recapitulated by unsupervised clustering. The two sets of patients did not differ by chronological age at diagnosis, but when investigating epigenetic aging - as captured by Horvath clock – we found that cluster 2 displayed a lower age acceleration in NAT compared to cluster 1. Among the subset of patients with phylogenetic trees constructed in our previous work, we observed cluster 1 had a higher frequency of patients (64.3%) that showed distinct evolutionary trajectories in matched NAT and tumor samples (multiple-tree group) compared to cluster 2 (33.3%). Consistent with previous findings, we found that cluster 1, enriched with multiple-tree NAT samples, was associated with lower proportions of CD14 cell population (P=1.6x10-6), monocytes (P=7.1x10-6), and M2 macrophages (P=2.3x10-7) compared to cluster 2. Interestingly, genomic features in the matched tumors such as PAM50 subtype, TP53

mutation status, or tumor mutational burden, did not vary significantly between clusters 1 and 2. Lastly, established BC risk factors examined in this study did not show significant differences by this methylation-based cluster assignment.
Conclusion: Using methylation data in an expanded dataset, we extended our previous analysis of paired tumor and NAT samples to characterize breast cancer field effect. Our main findings illuminate the diverse nature of the microenvironment surrounding the tumor and its potential influence on the genomic evolutionary path of the tumor.

P2-02-07: Different effects of gedatolisib versus single-node PI3K/AKT/mTOR pathway inhibitors on breast cancer cell metabolic functions

Stefano Rossetti, Aaron Broege, Jhomary Molden, Ross Kopher, Stephen Schulz, Ky McCracken, Igor Gorbatchevsky, Brian Sullivan, Lance Laing

Background: Currently approved treatment options for patients with HR+/HER2- advanced breast cancer include single-node PI3K/AKT/mTOR (PAM) inhibitors, such as everolimus (mTORC1), capivasertib (AKT), and alpelisib (PI3Kα), in combination with hormonal therapy. In a previous study, we found that comprehensive inhibition of multiple PAM pathway nodes by gedatolisib, a pan-PI3K/mTORC1/mTORC2 inhibitor, exerted greater growth inhibitory effect than single-node PAM inhibitors in breast cancer models, both in vitro and in vivo. Since increased activation of the PAM pathway plays a critical role in driving metabolic adaptations (e.g. increased glycolysis) required by cancer cells to sustain biomass production and proliferation, we hypothesize that gedatolisib exerts a greater effect on key metabolic functions in cancer cells relative to single-node PAM inhibitors.
Methods: A panel of breast cancer cell lines with mutated or non-mutated PAM pathway genes (e.g., PIK3CA, PTEN) were evaluated for their responses to gedatolisib versus single node PAM inhibitors (alpelisib, capivasertib, everolimus). Several metabolic functions were evaluated, including glucose consumption, lactate production (Biosen analysis), and real time oxygen consumption rate (OCR, Resipher analysis). Effects on PAM pathway activation, cell viability, and growth rate were evaluated in parallel by luciferase and flow cytometry assays.
Results: Gedatolisib strongly inhibited PAM pathway activity and reduced cell viability and growth rate in the cell lines tested. Consistent with these effects, significant inhibition of metabolic functions, such as glucose consumption, lactate production, and OCR was also found. Compared to the single-node PAM inhibitors, gedatolisib exhibited more potent and efficacious effects on these metabolic functions regardless of the cell lines' PI3K pathway mutational status. The metabolic changes induced by gedatolisib within the cancer cells also resulted in microenvironmental changes known to improve anti-tumor immune response, such as higher glucose levels, lower lactate levels, and increased oxygen concentration.
Conclusions: By targeting multiple PAM pathway nodes, gedatolisib, inhibited cancer cell metabolic functions more effectively relative to more narrowly targeted PAM inhibitors. A more comprehensive inhibition of PAM-controlled functions, including critical metabolic

functions required to sustain cancer cell proliferation, may explain the greater activity of gedatolisib relative to single node PAM inhibitors in breast cancer cells. Gedatolisib has previously demonstrated promising preliminary clinical efficacy and safety data in combination with hormonal therapy in advanced breast cancer. A Phase 3 study (VIKTORIA-1) evaluating gedatolisib plus fulvestrant with and without palbociclib is underway in patients with HR+/HER2- advanced breast cancer.

P2-02-08: Chromosome 8q gain-related transcription factor MYBL1 is a critical regulator in triple-negative breast cancer

Akihiro Fujimoto, Kazuhiro Ikeda, Keiichi Kinowaki, Hidetaka Kawabata, Akihiko Osaki, Satoshi Inoue, Kuniko Horie

Chromosome arm-level aneuploidies (CAAs) are a common consequence of genomic instability during the evolution of solid tumor and often associated with metastasis and therapy resistance. In breast cancer, chromosome arms 1q and 8q are the frequent amplified regions for CAAs. This study aims to investigate whether critical genes amplified in genomic regions with CAAs contribute to the pathophysiology in triple-negative breast cancer (TNBC). We established multiple long-term culturable TNBC patient-derived cells (PDCs) using spheroid culture technique, which is favorable for the enrichment of cell population with cancer stemness. We performed a genome-wide chromatin immunoprecipitation study for histone H3K27ac and super-enhancer analysis in these TNBC PDCs and cell lines. Super-enhancer analysis revealed that 8q is a substantial chromosomal region with a high density of super-enhancers. Among genes in the vicinity of 8q super-enhancers, we identified that MYB proto-oncogene like 1 (MYBL1) is a transcription factor with high abundance in TNBC PDCs as well as in basal-like BT549 cells. In TCGA breast cancer database, 8q gain is a common genomic feature in MYBL1 gene-amplified breast cancer tissues and MYBL1 expression is substantially correlated with 8q-related genes and proliferation-related genes like MKI67 and BUB1. In TNBC PDCs and cell lines, we showed that MYBL1-specific siRNAs significantly repressed TNBC cell proliferation and migration. RNA sequencing analysis in TNBC cells revealed that MYBL1 silencing substantially downregulated genes involved in the transcription machinery such as nuclear envelope, chromatin dynamics, and DNA replication. In immunohistochemical analysis of clinical TNBC tissues from a Japanese cohort, we showed that a positive MYBL1 immunoreactivity (IR) was significantly associated with shorter disease-free survival (DFS). Among candidate MYBL1 targets, we demonstrated that NCAPH, a subunit of condensin I complex, was a prognostic factor for patients with TNBC based on the immunohistochemistry of clinical TNBC tissues. Notably, patients with double IR positivity for MYBL1 and NCAPH showed shorter DFS than those with MYBL1 or NCAPH positivity alone. MYBL1 has been characterized as an essential transcriptional regulator in male meiosis and in female mammary gland development. Our findings indicate that MYBL1 plays an essential role in the pathophysiology for advanced breast cancers such as TNBC,

and MYBL1 and its downstream genes can be potential diagnostic and therapeutic targets for the disease.

P2-02-09: Novel Intramolecular BRCT Domain Interaction with ECT2 C-terminal Intramolecular Interaction Domain Regulates GEF Activity and Tumorigenesis

Jinyu Lu, Yong-Li Dong, Michelle Pirruccello, Hao Chang, Ming Wu, Thomas Ni, Jose Pastor-Pareja, Xiangyu Zheng, Deyou Zheng, Tian Xu

BRCT domain-containing proteins are a large family of regulators that control diverse biological processes through recruiting effector molecules. Regulation of BRCT effector recruitment is a key modulation step. ECT2 (Epithelial Cell Transforming sequence 2), a BRCT domain-containing Rho/Rac GEF and key regulator of cell division, is frequently upregulated in various human cancers, including breast cancer. Particularly, overexpression of ECT2 is strongly associated with poor prognosis and represents a putative independent prognostic factor for breast cancer.
Through an in vivo genetic screen for mutations driving tumor invasion and metastasis, we identified a group of special mutations in Pebble/ECT2. In this study, we report the discovery of a highly conserved, novel Intramolecular Interaction Domain (IID) in ECT2/Pebble (Pbl), which is frequently mutated in human cancers and contains conserved motifs shared by numerous BRCT domain-containing proteins. The binding of BRCT to RacGAP1 is promoted by intramolecular interaction of BRCT with IID, which is controlled by cell cycle-dependent phosphorylation of IID. Mutations in IID disrupt the intramolecular interaction and consequently prevent assembly of high-affinity Pbl/ECT2-RacGAP1 complexes, thereby causing abnormal Rac1 activation and tumor progression. Our study uncovers a novel intramolecular mechanism that governs the recruitment of BRCT domain effectors and provides potential target for developing novel therapeutic agents against breast cancers.

P2-02-10: Inhibition of the Kif11 mitotic protein kills TP53-mutant triple-negative breast cancer cells

Amanda Lanier, William Tahaney, Cassandra Moyer, Jamal Hill, Darrian Coleman, Banu Arun, Abhijit Mazumdar, Powel H Brown

Background: Triple-negative breast cancer (TNBC) has the poorest prognosis of the breast cancer subtypes, in part due to the lack of targeted therapy options. TNBCs lack the expression of targetable cell surface or nuclear receptors such as estrogen receptor (ER) or HER2 present in other breast cancers. Standard of care for TNBC patients includes surgery, radiation, and non-specific chemotherapy, and very few women with TNBC meet criteria to receive immunotherapy or PARP inhibitors. Thus, new targeted therapies are urgently needed for these aggressive cancers. Nearly all TNBCs harbor TP53 mutations, so our

laboratory has sought to identify molecular vulnerabilities of these TP53-mutant breast cancers. A drug screen identified that the Kif11 inhibitor SB-743921 preferentially killed TP53 mutant breast cancer cells (as compared to TP53-wild type cells). Kif11 is a motor protein critical for proper alignment of the mitotic spindle, and inhibition leads to mitotic defects and cell cycle arrest.
Hypothesis: We hypothesized that Kif11 inhibition causes TP53-mutant TNBC cells to undergo mitotic catastrophe leading to cell death.
Methods: Clinical Data: Data was accessed via cBioPortal or UCSD Xena Browser. Expression was compared between groups using Student's t-tests. For Kaplan-Meier curves, Kif11 expression was dichotomized at the median and survival curves were compared using the Log-Rank test. Cell Proliferation and Cell Death Assays: Cells were treated with SB-743921 or vehicle, stained with Hoechst 33342 and DRAQ7(2D) or Annexin V-FITC and PI (Flow), and imaged on the ImageXpress PICO or analyzed via flow cytometry. Immunofluorescence: Cells were treated with SB-743921 or vehicle, stained with anti-alpha tubulin-AlexaFluor488 and DAPI and imaged on the Nikon Ti2. Mouse xenograft experiments: Cells were injected into the mammary fat pads of nude mice. When tumors reached 50-100 mm3, mice were treated with the Kif11 inhibitor SB-743921 10mg/kg or vehicle i.p. 3 times weekly. Experiments were conducted under IACUC approval.
Results: The Kif11 mitotic kinesin is more highly expressed in TP53 mutant and TNBCs than in TP53 wild-type breast cancers, and high expression is associated with poorer survival. Kif11 inhibition leads to cell cycle arrest and growth suppression in both TP53-wild-type and TP53-mutant breast cancer cells. However, in TP53-mutant cells Kif11 inhibition causes mitotic dysfunction, formation of multinucleated giant cells, and caspase-dependent apoptotic cell death. Furthermore, knockout of TP53 in wild-type breast cancer cells sensitizes them to cell death following Kif11 inhibition. Kif11 inhibition also inhibits the growth of TP53-mutant TNBC xenografts in vivo.
Conclusions: In cells with TP53-mutation or loss, Kif11 inhibition results in mitotic dysfunction and cell death due to mitotic catastrophe, and Kif11 inhibitors suppress in vivo TP53-mutant TNBC growth. These results support further investigation of Kif11 inhibitors as a potential therapeutic in TP53 mutant TNBC.

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P2-02-11: Unveiling the subtype-specific role of PTPRK in triple-negative breast cancer: Implications for targeted therapies

Xiangyi Liu, Wen G. Jiang, Lin Ye

Introduction: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterised by the absence of estrogen receptor, progesterone receptor, and HER2 receptor, leading to limited treatment options and poor prognosis. Protein tyrosine phosphatase receptor type K (PTPRK) has been implicated in various cancer-related pathways, such as EGFR and BMPs, but its specific functions within TNBC subtypes remain poorly understood. Elucidating the subtype-specific roles of PTPRK in TNBC could provide crucial insights into its underlying mechanisms and identify potential therapeutic targets, paving the way for more effective and personalised treatment strategies for this challenging breast cancer subtype.
Methods: The transcript level of PTPRK was assessed in 11 breast cancer cell lines using PCR and qPCR. Additionally, its expression in subtype-specific breast tumour tissues from TCGA and in various breast cancer cell lines using Genearray data (E-MTAB-2770) was analysed. The overall survival analysis of PTPRK in different breast cancer subtypes was conducted using Kaplan-Meier test with TCGA RNA-Seq data. Relapse-free survival (RFS) and distant metastasis-free survival (DMFS) data for PTPRK across subtypes were obtained from the KM plotter website (https://kmplot.com/analysis/). Correlation analysis between PTPRK expression and TNM stages and its markers were performed using TCGA data. Lentiviral vectors were utilised to create PTPRK knockdown models in two TNBC cell lines, BT-549 and MDA-MB-468. Cellular function assays, including proliferation, adhesion, invasion, and migration, were conducted using these knockdown cell lines.
Results: PTPRK generally showed high expression in breast cancer cell lines, particularly in HER2+ and TNBC tumour samples from TCGA data, and in TNBC cell lines based on Genearray data. TCGA data revealed that higher PTPRK expression was correlated with longer overall survival in TNBC (P=0.049), while the opposite finding was observed in other subtypes. Similar trends were seen for RFS and DMFS, with high PTPRK expression associated with shorter survival in HER2+ subtypes (RFS: P=0.0058, DMFS: P=0.011) and longer survival in TNBC (RFS: P=0.031, DMFS: P=0.01). Advanced T stages (T3+T4) exhibited lower PTPRK expression compared to early T stages (T1+T2). No significant differences in PTPRK expression were observed with distant metastasis and lymph node status across all breast cancer subtypes. Positive correlations observed between PTPRK expression and CDK6 and KI67 were notably significant in the TNBC subtype. For cellular function assays with PTPRK knockdown models, significant changes were observed in adhesion and invasion tests in both BT-549 and MDA-MB-468 cell lines, with increased adhesive and invasive ability following PTPRK knockdown. Additionally, a significant increase in proliferation was observed in BT-549 PTPRK knockdown cells compared with the control cells, whilst this was not shown in MDA-MB-468 cell line.
Conclusion: PTPRK plays a subtype-specific role in TNBC, influencing survival of patients and cellular functions for cancer cells including adhesion, invasion, and proliferation. These findings highlight the potential of PTPRK as a therapeutic target in TNBC, warranting further investigation into its mechanistic pathways and therapeutic implications.

P2-02-12: Creating well-defined high-throughput breast cancer dormancy models with bioprinting for probing and targeting late recurrence

Lina Pradhan, Alex Wang, Rafael Castro, Dwayne Dexter, April M. Kloxin

Abstract: Late recurrences, especially in estrogen receptor–positive (ER+) breast cancer, often occur in distant metastatic sites like the bone marrow (BM) and are hypothesized to be triggered by local microenvironment interactions that stimulate the growth of dormant disseminated tumor cells (DTCs). Clinically, late recurrences have been observed after trauma, surgery, and aging, suggesting that changes in the microenvironment of dormant DTCs in conjunction with intrinsic factors (e.g., genetics, subtype) lead to reactivation and thereby cancer recurrence. While progress has been made in understanding this complex process, challenges remain in the detection, prevention, and treatment of late recurrences, leading to low survival rates. To enable studies for addressing these needs, in vitro human 3D culture models of breast cancer cell (BCC) dormancy have been developed. However, their broad use is limited by issues with their accessibility, throughput, and variance. Innovative technologies are needed to address several of these challenges and produce well-defined, accessible, high-throughput (HT) human models for mechanistic studies and identifying effective therapeutics to prevent late recurrence.
To address this need, our recent work established a tunable synthetic dynamic three-dimensional culture system for the indirect co-culture of ER+ BCCs with BM niche cells and identified the important role of soluble secreted factors in the dormancy and reactivation of DTCs, benchmarked versus a preclinical animal model. We now are translating this system to a HT human 3D co-culture model utilizing bioprinting technology, specifically the Inventia RASTRUM. Bringing bioprinting into the research framework brings forth three pivotal concepts. With the RASTRUM, we can create biologically-relevant 3D cell culture architectures inspired by the native microenvironment while providing rigor and reproducibility. The bioprinting further provides opportunities for HT cultures that enable screening of therapeutics and accessible methods for increasing model complexity with the integration of a range of BC and niche cells. We have successfully established an innovative dual and triple matrix co-culture systems using the RASTRUM and reproduced our existing ER+ BCC 3D model for the identification of pathways that regulate dormancy and reactivation and relevant drugs targeting these pathways for preventing late recurrence.
HIGHLIGHTS:

Established HT 3D co-culture models of breast cancer dormancy with increasing complexity for evaluating therapeutics to prevent late cancer recurrence.
Identified treatment strategies for modulating cancer cell dormancy/activation and preventing survival and re-activation of dormant BCCs in vitro and in vivo.

P2-02-13: Metformin prevents tumor cell growth and invasion of human hormone receptor positive breast cancer (HR+BC) cells via FOXA1 inhibition

SeungBaek Lee, Dawa Jung, Val Lowe, Tuba Kendi

Women with type 2 diabetes (T2D) have a higher risk of being diagnosed with breast cancer and have worse survival if they do develop breast cancer, than non-diabetic women. However, more research is needed to elucidate the biological underpinnings of these relationships. Here, we found that Forkhead Box A1 (FOXA1) and metformin (1,1-dimethylbiguanide hydrochloride), a medication used to treat T2D, may impact hormone receptor-positive (HR+) breast cancer (BC) tumor cell growth and metastasis. Indeed, fourteen diabetes-related genes are highly expressed in three HR+ breast cancer cell lines but not in other breast cancer subtypes using a 53,805 gene database obtained from NCBI GEO. Among the diabetes-related genes, FOXA1, MTA3, PAK4, FGFR3, and KIF22 were highly expressed in HR+ breast cancer from 4,032 breast cancer patient tissue samples using the Breast Cancer Gene Expression Omnibus. Specifically, the high expression of FOXA1 correlated to a worse overall survival for patients with ER+/PR+ breast cancer. Consistent with this, the loss of FOXA1 inhibited tumor proliferation and invasion of MCF-7 and T47D HR+ breast cancer cell lines in vitro. Metformin conspicuously inhibits the tumor cell growth in MCF-7 human hormonal breast cancer cells. Metformin or FOXA1 deletion enhanced tamoxifen-mediated tumor growth inhibition in HR+ breast cancer cell lines through the ex vivo three-dimensional (3D) organoid model. Therefore, metformin and FOXA1 inhibition might be a new treatment for patients with HR+ breast cancer when combined with tamoxifen, an endocrine therapy.

P2-02-14: C1QL1 inhibits breast cancer through HSP90α/VCP-ERS/UPR axis

Ningning Zhang, Qing shao, Tingxiu Xiang, Xiaohua Zeng

Our earlier research discovered that C1QL1 was expressed less in breast cancer (BrCa) tissues than in normal breast tissues by analyzing the gene profile of RNA sequences. However, up to now, the biological function of C1QL1 and its molecular mechanism in BrCa remains unknow. Public database analysis, qRT-PCR, western blot, immunohistochemistry, and quantitative methylation specific PCR were used to analyze C1QL1 expression and promoter methylation. The effects of C1QL1 on breast cancer proliferation, cell cycle, apoptosis, metastasis, were assessed using CCK8, flow cytometry analysis, TUNEL assays, transwell in vitro and nude mice experiments in vivo. LC-MS/MS, CoIP and western blot were performed to identify factors that mediate effects of C1QL1. In BrCa, C1QL1 is often silenced due to promoter methylation, and its expression is favorably connected with prognosis. Overexpression of C1QL1 inhibits BrCa cell proliferation, metastasis and promotes cancer cell apoptosis both in vitro and in vivo. Conversely, C1QL1 knockdown increases the proliferation and spread of BrCa cells. Mechanistically, C1QL1 is located at

endoplasmic reticulum (ER) and interacts with HSP90α and VCP to facilitate their ubiquitin-mediated degradation. This leads to the caspase-dependent apoptosis that occurs in breast cancer cells as a result of ER stress (ERS)/unfolded protein response (UPR). Our results support that C1QL1 can act as a tumor suppressor of BrCa by modulating C1QL1/HSP90α/VCP-ERS/UPR pathway, implying that the promoter methylation status of C1QL1 or the expression of C1QL1 may represent a potential marker for the diagnosis or prognosis of BrCa.

P2-02-15: Engineered macrophages secreting TRAIL achieve immunotherapy for triple-negative breast cancer by inhibiting the Wnt/β-catenin signaling pathway

Chuangui Song

Macrophages play a critical role in the body's defense against cancer by phagocytosing tumor cells, presenting antigens, and activating adaptive T cells. However, macrophages are intrinsically incapable of delivering targeted cancer immunotherapies. Engineered adoptive cell therapy introduces new targeting and antitumor capabilities by modifying macrophages to enhance the innate immune response of cells and improve clinical efficacy. TNF-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily that can either induce cell death or activate survival pathways after binding to death receptors (DRs) DR4 or DR5. In this study, we constructed engineered macrophages secreting mono-TRAIL and tri-TRAIL via viral transfection. The results showed that both types of TRAIL-secreting engineered macrophages could significantly kill triple-negative breast cancer (TNBC) cells and inhibit the proliferation of TNBC cells. The tri-TRAIL-secreting engineered macrophages (Tri-TRAIL-M) exhibited a more significant tumor-suppressive effect compared to the mono-TRAIL-secreting engineered macrophages (Mono-TRAIL-M). Additionally, the study results indicated that TRAIL-secreting engineered macrophages induced the M1 phenotype in macrophages by upregulating DR4 and downregulating DR5 expression. TRAIL, upon entering TNBC cells, could inhibit the tumor cell cycle by suppressing the Wnt/β-catenin signaling pathway. In conclusion, the tri-TRAIL-secreting engineered macrophages provide a novel strategy for constructing engineered macrophages for the immunotherapy of triple-negative breast cancer.

P2-02-16: Therapeutic Decision-Making in Breast Cancer: The Contribution of Artificial Intelligence

Vita Baldassara Leonardi, Fabio Aiello, Antonella Ussett, Giovanni Sortino, Salvatore Mirlocca, Marina Campione

Background: There is a growing effort to explore the benefits and risks associated with the application of large language models (LLMs) across various domains, including personalized medicine, clinical decision-making, and healthcare. This study aims to evaluate

the accuracy and interpretability of responses provided by artificial intelligence (AI) models, specifically in the context of metastatic breast cancer. By integrating advanced AI systems, our goal is to assess their potential impact on real-life patient care and their applicability in clinical practice. Our system integrates several cutting-edge components, including a GPT-4-based reasoning engine, a vector-based semantic search engine (QDrant), a specialized web search engine for PubMed (filtered for clinical studies), and utilizes the ReAct prompt paradigm. The Retrieval-Augmented Generation (RAG) facilitated the integration of the latest guidelines from the Italian Association of Medical Oncology (AIOM) and NCCN into QDrant.
Methods: A total of 43 patients with metastatic breast cancer treated at ARNAS Civico Hospital in Palermo, Italy, were randomly selected from clinical practice and included in the study. The median age was 68 years, with 58% of patients being older than 65 years. Comorbidities were present in 65% of patients, including cardiac problems (40%), hypercholesterolemia (14%), hepatitis (18%), and diabetes (17%). At diagnosis, 41% had metastatic cancer. Tumor types included luminal A (36%), luminal B HER2-negative (57%), and HER2-positive (8%). The AI model analyzed the patients' medical records to make therapeutic recommendations. The accuracy of these recommendations was assessed by comparing them to the AIOM guidelines. Three physicians independently reviewed and compared the AI model's therapeutic recommendations against the AIOM guidelines, rating the explainability of the model's responses on a scale from 1 (poor) to 5 (excellent).
Results: For 81% of patients, questions concerned first-line metastatic treatment, while 18% were in later lines of therapy. The concordance rate with AIOM guidelines was 91%, and the overall explainability score given by the physicians was 4.7. LLMs enabled the provision of concise summaries of patients' electronic medical records, generating comprehensive synopses of the patient's current condition by summarizing their therapeutic journey. The model made considerations regarding the patient's comorbidities, issuing alerts about therapy continuation for patients with critical test values. Notably, the AI model considered the patient's clinical course, adjusted treatment based on comorbidities, changed therapy at disease progression, and provided recommendations for complementary (e.g., radiotherapy) and supportive care.
Conclusion: The AI model demonstrates high concordance with clinical guidelines and provides explainable, comprehensive treatment recommendations that account for patient comorbidities and disease progression. These findings suggest a potential role for AI in supporting oncological treatment decisions in metastatic breast cancer.

P2-02-17: Race and outcomes in HR+/HER2- breast cancer: how tissue morphology and molecular pathways contribute to worse outcomes in Black women

Daniel Cook, Zach Cacini, John A. Cole, Kimberly A. Kelly, Yara Abdou

Background: Black women with breast cancer (BC) have a 40% higher relative risk of BC death than White women. Previous analyses from the RxPonder study have shown that

differences in tumor biology, particularly higher proliferation axis scores in tumors from Black women, may contribute to these poorer outcomes (Abdou et al. ASCO24). However, a comprehensive understanding of the underlying tumor biology necessitates further investigation. To this end, we conducted an in-depth analysis of tissue morphology and molecular pathways in Black and White women with early-stage hormone receptor-positive (HR+) BC, aiming to elucidate the biological factors driving these disparities.

Methods: We used the SIMBIOSYS PhenoScope Discovery software suite to investigate (HR+) BCs using paired MRIs and gene expression data from the ISPY-2 trial (N = 309 patients, 272 White and 37 Black). PhenoScope Discovery uses an AI-based algorithm to automatically segment tumors and surrounding tissues from MRIs and calculates morphological features associated with tumor biology. These morphological features, including features describing the tumor size, shape, and vascularity are then integrated via a machine learning model into a composite score called the the TumorSight Risk score, which has been shown to be prognostic of event-free survival (EFS) in early-stage breast cancer. Additionally, PhenoScope Discovery uses genome-scale metabolic modeling to simulate tumor metabolic pathway usage and dependency from gene expression data. We integrated the multi-scale outputs of PhenoScope Discovery to compare tumor morphology, simulated metabolism, and risk of recurrence amongst Black and White patients.

Results: We used the TumorSight Risk score to stratify patients based on their tumor morphology characteristics into recurrence high-risk and low-risk groups. We found Black women with (HR+) BC are more likely to have high-risk tumors than White women with (HR+) BC – 43% of tumors in Black women were high-risk; compared to only 29% of tumors in White women. Furthermore, integrating results from our metabolic modeling, we found that high-risk tumors were more likely to be dependent on mitochondrial fatty acid metabolism, suggesting a mechanistic connection between recurrence risk and breast adiposity.

We also directly compared tumor metabolism simulated from our metabolic model between Black and White women. We found that the tumors in Black women had a higher proliferation rate than tumors in White women ($p = 0.001$), indicating more aggressive tumors in Black women. This increased proliferation rate was driven by an increase in Warburg metabolism (characterized by higher glucose uptake, more use of the glycolysis pathway, and higher lactate secretion in tumors in Black women).

Conclusions: In this cohort of patients with (HR+) BC, tumors in Black women had a higher proliferation rate and a higher likelihood of being classified as high-risk of recurrence (using TumorSight Risk). Additional analyses of cellular signaling, tumor vascularity, and tumor perfusion are underway to try to further understand these disparities.

P2-02-18: Agreement Across 10 Artificial Intelligence Models in Assessing HER2 in Breast Cancer Whole Slide Images: Findings from the Friends of Cancer Research Digital PATH Project

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Recent successes of HER2 antibody-drug conjugates (ADCs) have expanded patient eligibility for HER2-targeted therapy; therefore, accurate and consistent identification of patients who may benefit from ADCs is more critical than ever. Previous studies of agreement between pathologists highlight areas of discordance, but little is known about the reproducibility of assessments by emerging artificial intelligence (AI) models, particularly at low levels of HER2 expression. These models have the potential to deliver more quantitative and reproducible HER2 assessments than visual scoring by pathologists, but large-scale comparative evaluations to understand their variability are lacking.
Friends of Cancer Research created a research partnership to describe and evaluate the agreement of HER2 biomarker assessment across independently developed AI models. Both H&E and HER2 IHC whole-slide images (WSIs, N=1,124) from 733 patients diagnosed with breast cancer in 2021 were obtained from a single laboratory (ZAS Hospital, Antwerp, Belgium). Available pathology and specimen data include three pathologists' HER2 readings and details on slide processing and digitization. Ten AI models assessed HER2 status on all cases. Blinded, independent analyses were performed by statisticians from the National Cancer Institute.
Of the 10 AI models, seven used HER2 IHC WSIs, two used H&E WSIs, and one used both stains as inputs to determine HER2 score and/or status. The primary analysis focused on the seven models (6 using IHC, 1 using IHC and H&E) providing HER2 scores based on the ASCO/CAP 2018 categories (0, 1+, 2+, 3+). Absent a defined reference standard, agreement was evaluated for all possible pairings of models across all samples, resulting in a median (interquartile range, IQR) pairwise overall percent agreement (OPA) of 65.1% (60.3-69.1%) and unweighted Cohen's kappa of 0.51 (0.45-0.55). When defining binary HER2 scores as 3+ vs. not 3+, the median (IQR) pairwise agreement measures were: OPA 97.3% (95.9-97.9%), average positive agreement (APA) 87.3% (84.1-90.9%), average negative agreement (ANA) 98.5% (97.7-98.8%), and kappa 0.86 (0.82-0.90). Conversely, when defining HER2 scores as 0 vs. not 0, the median (IQR) pairwise measures were: OPA 85.6% (82.4-88.0%), APA 91.3% (87.4-92.6%), ANA 65.2% (59.9-69.7%), and kappa 0.57 (0.51-0.61). Ongoing analyses aim to assess the association of between-model agreement with patient, specimen, and model characteristics as well as the agreement between models and pathologist readings.
These findings highlight variability in HER2 biomarker scoring across models, with the least

variability and a higher level of agreement in reporting 3+ cases and larger inter-model variations in evaluating HER2 low tumors, similar to agreement measures between pathologists observed in published studies. Further work is needed to understand the variability in ascribing lower HER2 scores and to evaluate performance in the context of clinical application, especially given the evolving treatment landscape and clinical implications of HER2 scores. This ongoing research partnership will enable a greater understanding of the variability in AI models and support best practices for using these models for measuring and reporting AI driven biomarker assessments in drug development and clinical practice. This dataset also has potential value for creating reference sets for future model development.

P2-02-19: Virtual Multiplex Immunofluorescence Identifies Lymphocyte Subsets Predictive of Response to Neoadjuvant Therapy

Anran Li, Madeleine Torcasso, Anna Woodard, Hanna Hieromnimon, James Dolezal, Rebecca Abraham, Marcus R. Clark, Yuanyuan Zha, Alexander T. Pearson, Frederick M. Howard

Background: Multiplex immunofluorescence (mIF) captures spatial relationships within the tumor microenvironment and predicts response to therapy. However, cost and complexity limit real-world clinical application. We evaluated generative artificial intelligence approaches to recreate virtual mIF images from routine hematoxylin and eosin (H&E) stained pathology.
Methods: Core-needle biopsies from a cohort of triple-negative breast cancer underwent staining and mIF imaging. H&E and a composite mIF image for DAPI, pan-CK, CD3, CD4, CD8, and CD20 were aligned. We designed a pipeline, multiplex Synthetic Immunofluorescence Generated through H&E Translation (mSIGHT), which integrates a trainable registration network to overcome alignment issues in pairs of H&E and mIF images. Pairs of image tiles were extracted to train the mSIGHT pipeline, and the results were compared to Pix2Pix and CycleGAN image translation approaches. The mean squared error (MSE) between the six image channels was compared across generators using a paired t-test in the validation cohort, and Frechet Inception Distance (FID) was also compared. Cells were segmented and clustered into tumor cells, B-cells, CD4+ T-cells, and CD8+ T-cells. The accuracy of cell classification was measured using Spearman's correlation coefficient between real and generated classifications, as well as the area under the receiver operating characteristic curve (AUROC) for the prediction of real cell density in the top tertile of images from the validation set. The association of predicted immune cell subsets with pathologic complete response (pCR) was evaluated using an adjusted logistic regression model in a retrospective cohort of patients receiving neoadjuvant therapy, controlling for hormone receptor status and grade.
Results: Fifteen core needle biopsies yielding 418 image tiles were used for training, and two biopsies yielding 160 images were used for validation of image generation and cell classification. The mSIGHT pipeline (MSE 0.11, 95% CI 0.10 - 0.12, FID 125.7) outperformed Pix2Pix (MSE 0.31, 95% CI 0.28 - 0.33, $p < 0.001$ vs mSIGHT; FID 322.0) and CycleGAN (MSE

0.14, 95% CI 0.13 - 0.15, p = 0.019 vs mSIGHT; FID 167.0) in average pixel level accuracy across all channels. Generated images provided significant correlations for all immune cell subtypes, although accuracy ranged from Spearman rho 0.49 (95% CI 0.37 – 0.60), AUROC 0.77 (95% CI 0.69 – 0.85) for CD4+ T-cells to Spearman rho 0.20 (95% CI 0.05 – 0.35), AUROC 0.61 (95% CI 0.51 – 0.71) for CD8+ T-cells. In a cohort of 310 cases treated with neoadjuvant therapy with digital H&E images available, the predicted density of CD8+ T-cells was significantly associated with pCR (adjusted odds ratio [aOR] 1.34, 95% CI 1.16 – 1.62), whereas among spatial metrics derived from virtual mIF, proximity of CD4+ T-cells within 20 microns of B-cells was negatively associated with pCR (aOR 0.63, 95% CI 0.44 – 0.91).
Conclusions: The mSIGHT pipeline may be used to gain insight into clinically relevant spatial immune features directly from H&E images. Compared to other image translation pipelines, mSIGHT demonstrated superior predictive accuracy for identification of immune cell subsets. Additional study of this approach using larger datasets is ongoing.

P2-02-20: Prediction of Axillary Lymph Node Metastasis in Breast Cancer using Intraoperative Fluorescence Multi-modal Imaging

He Sun, Xiaobo Zhu, Jiaqian Li, Zhenyu Liu, Yu An, Jie Tian

Axillary lymph node (ALN) status serves as a crucial prognostic indicator in breast cancer (BC). Currently, sentinel lymph node biopsy (SLNB) and axillary lymph node dissection (ALND) are the standard methods for predicting and evaluating ALN status; however, intraoperative frozen section and pathological analysis are both time-consuming and laborious, and there is a certain false-positive rate. Therefore, there is a need for a method that can rapidly and accurately assess the status of ALN metastasis (ALNM) during surgery. To address the above clinical needs, this study combined near-infrared fluorescence imaging technology during BC surgery with advanced deep learning strategies to build a predictive model called "Multi-modal Fluorescence Imaging Feature Fusion Prediction" (MFI-FFP). For the multi-modal imaging data obtained during BC surgery, including white light imaging (WLI), near-infrared fluorescence imaging (FI), and pseudo-color imaging (PCI), we selectively customized feature extraction networks for each imaging modality to fully explore and utilized the complementary information between different modalities. We designed a multi-modal feature fusion module that effectively integrates features extracted from each modality by combining global and local information. Additionally, due to the common issues of class imbalance and classification challenges in real-world clinical datasets, we also designed a novel loss function to enhance the recognition of minority class instances. Through the above design, MFI-FFP can quickly and accurately determine the positive or negative of ALNM in surgery, reducing diagnostic time and costs, and ultimately improving patient outcomes.
The BC lymph node image database used in this study was derived from intraoperative fluorescence videos of 93 patients. During the surgical procedure, 312 excised lymph nodes were obtained from these 93 patients. with 35 lymph nodes testing positive for metastasis.

The ratio of negative samples to positive samples was approximately 8:1. During data preprocessing, key frames of each lymph node in the videos were captured and extracted as intraoperative fluorescence images. Each captured frame contained the three modes of data which include WLI, FI, and PCI. A total of 936 imaging data were captured, with 312 images corresponding to each mode.
Experimental evaluations demonstrated that the MFI-FFP model achieved remarkable performance in predicting ALNM, with an area under the ROC curve (AUC) of 0.7512 and an accuracy (ACC) of 0.8617, significantly outperforming single-modality models (WLI: AUC 0.5810; FI: AUC 0.5226; PCI: AUC 0.5601) and dual-modality models (WLI+FI: AUC 0.6786; FI+PCI: AUC 0.6089; WLI+PCI: AUC 0.6571). Given the absence of dedicated multimodal models for intraoperative fluorescence data from BC lymph nodes, we concatenated multi-modal features and input them into models that excel in natural image classification tasks, including resnet18, swin_transformer, convNeXt, efficientnet_v2, mobilenet_v3. Experimental results indicated that our model significantly outperforms the best-performing model in natural image classification, MobileNet_v3, with an AUC value of 0.6595.
This study not only validated the precision and reliability of the MFI-FFP model in predicting the status of ALNM in BC but also highlighted its substantial potential in assisting clinical decision-making and enabling real-time diagnosis of ALNM.

P2-02-21: Predicting HER2-targeting antibody-drug conjugate dosing regimens and quantifying immune system contributions to efficacy using agent-based computational modeling

Melissa Calopiz, Greg M. Thurber, Jennifer J. Linderman

Antibody-drug conjugates (ADCs) have proven to be successful in the clinic with 12 FDA approvals, including 7 approved within the last 5 years. ADCs combine a targeting monoclonal antibody backbone conjugated with a cytotoxic payload via a specific linker. Every component of an ADC must be designed and optimized for a particular target to ensure efficacy. The complexity of ADC design makes it challenging to study experimentally, as variability across experimental models, target expression, and heterogeneity make design and optimization both time-consuming and expensive. Computational modeling can be advantageous in ADC design and testing due to the ability to simulate a large range of ADC features to narrow down the optimal properties, and here we use our validated computational model, SimADC, to evaluate the efficacy of several ADCs. Specifically, we study trastuzumab, a HER2-targeting antibody used in 2 FDA-approved ADCs, conjugated to several types of payloads in different environments including ADC-induced immune responses.
SimADC captures heterogeneous ADC distribution, individual cell responses, and ADC efficacy, allowing us to study a wide variety of conditions and parameters, and generate/test new hypotheses efficiently. In this work, we first simulate different ADCs with a trastuzumab backbone to study the impact of a carrier dose (co-administering the ADC

with its unconjugated antibody backbone) on ADC intratumoral distribution. Studies have shown that ADCs tend to localize around perivascular areas but leave other areas of the tumor untreated, thereby reducing efficacy. Adding a carrier dose creates partial competition with the ADC for binding sites, which can improve distribution and therefore efficacy. For the ADCs tested, it was determined that co-dosing the ADC with enough carrier to saturate all cell receptors in the tumor results in the best possible efficacy.
Next, we used SimADC to study the impact of and immune response during ADC therapy. Data support a role for the immune system in ADC efficacy, although the relative importance and mechanisms operating between ADCs and immune cells are currently unclear. We build upon SimADC by incorporating the immune system, specifically CD8+ T cells and macrophages, to quantify the contribution of immune cells when dosing Enhertu (trastuzumab deruxtecan) in low-expressing systems. Enhertu is known to be efficacious in these low-expressing systems, but the role of the immune system versus payload cell killing is unclear. Using SimADC, we can set bounds on the HER2 expression level needed (receptors/cell) to attribute efficacy to payload cell killing versus the need for an immune response. The relative role of payload release from macrophages and systemic uptake of free payload is also included. Understanding the relationship between ADCs and the immune system can allow for the design of treatment regimens that target immune cell activation to aid in tumor treatment instead of relying on high doses of drug, resulting in less drug toxicity for the patient.

P2-02-22: BCM PDX Insights Portal: An Intuitive Web-based Tool for Patient-Derived Xenograft Collection Management to Facilitate Pre-clinical Studies in Breast Cancer

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Objective: Mouse Patient-Derived Xenograft (PDX) models are essential tools for evaluating experimental therapeutics. Baylor College of Medicine (BCM) established a PDX Core to provide technical support and infrastructure for PDX-based research. To manage PDX collections effectively, de-identified patient clinical and omics data, as well as PDX-related information and omics data, must be curated and stored. Data must then be analyzed and visualized for each case. To enhance PDX collection management and data dissemination, the BCM Biomedical Informatics Core created the BCM PDX Insights portal (https://pdxportal.research.bcm.edu/).
Materials and Methods: Patient clinical data are abstracted from medical records for each PDX and stored in a central database. Annotations are reviewed by a clinician and de-identified. PDX development method and biomarker expression are annotated. DNAseq, RNAseq, and proteomics data are processed through standardized pipelines and stored. PDX gene expression (mRNA/protein), copy number alterations, and mutations can be

searched in combination with clinical markers to identify models potentially useful as a PDX cohort.
Results: PDX collection management and PDX selection of models for drug evaluation are facilitated using the PDX Portal. Users identify models for studies by querying DNA mutation, copy number variations and gene expression. Model selection is further narrowed by viewing the patient clinical timeline, indicating staging, disease progression and response to treatments. A large breast cancer collection of 300 PDX models is accessible in the portal from multiple institutions including BCM, MD Anderson, and Huntsman Cancer Institute. Models from this collection with the assistance of data collected in the portal have been utilized to execute drug sensitivity studies with breast cancer avatars presenting specific molecular signatures and patient resistance to first line therapies resulting in evidence for alternative treatments.
Discussion: Successful preclinical studies provide critical evidence for well-designed clinical trials. To closely simulate real world conditions in preclinical PDX studies, methods that capture and display multiple features of the patient clinical and molecular data are needed which operate at scale. Selection of models for studies should be representative of the patient cohort from which they originated while also being standardized to allow for aggregation of models from various sources to achieve an adequate representation of uncommon cancer subtypes. At BCM, our PDX core and collaborators across Texas have found value in using the PDX Insights portal to manage, standardize, query, aggregate and disseminate PDX annotations.
Conclusion: The BCM PDX Insights portal is a highly effective PDX collection management tool allowing data access in a visual, intuitive manner which has been shown to be effective in facilitating breast cancer preclinical studies.

P2-02-24: Evaluating ChatGPT as an educational resource for patients with Breast cancer: A preliminary investigation

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Introduction: Breast cancer (BC) is the most commonly diagnosed cancer among women in the United States, representing about 30% of new cases annually. Patients with BC encounter various challenges, such as understanding the disease, exploring treatment options, managing prognosis, coping with side effects, and accessing supportive care. Educating patients is crucial for empowering them to make informed healthcare decisions. With the evolution of artificial intelligence (AI), there is potential to leverage these technologies for patient education. ChatGPT, an AI-based language model, is a promising avenue in this regard. While ChatGPT has been applied across diverse domains, its potential in medicine is currently under exploration. This study aims to evaluate ChatGPT's effectiveness as an educational tool for BC patients, assessing its accuracy and safety in delivering medical information.
Methods: We designed a comprehensive questionnaire with 22 questions covering various

aspects of breast cancer—from diagnosis to treatment options and prognosis. This questionnaire served as the prompt for our study utilizing OpenAI's ChatGPT version 3.5.0 to generate responses. The accuracy of these responses was meticulously evaluated by nine Breast Medical Oncologists (BMOs): four from Roswell Park Comprehensive Cancer Center, four from Ohio State University, and one from the Medical College of Wisconsin. Each expert independently assessed the responses provided by ChatGPT and categorized them as accurate, inaccurate, or harmful.
Results: We found that sixteen of these questions (16/22) 73% received unanimous agreement from all BMOs for accuracy. Only one question (1/22) 4% was deemed harmful, and five questions (5/22) 23% graded as inaccurate by some of BMOs due to insufficient or misleading information. The response to questions that received criticism were “Are there any alternative or complementary therapies that may help with breast cancer?” it was flagged harmful by (2/9) 22% BMO and inaccurate by (2/9) 22%. Another question, asking about the different types of breast cancer, was graded inaccurate by (6/9) 67% of experts as it was missing a few breast cancer types. Additionally, (3/9) 33% of BMOs found the response regarding dietary recommendations for breast cancer patients inaccurate, highlighting the lack of evidence supporting dietary interventions in metastatic breast cancer. The advice on preserving hair during chemotherapy was rated as inaccurate by (3/9) 33% of experts due to concerns that some of the recommendations, such as maintaining a good diet and using a mild shampoo, do not effectively prevent chemotherapy-induced alopecia and could potentially raise false hopes among patients. Similarly, explanations about HER2-positive breast cancer and its prognosis were labeled as inaccurate by (2/9) 22% of BMOs due to misleading statements about recurrence rates. Lastly, (3/9) 33% of experts criticized the discussion on potential side effects of treatment modalities for omitting important information, such as cardiovascular toxicity associated with chemotherapy.
There are some limitations to our study, such as small sample size, subjective evaluation criteria, and rapid evolution of ChatGPT and other large language models.
Conclusion: While ChatGPT shows potential as an educational resource for BC patients with 73% of answers graded accurate by all BMOs, it is essential to recognize its limitations and the indispensable role of human medical expertise. These findings underscore the variability in accuracy and appropriateness of AI-generated responses in medical contexts, highlighting the importance of refining and validating AI tools for patient education and information dissemination. A few of the safety considerations are the use of complex medical terminology in responses and, the dissemination of sensitive information without empathy and emotional support.

P2-02-25: Predicting the response of triple negative breast cancer to neoadjuvant systemic therapy via biology-based modeling and habitat analysis

Casey Stowers, Chengyue Wu, Zhan Xu, Clinton Yam, Jong Bum Son, Jingfei Ma, Gaiane M. Rauch, Thomas E. Yankeelov

Introduction: Neoadjuvant systemic therapy (NAT) is the standard of care for the treatment of locally advanced triple-negative breast cancer (TNBC) [1]. Unfortunately, only 50-65% of TNBC patients who receive NAT attain a pathological complete response [2]. Our goal is to improve methods for predicting treatment response early during NAT to allow for better patient outcomes. Biology-based mathematical models calibrated to magnetic resonance imaging (MRI) data have proven accurate in predicting NAT response, but they require hundreds of locally calibrated parameters to achieve highly accurate predictions of voxel-wise tumor cellularity [3]. Fitting so many parameters is computationally expensive and may suffer from overfitting of the data. Here, we combine biology-based mathematical models with habitat analysis to dramatically reduce the number of parameters to be calibrated within our biology-based model, and then test the accuracy of the subsequent predictions.

Methods: Data acquisition – This study used 138 patients from the ARTEMIS trail (NCT02276443) who received Adriamycin/Cytoxan (A/C) followed by Paclitaxel. Each patient received MRI pre- (V1), post two cycles (V2), and post four cycles A/C (V3).

Defining habitats –We applied habitat analysis on the apparent diffusion coefficient (ADC) map obtained from diffusion weighted MRI and the positive enhancement integral (PEI) map obtained from dynamic contrast enhanced MRI. To do so, we constructed a vector of ADC and PEI at V1 and V2 for every tumor bearing voxel in every patient. We then pooled these vectors across the cohort and applied k-means clustering to identify habitats.

Mathematical model - Our biology-based mathematical model captures the spatio-development of tumor cellularity at each voxel location within the breast as the sum of tumor cell diffusion (controlled by a fixed diffusivity), proliferation (controlled by a calibrated proliferation rate), and death due to NAT (controlled by a calibrated drug efficacy rate). These parameters can be calibrated globally (one value per tumor) or locally (one value per tumor voxel). We calibrated the models to V1 and V2 data, after which the calibrated model is used to predict tumor status at V3.

Habitat-informed model calibration – Habitats allow us to calibrate one drug efficacy value per habitat, as opposed to one value per tumor voxel. We first identified and calibrated for two habitats, and then gradually increased the number of habitats up to 16. We report preliminary results for three models, all of which calibrated for a global proliferation rate, but differ in how they handled drug efficacy: (A) global drug efficacy, (B) an efficacy value for each of ten habitats, and (C) local drug efficacy.

Results: To evaluate the quality of the model calibrations we compare the median (interquartile range) of the absolute difference between the modeled and measured percent change in total tumor cellularity (%ΔTTC) across the cohort for each calibration. When calibrating from V1 to V2, we obtained %ΔTTC's of 19 (5.0-25) %, 12 (7.7-15) %, and 2.0 (0.42-4.1) % for models A, B, and C, respectively. We repeat the analysis for predicting

%ΔTTC at V3 and obtained 6.5 (0.90-20) %, 5.5 (2.8-12) %, and 15 (5.8-23) %, for models A, B, and C, respectively.

Summary and Discussion: The accuracy of the calibrated V1 to V2 %ΔTTC increased as we increased the number of calibrated drug efficacy parameters due to the larger number of free parameters. However, the accuracy of models A and B for predicting %ΔTTC at V3 is superior to model C, which is also more computationally expensive. The more accurate and efficient models could support personalized treatment optimization, in which the calibrated model is used to predict patient response to a variety of treatment schedules.

References
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P2-02-26: Development of AI diagnosis of immunohistochemistry for ER and HER2 in breast cancer.

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Background: Breast cancer is the most common cancer among women worldwide, and subtype estimation by immunohistochemistry (IHC) is essential in the process of treatment decision making. In recent years, the importance of basic IHC diagnosis has further increased, as multigene assays have been used to predict the efficacy and prognosis of chemotherapy in hormone receptor-positive human epidermal growth factor receptor 2 (HER2) -negative breast cancer and HER2 low diagnosis is used to determine the indication for T-DXd. On the other hand, however, the effort required for routine IHC diagnosis is enormous and complicated, and there is a need to reduce effort in clinical practice. Therefore, we developed an automated diagnosis system using artificial intelligence (AI) for IHC of estrogen receptor (ER) and HER2, which is necessary to determine the treatment strategy for breast cancer. Methods: We retrospectively extracted 236 IHC preparate of ER and HER2 of breast cancer in Akita University Hospital consecutively. Each pathology image was divided into patches, the data was expanded for each patch, the expanded patches were trained by Convolutional Neural Network (CNN), and Train, Validation, and Test were performed to calculate sensitivity, specificity, positive diagnostic rate, and AUC for ER with more than 50% and less than 50% occupancy and for HER2 Sensitivity, specificity, positive diagnostic rate, and AUC were calculated for four classes based on the HER2 score for HER2; for HER2, training and evaluation were also performed using open data. In addition, the time required to diagnose one image was examined. Results: In ER, based on 3536 training data and 884 test data, sensitivity was 96%, specificity was 93%, accuracy was 95%, and AUC was 0.99 for less than 50% of ER expression and 0.99 for more than 50% of ER expression, based on 3272 training data and 818 test data, sensitivity was 82%, specificity was 87%, accuracy was 71%, and AUC was 0.79 for HER2 score 0,1, 0.80 for HER2 score 2,

and 0.89 for HER2 score 2. Diagnostic accuracy was slightly lower in the low score situation. On the other hand, in the open data study (data: score 0;65, score 1;60, score 2;101, score 3;113), the accuracy was 94.2% when scores were divided into 4 classes. It took less than one second to diagnose one image. Conclusions: In this study, we have developed an AI diagnosis system that speedily assists pathologists in diagnosing IHC. In breast cancer treatment, IHC diagnosis of ER and HER2 is indispensable for treatment decision making, and rapid and precise diagnosis is desired. On the other hand, it is desirable to reduce the potential labor required to perform complicated IHC diagnosis of ER and HER2 in a large number of breast cancer patients. The present AI system for IHC diagnosis of ER and HER2 showed a certain high accuracy as a numerical value, although no similar study has been reported in the past as far as we could find. Although discrepancies in the diagnosis of HER2 between pathologists have been a problem, more reproducible diagnosis is needed to determine the indication of T-DXd for HER2 low. In this study, high accuracy was obtained by AI diagnosis based on open data. The results of further additional analyses will also be reported. Furthermore, we are currently marketing an in vitro diagnostic device system that enables IHC in a short time with reproducibility. As a future prospect, we are developing a system to enable faster and more accurate diagnostic support in combination with this AI diagnosis.

P2-02-27: Automating Breast Cancer Relapse Identification in Pathology Reports Using Natural Language Processing

Jaimie Lee, Andres Zepeda, Gregory Arbour, Kathryn Isaac, Raymond Ng, Alan Nichol

Background: Relapse is a major concern for breast cancer survivors and oncologists. However, population-wide cancer registries fall short in systematic relapse monitoring due to logistical challenges and prohibitive costs. Recent advancements in Natural Language Processing (NLP) have created new opportunities to address these limitations. Hence, we investigated Bidirectional Encoder Representations from Transformers (BERT), a state-of-the-art NLP language model, to automate relapse identification in the text of pathology reports. Methods: Follow-up pathology reports from breast cancer patients diagnosed between January 1, 2005, and December 31, 2014, were included. These reports were gathered within a 120-day window of a known relapse finding. The presence or absence of local, regional, and distant breast cancer relapses were annotated by trained staff. We selected BlueBERT as our base model, a transformer pre-trained on PubMed abstracts and MIMIC-III clinical notes that is commonly used for medical text classification. To fine-tune our BlueBERT models, pathology reports were divided into training (80%), validation (10%), and test (10%) sets. Model performance was evaluated using accuracy, sensitivity, specificity and respective 95% confidence intervals [CI]. Results: The study cohort comprised 1,375 breast cancer patients with a median age at breast cancer diagnosis of 58 years (range 23-94 years). A total of 1,896 pathology reports were included from these patients, with 673 (35.5%) describing local relapses, 295 (15.6%) regional relapses, and 643 (33.9%) distant relapses. The local-relapse model achieved 93.1% [91.8-94.4] accuracy,

91.3% [89.0-93.6] sensitivity, and 94.2% [92.7-95.7] specificity. The regional-relapse model showed 91.4% [90.0-92.8] accuracy, 81.9% [77.0-86.8] sensitivity, and 93.2% [91.8-94.6] specificity. Finally, the distant-relapse model demonstrated 90.6% [89.1-92.1] accuracy, 82.8% [79.3-86.3] sensitivity, and 94.1% [92.7-95.5] specificity. Conclusion: We used BlueBERT-based NLP models to identify breast cancer relapse in pathology reports. These models demonstrated excellent performance across all relapse types. When applied retrospectively, the automation of breast cancer relapse identification has the potential to enrich cancer registries with information on patient outcomes. When used prospectively, this technology holds the potential to enhance patient care by facilitating timely relapse identification.

P2-02-28: Challenges and Opportunities in Early Diagnosis of Breast Cancer: Utilizing Clinical Decision Support Platforms for Improved Detection

Bea Bakshi, Seema Dadhania, Brian Herrick, Edwin Lee, Greg Joondeph-Breidbart, Miles Payling

Introduction: Breast cancer is the most common cancer diagnosed worldwide. Despite a widely adopted screening program in most countries, many patients are missed and present symptomatically. In the US, symptomatic breast cancer patients are reported to represent 30-40% of patients with breast cancer. However, while the percentage between screen-detected cancer and symptomatic breast cancer has been improving over the last three decades, evidence suggests the total number of patients diagnosed with symptomatic breast cancer has increased by over two thirds during this period. In addition, the number of patients with late stage disease has not significantly fallen. This therefore highlights that early detection of symptomatic breast cancer remains a critical challenge in healthcare in order to optimize outcomes.
Utilizing the extensive data available in Electronic Medical Records (EMRs) offers a valuable opportunity to pinpoint high-risk patients with symptomatic breast cancer for early intervention. In this study, we investigate the effectiveness of utilizing the C the Signs AI Prediction Model to identify patients at risk of breast cancer and explore the time to diagnosis relative to primary care physician detection.
Methods: A retrospective analysis was conducted on 900,601 patients within the Mayo Clinic Data Platform between 1st January 2002 and 31st December 2021. 19,879 individual patients were diagnosed with breast cancer during this interval. We utilized the C the Signs AI Prediction Model to match relevant factors from the EMR record (e.g. breast lump, nipple changes etc) and calculate if a patient was at risk of breast cancer according to the model's algorithms.
A sensitivity analysis was performed to evaluate the platform's effectiveness in early detection. In addition, we examined the timing of breast cancer diagnosis relative to primary care physician detection to assess the potential for earlier identification. Staging data for patients with breast cancer diagnosed was not available so could not be assessed.

Results: The sensitivity of the C the Signs platform for detecting breast cancer was 76.8%, with a specificity of 79.5%. The positive predictive value was 7.8% and the negative predictive value was 99.3%. Remarkably, 20.7% of patients with breast cancer were diagnosed up to 5 years before their diagnosis by primary care physicians. Of interest, with the sensitivity being at 76.8%, the vast majority of patients with breast cancer within the Mayo Clinic Data Platform were symptomatic at presentation which is at odds with previously reported screening detected cancer proportions (which the C the Signs AI Prediction Model would not have been able detect in this configuration).
Conclusion: Our findings establish the potential of AI Prediction Models, such as C the Signs, to address the challenge of identifying patients with symptomatic breast cancer at the earliest stages of the disease. By leveraging EMR data and advanced algorithms, these platforms can enhance the identification of at-risk patients and enable earlier intervention. The evidence of this study may also suggest that the proportion of asymptomatic patients who are currently detected through mammography alone, may be much smaller than previously assumed (<30% compared to the currently assumed 60-70%). Further research and integration of such platforms into clinical practice are warranted to realize their full potential in improving breast cancer diagnosis and confirm stage shift.

P2-02-29: AI-based Clinical Decision Support System (CDSS) for predicting response to primary systemic therapy in early breast cancer: multimodal modeling and pathology contribution.

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$ The BreaCS Consortium is a collaborative research effort between four Belgian EUSOMA-certified hospitals and Endare as external partner.

Background: Accurate prediction of tumor response to primary systemic therapy (PST) in early breast cancer is crucial for optimizing treatment decisions. The Breacs consortium developed an AI-based CDSS that integrates digital pathology data and pre-operative clinical variables to predict pathological complete response (pCR) in early breast cancer patients. Deciphering the factors on which the model relies to make these predictions may provide valuable new insights.

Material and methods: A multimodal predictive model was developed using data from the BreaCS consortium, encompassing a cohort of 550 patients who underwent PST.
The dataset included digital pathology data from biopsies and more than 100 pre-operative clinical variables from the standardised EUSOMA database.
The data was split into a training and a test sets (80-20 stratified split on patient level).
Model evaluation metrics included the area under the operator curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Quantitative cell segmentation analysis was conducted on the test set to identify histopathological features influencing the model's predictions. Cell type fractions were compared across 4 different groups (true positives, false positives, false negatives, true negatives) within the confusion matrix.

Results: The unimodal model based on pathology data achieved an AUC of 0.70 (training) and 0.81 (test). The model using only EUSOMA clinical data scored an AUC of 0.88 (training) and 0.82 (test). The multimodal model, incorporating both pathology and clinical data, demonstrated superior performance with an AUC of 0.91 (training) and 0.84 (test).
The multimodal model demonstrated a significant improvement in sensitivity, NPV and PPV compared to the unimodal models, with specificity remaining relatively unchanged.
The test set confusion matrix showed 72 true negatives (TN), 11 false negatives (FN), 14 true positives (TP), and 9 false positives (FP).
The cell segmentation in the group of the TP (predicted and obtained complete remission) shows an excess in the fraction of tumor cells compared to the TN. The TN (non-responders) on the other hand have an excess in the fraction of connective tissue compared to the TP.

Conclusions: The AI-based CDSS demonstrates promising predictive capabilities for response to PST in early breast cancer, with the multimodal approach showing enhanced predictive accuracy. This study underscores the importance of integrating multimodal data in predictive models.
The observed differences in cellular composition between TP and TN reveals distinct cellular compositions and may suggest potential refinements for improving model accuracy.

P2-03-01: Sexual health in unpartnered women after breast cancer: Report from a joint analysis of two international multi-site prospective cohorts of young breast cancer survivors

Sharon Bober, Eleonora Pagan, Monica Ruggeri, Shari Gelber, Vincenzo Bagnardi, Ann Partridge, Olivia Pagani, Shoshana Rosenberg, Karin Ribi

Objectives: Sexual dysfunction is a distressing potential side effect of breast cancer (BC) treatment. However, little is known about the experience of unpartnered young women whose concerns may differ from partnered peers. Helping Ourselves, Helping Others: The Young Women's Breast Cancer Study (YWS-HOHO) investigates gaps in knowledge about young BC survivors in 2 multi-site prospective cohorts. Methods: The North American (USA, Canada) and European (Italy, Switzerland) YWS-HOHO cohorts jointly enrolled 1602 women. Participants were ≤40 yrs. and diagnosed with stage 0-IV BC <6 months before enrollment. Serial surveys were completed every 6 months for the first 3 yrs. and annually thereafter. Surveys included assessment of body image, sexual health, menopausal symptoms (using the CARES and BCPT scales, score range: 0-4, with higher scores indicating greater severity) and menopausal concerns. Unpartnered and partnered women

were compared at baseline and 2-yrs. post-enrollment using T-tests. Results: A total of 1342 women were included in this analysis (1054 from N.A., 288 from Europe). The median age at enrollment was 37 yrs. (IQR 33-39), 81% of women had stage 0-2 disease. Most women (n = 1000, 74.5%) had received chemotherapy, 63.8% (n = 856) had undergone mastectomy. At baseline, 13% of women were unpartnered (n=180). Unpartnered young women reported significantly higher body image concerns than partnered peers at baseline (mean CARES body image scale 1.28 vs 1.09, p-value=0.04) but not at 2-yrs (1.17 vs 1.08, p-value=0.35). Concern about general sexual function was comparable for unpartnered and partnered women at baseline (1.97 vs 1.81, p-value=0.11) and at 2-yrs (1.43 vs 1.42, p-value=0.92). For example, >47% of both unpartnered and partnered women endorsed a loss of sexual interest at baseline and >36% of both groups also at 2-yrs. Unpartnered women reported fewer vaginal symptoms (e.g., vaginal dryness, pain with intercourse) than partnered women at both timepoints (mean BCPT vaginal scale at baseline 0.53 vs 0.83, p-value<0.001; at 2-yrs 0.74 vs 1.01, p-value=0.002), while there were no differences in hot flashes and urinary symptoms at either timepoint. Greater than 50% of both unpartnered and partnered women reported concern about becoming post-menopausal at both timepoints. Approximately half of unpartnered women reported difficulty with dating at both timepoints, including difficulty with initiating contact with potential partners and difficulty with discloser about their cancer experience. Conclusion and Implications: Unpartnered young BC survivors report similar levels of sexual dysfunction as partnered peers as well as distinct concerns about body image and dating. These findings underscore the need to routinely address sexual health regardless of partner status and to better investigate specific concerns of unpartnered women such as dating and relationship-building.

P2-03-02: Fertility and ovarian function preservation in young women with breast cancer: A comparative analysis of two prospective cohort studies in Mexico and Italy

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Background: Due to their age and life stage, young women with breast cancer (YWBC) encounter distinct challenges related to their diagnosis and treatment. Unmet parity along with potential premature ovarian insufficiency represent a notable concern among this group of patients. Effective strategies to address this issue include fertility preservation techniques, as well as temporary ovarian suppression with gonadotropin-releasing hormone agonists (GnRHa) for ovarian protection during cytotoxic treatments.
Here we report a comparative analysis of two prospective studies describing the rates and factors associated with fertility and ovarian function preservation choices in YWBC from two countries.

Methods: Women with BC at an age ≤40 years were prospectively accrued in the Joven & Fuerte and PREFER multicenter cohorts in Mexico and Italy, respectively. These studies provide early referrals to fertility and/or ovarian function preservation strategies at diagnosis as needed. This analysis comprised YWBC diagnosed from 2014-2019 whose treatment included chemotherapy (CT) with the objective of identifying their uptake of fertility and ovarian function preservation options. Patients' characteristics within and across cohorts were compared using chi-squared, Fisher's exact, and Wilcoxon tests. Simple logistic regression was conducted to calculate the likelihood of undergoing fertility preservation.

Results:In total, 485 patients were included: 361 (74%) in Mexico and 124 (26%) in Italy. Median age at diagnosis was 35 years (IQR 32-38) in both cohorts. A higher proportion of Mexican patients had a partner compared to Italian patients (65% vs 59%, p=.04). Patients' median number of children at diagnosis was also higher in Mexico than in Italy (1 [IQR 0-2] vs 2 [IQR 1-3], p<.001). Patients' distribution by clinical stage (p<.001), timing of CT (p=.01), hormone receptor status (p=.04), and HER2 status (p=.002) also differed between cohorts: Mexican patients were more frequently diagnosed with stage III BC and received neoadjuvant CT, while Italian patients more commonly had positive hormone receptors or HER2 overexpression.

Regarding fertility preservation, a technique was used in 8% and 25% of patients in Mexico and Italy, respectively (p<.001). Methods comprised oocyte (50% in Mexico vs 87% in Italy), embryo (53% vs 0%; this strategy is forbidden by law in Italy), and ovarian tissue cryopreservation (0% vs 16%). GnRHa for ovarian protection were used in nearly all Italian patients (98%) but in a minority of Mexican patients (6%).

Not undergoing fertility preservation was mainly due to lack of interest (47-50%), urgency to start treatment (4-6%), and personal reasons (3-5%). Fertility preservation uptake was associated with younger age (OR 1.2, 95%CI 1.1-1.2), unpartnered status (OR 3.4, 95%CI 1.9-5.9), childlessness (OR 21.8, 95%CI 10.0-47.6), private healthcare coverage in Mexico (OR 3.0, 95%CI 1.1-8.1), stage I-II BC (OR 3.1, 95%CI 1.5-6.3), positive hormone receptors (OR 2.3, 95%CI 1.2-4.6), and adjuvant CT (OR 2.4, 95%CI 1.4-4.3).

Conclusions: This comparative analysis of two prospective studies in Mexico and Italy showed that a significant distinct proportion of YWBC had access to the available fertility and ovarian function preservation techniques in these countries, possibly reflecting the different social and healthcare contexts.

Although all women should receive a complete oncofertility counseling, patients who are younger, unpartnered, childless, and have earlier-stage BC appear to be those who particularly benefit from being offered fertility preservation options. This study underscores the need to enhance awareness and access to oncofertility services in order to provide comprehensive, patient-centered care to this young population.

P2-03-03: Aromatase inhibitor (AI) use in a phase I, single arm, prospective study to evaluate the treatment of genitourinary syndrome of menopause (GSM) with platelet rich plasma (PRP) in women with a history of breast cancer.

Anita Chen, Johnny Yi, Emanuel Trabuco, Jacqueline Thielen, Jeffrey Cornella, Shane Shapiro, Reagan Dukes, Jennifer Arthurs, Michael Heckman, Sophia Blumenfeld, Saranya Chumsri

Background: GSM is a constellation of symptoms and signs associated with a decrease in estrogen involving changes to the external genitalia, vagina, urethra, and bladder. The syndrome may include dryness, burning, and irritation of the vagina and vulva. Sexual dysfunction may be caused by lack of lubrication, discomfort or pain, and impaired function. Urinary symptoms of urgency, dysuria, and recurrent urinary tract infections are also commonly described with GSM.

Breast cancer is the most common cancer among women worldwide, and GSM is an extremely challenging, unique issue for breast cancer survivors especially those taking AI where the goal is to lower estrogen levels. It is estimated that more than 2 million US survivors of breast cancer are affected by GSM, the majority receiving no treatment. There is overwhelming evidence to support the use of vaginal estrogen to treat GSM, but many patients with breast cancer and their providers are hesitant due to the concern of exogenous hormones. In addition, these painful symptoms may result in discontinuation of adjuvant endocrine therapy (ET). GSM symptoms typically do not resolve without treatment and may worsen with time.

PRP is theorized to induce angiogenesis and restore the effects of growth factors. It has been used for various conditions including GSM.

Objective: To assess the effect of AI treatment on breast cancer survivors who underwent injection of autologous PRP to the vaginal canal for treatment of GSM.
Design: Breast cancer survivors (stage 0-III) who reported GSM symptoms of vaginal dryness with or without dyspareunia underwent a single treatment of autologous PRP injected throughout the vaginal canal and posterior fourchette. The primary outcome was to assess the safety and feasibility of the treatment. Secondary outcomes included the efficacy of treatment. Vaginal atrophy was measured by vaginal maturation index (VMI) and vaginal health index (VHI), vaginal and vulvar symptoms with assessment scales (VAS/VuAS), quality of life with the day-to-day impact of vaginal aging questionnaire (DIVA), sexual function with the female sexual function index (FSFI), and urinary symptoms with the urogenital distress index (UDI-6). VMI was obtained at baseline and 6 months. All other measures were obtained at baseline, 1, 3, and 6 months. Patient global impression of improvement (PGI-I) was assessed with a 7- point Likert scale.
Results: Mean age was 53 years (range 40-66) and body mass index was 27.2 kg/m2 (range 20-36.6). All completed the planned injection protocol with a mean visual analogue scale

(VAS) pain score of 3.9(range1-7). Of the 20 participants, 11 were on endocrine therapy with an aromatase inhibitor. There was no statistical difference between the AI group and no AI group in regards to safety, feasibility, or efficacy. Interestingly, 81.8 % in the AI group noted “much better” and “very much better” on PGI-I versus 66.6% in the no AI group. All patients remained compliant with adjuvant ET.
Conclusion: Adjuvant ET is proven to reduce the risk of recurrence and mortality in patients with hormone receptor positive breast cancer; however up to 20% of patients discontinue treatment prematurely due to gynecologic side effects. In this phase I trial sub-analysis of participants on AI, we found that PRP treatment significantly improved GSM symptoms, sexual function, urinary symptoms, and quality of life in breast cancer survivors regardless of AI status. There was a trend towards more AI participants reporting improvement of symptoms. All participants demonstrated adherence to AI treatment, a key component to improved disease-free survival. Future randomized controlled Phase 2 trials are warranted to further validate these promising findings.

P2-03-04: Assessing and Addressing the Distinct Psychosocial Needs of Young Women with Breast Cancer

Shari Goldfarb, Alanna Jamner, Cassandra Chang, Padmapriya Subramanian, Jeannie Englehardt, Morgan Moy, Mary Gemignani

Background/Objective: Although young women under 45 represent only 11% of newly diagnosed breast cancer patients annually in the US, their physical and psychosocial experiences are distinct. Diagnosis often raises concerns about fertility, family planning, sexual function, body image, acceptance by partner(s) and family, and career trajectory. In 2023, MSKCC launched the Young Women with Breast Cancer Program (YWBCP) to provide streamlined comprehensive care, support, education, and research opportunities tailored for women 45 and younger.
Methods: The YWBCP administered an online 35-question patient-reported intake survey to women 45 and younger with newly diagnosed breast cancer. It assesses psychological status, fertility and family history, sexual health, and body image before treatment. All women registered at MSKCC for new appointments received the option to participate. Quantitative analysis was performed.
Results: From 5/2023- 5/2024, 72.2% (600/820) of women with stage 0-IV breast cancer ages 19-45 completed the YWBCP Intake Survey on MSK Engage within six weeks of their initial visit. 78.2% (469/600) remained at MSKCC for primary treatment. The mean and median age of participants was 38.7 and 40 years, respectively.
51% (305/595) of women reported experiencing high levels (very much or quite a bit) of anxiety in the previous 28 days. 58% (171/298) reported being very much or quite a bit worried about how cancer will affect their friends/family and 52% (76/180) were very much or quite a bit worried about discussing cancer with friends/family. 46% (71/155) were very much or quite a bit worried about trouble with school or work and 43% (48/110) were very much or quite a bit worried about trouble parenting. 41% (38/92)

were very or quite a bit worried about relationships and 58% (98/169) about money problems. 22% (130/586) reported wanting to speak to a social worker about how to discuss their diagnosis with their children, parents, and friends. Patients reported preference for individual therapy 34%(206/600), support groups 32% (189/600), support for family 30% (177/600), recreational programs 19% (112/600), peer-to-peer programs 18% (109/600), and other mental health resources 17% (103/600).
37% (224/581) of women were very or quite a bit concerned that their cancer would affect their ability to be physically intimate. Regarding fertility, 17% (100/595) already protected future fertility, 27%(159/595) had not yet, but wanted to and 56% (336/595) were not interested. 41% (245/600) wanted more info about having children in the future.
Conclusion: The YWBCP tailors its services to meet the needs reported by newly diagnosed breast cancer patients ages 45 and younger. Expert social work and educational services were developed to address the needs of these women given their significant anxiety and distress. Likewise, reproductive and sexual health counseling are key components of our program. The YWBCP will continue to develop programs and services to help address the concerns of these young women. Further analysis of the six-month and one-year surveys will be crucial in identifying programmatic and service gaps and guide the development of evidence-based interventions.

P2-03-05: AI-Powered Breast Cancer Survivorship Support: A Comparative Analysis of ChatGPT and Gemini in Providing Evidence-Based Lifestyle Guidance

Jasmin Hundal, Asfand Yar Cheema, Amna Zaheer, Mishaal Munir, Baidehi Maiti

Background: Breast cancer survivors are a growing population in the United States, with an increasing number of women facing challenges in navigating the complexities of post-treatment care. However, the transition to survivorship is often challenging, as many women struggle to adhere to recommended lifestyle guidelines, which are crucial for long-term health and well-being. As highlighted in a recent survey study of cancer survivorship programs accredited by the American College of Surgeons Commission on Cancer (CoC), there are significant gaps in the provision of certain services, particularly those related to sexual health and fertility1. Additionally, low patient awareness and lack of referrals remain barriers to accessing available resources. Integrating innovative solutions like large language models (LLMs) into breast cancer care could address these challenges and empower survivors to actively participate in their health and wellness journeys. This study aims to evaluate the potential of AI-powered chatbots, specifically ChatGPT and Gemini, to provide personalized, evidence-based guidance on exercise, diet, and weight management, consequently improving long-term health outcomes for breast cancer survivors.
Methods: This study utilized the Exercise, Diet, and Weight Management During Cancer Treatment guidelines from the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) to formulate 22 questions focused on preventive health, physical activity, nutrition, and weight management for cancer survivors.

These questions were posed to ChatGPT-4 and Gemini. Responses were evaluated independently by 2 physicians who graded each response on 5 criteria: factual accuracy, relevance, completeness, clarity, and coherence, using a scale from 1 (poor) to 5 (excellent). Grading was analyzed and compared to determine their alignment with ASCO and NCCN guidelines.
Results: ChatGPT demonstrated high performance in factual accuracy, with an average score of 4.52/5. Gemini exhibited a lower average score of 4.38 but achieved 75% of responses rated 4 or higher. In terms of relevance, ChatGPT maintained an average score of 4.43/5. Gemini performed well in relevance, with an average score of 4.29. For completeness, ChatGPTachieved an average score of 4.38/5. Gemini showed slightly higher performance in this criterion, with an average score of 4.48/5. Both models excelled in clarity, each attaining an average score of 4.57/5, with high ratings and minimal ambiguity in their responses. For coherence, both ChatGPT and Gemini demonstrated logical structuring, with average scores of 4.33/5.
Conclusion: The findings highlight the potential of integrating LLMs into oncology for survivorship care. Both models demonstrated robust performance across all criteria. ChatGPT excelled in factual accuracy and relevance, while Gemini showed slightly better completeness. Both models achieved high clarity and coherence scores, indicating their ability to provide clear, comprehensive, and logically structured responses. This integration can significantly enhance adherence to survivorship guidelines, offering personalized, real-time support that improves patient education, risk communication, behavior modification, and systematic follow-up. Continued development and refinement of these models, with a focus on addressing specific needs and concerns of breast cancer survivors, could revolutionize survivorship care, leading to improved adherence to guidelines, better quality of life, and, improved long-term outcomes.
Reference:
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P2-03-06: Factors Associated with Pregnancy and Postpartum Treatment After Endocrine Therapy Interruption in Breast Cancer Patients

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Background: We previously retrospectively analyzed the long-term prognosis of patients who interrupted endocrine therapy in an attempt to achieve pregnancy after breast cancer treatment. We reported that there was no significant difference in the risk of breast cancer related events between the endocrine therapy interruption and continuation groups (at 110 months postoperatively were 25.7% in the continuation group vs. 20.0% in the interruption group, $p = 0.5$). The study also reported that 58% of the patients in the interruption group became pregnant (14.6% in the continuation group, $p<0.05$), indicating that interruption of

endocrine therapy is significantly increases pregnancy rates. In this study, we aimed to examine factors that predispose to pregnancy after interruption of endocrine therapy and postpartum breast cancer treatment.

Methods: This was a retrospective study using surveying of medical chart data between June 2007 and November 2015 at St. Luke's International Hospital. Patients with stage I-III ER-positive breast cancer who visited a reproductive center before starting treatment for breast cancer and interrupted endocrine therapy for less than 120 months were included in this study. Patients who attempted to conceive after discontinuing endocrine therapy were divided into two groups: those who became pregnant and those who did not. The factors associated with achieving pregnancy and postpartum treatment were compared between the two groups. Statistical analysis was performed using the chi-square test and t-test.[Result] Patients who interrupted treatment within 120 months of endocrine therapy and became pregnant were 20/33 patients (60.1%). The median age was 35 years in the pregnancy group and 40 years in the non-pregnancy group. In the pregnancy group, 18 patients (90%) were 39 years old or younger, and in non-pregnancy group, 7 patients (53.8%) were 39 years old or younger ($p<0.05$). The number of patients who were married or had a partner was 16 (80%) in the pregnancy group and 11 (85%) in the non-pregnancy group. Chemotherapy was administered in 9 (45%) of the pregnancy group and 6 (46.2%) of the non-pregnancy group and GnRHa for fertility purpose during chemotherapy was used for four patients (4/9, 44.4%) in the pregnancy group and two patients (2/6, 33%) in the non-pregnancy group. Fertility procedures included duplicate cases: 17 cases preserved embryos (85%) and 5 preserved oocytes (25%) in the pregnancy group, 8 cases preserved embryos (61.5%), 3 preserved oocytes (23.1%), 2 preserved ovaries (15.4%) and 1 (7.7%) could not be frozen in the non- pregnancy group. Of the 20 cases in the pregnancy group, 18 had delivered and 20 children were born. (Two cases gave birth twice.) and two were currently in the process of conceiving. The median time from interruption of endocrine therapy to delivery was 23 months (median); 21 months for those under 35 years of age and 27 months for those 35-40 years of age. Endocrine therapy was resumed after delivery in 9 cases (9/20, 45%), with a median of 8 months (0-19 months) between delivery and resumption of endocrine therapy. The 11 cases who did not resume endocrine therapy after childbirth included 6 cases who had completed 5 years of endocrine therapy, 2 cases who did not resume endocrine therapy because their hormone receptors were originally weakly positive, and 3 cases for unknown reasons.

Discussion and Conclusion:Pregnancy after interruption of endocrine therapy was significantly associated with age <35 years at the time of fertility preservation. This age factor was not related to other factors. While many patients resumed endocrine therapy after delivery, there were instances of non-resumption, highlighting the need for clearer guides and communication regarding postpartum treatment decisions. The findings can inform clinical practice and patient counseling regarding fertility preservation and treatment decisions.

P2-03-07: Neoadjuvant (Z)-endoxifen for Premenopausal Estrogen Receptor (ER)+, Human Epidermal Growth Factor Receptor 2 (HER2)- Breast Cancer (BC): Evaluation of Quality of Life (QOL) measures in the EVANGELINE Study

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Background: Premenopausal women (PrW) with ER+, HER2- breast cancer (BC) are commonly treated with aromatase inhibitors (AI) with ovarian function suppression (OFS) or tamoxifen (tam) +/- OFS. Common adverse effects (AE) include musculoskeletal symptoms, hot flashes, night sweats, mood changes, and sexual side effects that may seriously impact quality of life (QOL). In SOFT, compared to tam alone, pts treated with tam + OFS had more hot flashes, loss of sexual interest, sleep disturbances, and vaginal dryness. In the combined SOFT/TEXT analysis, pts receiving exemestane + OFS had greater bone/joint pain, vaginal dryness, and loss of sexual interest, whereas pts receiving tam + OFS had more hot flushes and sweats. In the premenopausal setting, E1/E2 levels differ based on type of endocrine therapy regimen, with menopausal E1/E2 levels following OFS, but increases with tam monotherapy. EVANGELINE (NCT05607004) is an ongoing phase 2 multicenter neoadjuvant study assessing (Z)-endoxifen (ENDX), a potent tam metabolite that dually targets ERα and PKCβ, in PrW with ER+/HER2- BC. Prior to the randomized phase II, a PK run-in was designed to identify a dose wherein 28-day ENDX Css > 500 ng/ml in ≥ 5/6 pts and to assess antitumor activity and toxicity when ENDX is dosed at 40 mg/day (monotherapy) and 80 mg/day (+/- OFS). Pts with endocrine sensitive disease (ESD) defined as 4-week Ki67 ≤ 10% continue treatment (tx) for 24 weeks followed by surgery. Here, we report the menopausal symptoms for pts enrolled onto 40 mg/day cohort. Methods: All eligible pts who completed baseline and at least one tx questionnaire or toxicity evaluation are included. Data captured by the medical team using Common Terminology Criteria for Adverse Events (CTCAE) and self-reported symptoms are reported in pts menstrual diary or on the Menopause-Specific Quality of Life (MENQOL) questionnaire administered at baseline, end of week 4, 12, and 24. MENQOL bothersomeness level was categorized as mild (score 0-1), moderate (2-4), and high (5-6). Estradiol and estrone levels were assessed for the 40 mg/day dose at week 4 and 24. Results: For the 40 mg/day cohort, 7 PrW (6 White, 1 Asian) aged 28-51 (median 46) received ENDX. One pt discontinued due to week 4 Ki-67 > 10%. The remaining 6 had ESD, and after 24 weeks underwent surgery. Over the course of tx, relevant AEs reported as possibly, probably or definitely attributed to ENDX included: amenorrhea (G2-n=5); dysmenorrhea (G2-n=1; G1-n=1), hot flashes (G2-n=1; G1-n=5), hyperhidrosis (G1-n=1), hypomenorrhea (G1-n=1), irregular menstruation (G1-n=2), and libido decrease (G2-n=1). Menses did not occur on tx for 4 pts. No dose reductions occurred. Among the remaining 3

pts, dysmenorrhea was described as mild/moderate. MENQOL after 4 wks of tx revealed that hot flashes developed in 4 pts (moderately bothersome -3 pts, not bothersome -1 pt). One pt began venlafaxine for hot flashes. Three pts reported 'being impatient with others' as moderately bothersome. The median (range) baseline estrone (n=5) was 54 pg/mL (19-114) with median (range) fold increase from baseline of 9.0 (1.3-23.2 pg/mL) at wk4 and 4.7 (0.4 - 25.9 pg/mL) at wk24. The median baseline estradiol level (n=5) was 29 pg/mL (19-209) with median fold increases from baseline of 17.9 (0.4-57.0 pg/mL) at wk4 and 8.1 (0.04 - 56.6 ng/mL) at wk24. Currently, 12 pts are enrolled to either 80 mg/day monotherapy or 80 mg/day + OFS.
Conclusions: This is the first report of menopausal side effects in PrW women treated with ENDX 40 mg/day without OFS. Most side effects were low grade and amenorrhea was common. ENDX induced marked increases in E1/E2 levels that peaked at 4 weeks and then declined. MENQOL data from the 80 mg/day monotherapy and 80 mg/day + OFS cohort will be reported at the meeting.

P2-03-08: Influence of Work Schedule Flexibility on Nutrition and Physical Activity Among Employed Breast Cancer Survivors

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Introduction: Healthful dietary and physical activity behaviors are essential for breast cancer survivors after treatment to enhance recovery and perception of physical well-being. These interventions are critical because they can reduce fatigue, improve cardiorespiratory fitness, strength, health-related quality of life, and reduce morbidity and mortality among breast cancer survivors. Prior studies suggest that survivorship care plans should incorporate physical activity, as even moderate activity can be vital for improving health-related quality of life. However, it is unknown for those in the workforce if employment-related stressors affect compliance and/or self-perception of overall health. This is the first study to evaluate these factors among employed breast cancer survivors.
Methods: We analyzed cross-sectional survey data (self-completed) from 156 employed breast cancer survivors in central Connecticut. Age-adjusted linear regressions were performed using Likert scale questions (5-point or 10-point). Independent variables included adherence to nutrition recommendations (e.g., half of each meal consisting of fruits and vegetables) and physical activity (e.g., strength training twice per week plus other activities to raise heart rate several days per week). The dependent variable was the perception of overall physical health. We conducted stratified analyses based on a dichotomized Likert variable representing the perceived level of control over one's work schedule (Agree/Strongly Agree vs. Disagree/Strongly Disagree, with the central neutral value assigned to Agree). Future analyses are planned to examine overall mental health as the dependent variable using multivariate linear regression and a structural equation model with multiple predictors.
Result: Among the full sample, age-adjusted linear regression revealed a strong positive correlation between adherence to nutrition recommendations and the perception of

physical health (B=0.61, p<0.001). In stratified analyses, we observed that those who reported more control over their work schedule exhibited a slightly stronger relationship compared to those who reported less control (B=0.64, p<0.001; B=0.53, p=0.08, respectively). Similarly, the correlation between adherence to physical activity guidelines and perceived overall physical health was high in the full sample (B=0.61, p<0.001). A notable difference in this association was observed between those who perceived more control over their work schedule compared to those who did not (B=0.72, p<0.001; B=0.32, p=0.18, respectively).
Conclusion: Reduced control over work schedules might significantly affect compliance with healthful behaviors and, concomitant perception of health, particularly for engagement in physical activity. This presents a crucial point of intervention for health care providers. Physicians should recognize and address the impact of work-related stressors on health behaviors to better support their patients in maintaining healthy lifestyles post-treatment. Tailoring survivorship care plans for healthful behaviors that account for work schedules can potentially improve both health outcomes and quality of life in this patient group. Further research is needed to explore the underlying mechanisms and develop interventions to mitigate the negative effects of work-related stress on adherence to healthful behaviors.

P2-03-09: Breast Cancer Surgery and Its Quality-of-Life Outcomes: A Study of São Paulo Public Employees

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Objective: To evaluate the quality of life (QoL) of breast cancer patients' post-surgery, compare conservative, radical, and oncoplastic surgical outcomes, and identify symptoms and treatment-related factors that negatively impact QoL.
MethodsThis observational, cross-sectional, descriptive study was conducted at the Hospital do Servidor Público Estadual de São Paulo (HSPE) from October 2021 to December 2022. We assessed the QoL of female state public servants presenting in situ or non-metastatic invasive breast cancer who underwent surgical treatment and completed the EORTC QLQ-C30 questionnaire. Inclusion criteria encompassed female patients over 18 years, diagnosed with breast cancer, undergoing surgical treatment, employed as public servants in São Paulo, and actively working at diagnosis. Exclusion criteria included refusal to respond to the questionnaire and metastatic breast cancer at the study's outset or progression within six months of diagnosis. Ethical approval was obtained from the HSPE Research Ethics Committee (CAAE 68337823.4.0000.5463), and all participants signed informed consent forms.
Results: The study included 300 patients with a mean age of 56.6 years. Most participants had a partner (55.69%) and higher education (72.15%). The EORTC QLQ-C30 scores

indicated a reasonable overall quality of life, with an average score of 70.6. Physical (76.6), social (87.1), and role functioning (78.3) scored high. Most patients did not experience bed confinement, did not need assistance with daily activities, and were able to maintain their leisure activities. Emotional functioning had the lowest score (65.1), indicating some level of tension, irritability, depression, or worry. On the symptom scale, insomnia (33.3), pain (30.0), and fatigue (28.3) were the most prevalent, while nausea and vomiting (5.7), dyspnea (7.2), and diarrhea (8.0) scored low, suggesting little interference with daily activities. There was some financial difficulty due to the physical condition and treatment (15.6). In the QLQ-BR23, the score for side effects was 18.5, indicating few adverse effects from systemic treatments. Concerns about hair loss (35.9), arm symptoms (25.2), and breast symptoms (22.4) were the most affected. Body image acceptance was good (74.9), indicating that most patients did not feel less attractive or feminine. Sexual function scored high (82.5), but sexual satisfaction was lower (57.4), showing that although sexual activity continued, satisfaction was impaired. Patients who underwent conservative surgery had better overall quality of life (73.2) compared to radical (66.7) and oncoplastic surgery (55.2) ($p=0.033$). Cognitive function was better preserved in conservative surgery (79.5) compared to oncoplastic (56.3) ($p=0.045$). Social function was also better with conservative surgery (90.7) compared to oncoplastic (70.8) and radical (78.3) ($p=0.017$). More advanced clinical staging was negatively correlated with quality of life, especially in role functioning ($p=0.006$), social function ($p=0.004$), and body image ($p=0.002$). Patients with triple-negative tumors reported greater limitations in activities, while those with HER-2 expression had better role functioning scores ($p=0.094$). Neoadjuvant chemotherapy was associated with lower body image satisfaction (60.3 vs. 78.9; $p=0.033$) and greater concern about hair loss (58.3 vs. 30.1; $p=0.014$). Adjuvant radiotherapy was associated with better overall quality of life (73.0) and social function (89.3) scores ($p=0.007$ and $p=0.033$, respectively), as well as lower pain symptoms (8.5 vs. 25.0; $p=0.007$).
Conclusion: Breast cancer patients demonstrated that while the overall quality of life was reasonable, emotional functioning remained a challenge. Conservative surgery yielded better outcomes in quality of life, cognitive, and social functions compared to radical and oncoplastic surgeries. Advanced clinical staging and triple-negative tumors were linked to greater limitations, whereas HER-2 positive patients showed better role functioning. Neoadjuvant chemotherapy negatively impacted body image and increased concern about hair loss, while adjuvant radiotherapy improved overall quality of life and reduced pain. These findings underscore the need for tailored supportive care to address the emotional and specific functional challenges faced by breast cancer patients.

P2-03-10: Functional Disability and Adverse Mental Health Outcomes among Breast Cancer Survivors: A US National Survey Study

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Background: Breast cancer diagnosis and treatment can have a negative impact on patients' mental health and well-being. Disability adds another layer of adverse effects to

survivorship, and cancer survivors with disabilities may bear heavier burdens of mental health symptoms. However, little is known about the relationships between disability and adverse mental health outcomes among breast cancer survivors in the US.
Methods: We analyzed data from the 2022 National Health Interview Survey that used multistage probability sampling to interview US adults. This study was restricted to adults with a breast cancer history. Any disability (yes/no) was defined by the Washington Group Composite Disability Indicator measuring the self-reported level of difficulty in 6 functional domains: vision, hearing, mobility, communication, cognition, and self-care. Ever having anxiety was per self-report (yes/no), and level of severity in the past 2 weeks was assessed using the 7-item Generalized Anxiety Disorder scale (none-minimal, mild, moderate, and severe). Participants self-reported ever having depression (yes/no), and level of severity in the past 2 weeks was assessed using the 8-item Patient Health Questionnaire depression scale (none-minimal, mild, moderate, and severe). We compared weighted proportions using Rao-Scott Chi-squared tests and calculated P-trends using the Cochran-Armitage test. Multivariable weighted logistic regression was used to estimate adjusted odds ratios (AOR). All statistical analyses accounted for complex design and survey weights.
Results: The unweighted sample size was 644, representing a weighted sample of 4,234,520 US breast cancer survivors. The mean age was 68 years; 79.8% were White, followed by 9.7% Black, 6.3% Hispanic, and 3.6% Asian. Overall, 21.2% had any disability, and they were older than those without disabilities (73 vs. 67 years). Black survivors reported a higher proportion of having any disability than White survivors (27.3% [95% CI: 13.9-40.6%] vs. 21.8% [95% CI: 17.8-25.7%]). Of the total, 25.9% (95% CI: 21.8-30.1%) and 21.3% (95% CI: 17.5-25.1%) have ever experienced depression and anxiety disorder, respectively. Survivors with any disability reported a higher percentage of depression than those without (35.3% [95% CI: 25.3-45.2%] vs. 23.5% [19.0-27.9%], P=.023). The proportion of anxiety was higher among survivors with any disability than those without (32.3% [95% CI: 22.5-42.1%] vs. 18.3% [14.3-22.3%], P=.004). After controlling for demographic and socioeconomic factors, survivors with any disability had greater odds of depression than those without (AOR 1.90, 95% CI: 1.08-3.36). The odds of anxiety were much greater among survivors with any disability than those without (AOR 2.47, 95% CI: 1.35-4.50). In addition, compared with survivors without disabilities, those with any disability were more likely to experience mild (25.1% vs. 15.5%), moderate (20.3% vs. 4.1%) or severe (2.7% vs. 0.8%) depression (P-trend<.001). Similarly, survivors with any disability tended to experience mild (19.6% vs. 8.9%), moderate (8.4% vs. 3.4%), or severe (4.1% vs. 2.0%) anxiety than those without (P-trend<.001).
Conclusions: In this US national sample of breast cancer survivors, 1 in 5 had any functional disability, 1 in 4 experienced depression, and 1 in 5 experienced anxiety. Survivors with any disability had a greater likelihood of lifetime depression or anxiety and an increased level of severity of recent mental health symptoms than those without. Early and routine screening for these symptoms in this population is needed. Oncology programs should identify unmet needs, provide appropriate supportive care or services for cancer-related disability, and improve mental health among breast cancer survivors, particularly those with functional disabilities.

P2-03-11: Health-Related Quality of Life in BREAST trial (Brazilian outcome for Metastatic Breast Cancer): clinical outcomes of HR+, HER2+ breast cancer and its relationship with access to healthcare in Brazil

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Introduction: Cyclin-dependent kinase 4 and 6 inhibitors (CDK 4/6i) in combination with endocrine therapies have become standard of care in hormone receptor positive (HR+) and human epidermal growth factor receptor 2 negative (Her2-) advanced/metastatic breast cancer. However, in Brazil, women have limited access to CDK 4/6i, notably in public healthy system. Evaluation of health-related quality of life (HR-QoL) among cancer patients has gained an increasing importance and is now a key determinant of anticancer treatments' value. Several trials show that HR-QoL was generally maintained with CDK4/6i. Objective: This study evaluated HRQoL by EQ-5D questionnaire in the first 12 months follow up in patiens from the BREAST trial, comparing their results in the public and private health care systems. Methods and Materials: BREAST trial is a multicenter, prospective observational study that included 300 patients divided into two groups: the public and the private health system, with the ratio of 1 patient in the private for every 2 patients in the public health system. For evaluating HRQoL, the EQ-5D questionnaire was applied by telephone contact at baseline, 3,6, 12 and 24 months. Results: A total of 300 patients were evaluated, with 199 (66.3%) in the public and 101 (33.7%) in the private service. The mean age was 58 years and 76.2% were postmenopausal. In terms of metastatic sites, 84.7% had non-visceral disease, predominantly with bone as the main site. The use of CDK 4/6i at any point of the treatment was 6% in the public health system versus 90.1% in the private setting. At baseline, the EQ5D score was similar between the groups, with median of 0.69 in public (0.52-0.79) and 0.74 (0.64-1) in private system. When evaluation the burden of symptoms, a significant higher proportion of patients from the public system reported problems in one or more EQ-5D dimensions at baseline, most frequently pain/discomfort (70.3%), followed by anxiety/depression (61%), usual activities (42.1%), mobility (34.9%), and self-care (17.9%). At 12-months follow up, the score remained stable in both groups, with median of 0.69 in public (0.54-0.79) and 0.79 (0.65-1) in private; no deterioration was seen in the private setting, despite the fact that the patients received a more intense treatment. In longitudinal analysis, the changes in domains between baseline and 12-months showed an improvement for about 1/3 of the patients, regardless of the health care system, notably for pain and discomfort, this item, however, more prounounced in patients receiving CDK4/6i. The global burden of symptons remained higher at the patients from the public system. Of note, with a median follow up of 31 months, a difference in overall survival was demonstrated, with a mortality rate of 8.9% in the private sector and 19.6% in the public system (p=0.02). Conclusions: The results of our study highlights the disparity in the treatment of metastatic breast cancer among patients in relation to access to health care, with patients dependent on the public system experiencing higher mortality

rates and worse HRQol regarding the burden of symptons at diagnosis and at 12-months follow up.

P2-03-12: Reducing Stress and Improving Quality of Life for Adult Breast Cancer Patients via delivery of monthly non-medical essential services for up to six months during treatment

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Introduction: Ellie Fund provides essential support services for breast cancer patients to ease the stresses of everyday life, allowing the focus to be on family, recovery and healing. In 2019, Ellie Fund launched an Outcomes Measurement Program to empirically measure the impact of its services on patients' physical, emotional and financial health and show improved quality of life (QOL).
Methods: Patients apply for services via an online application, selecting 2 services such as grocery gift cards, prepared/delivered meals, transport to/from medical appointments, childcare, housekeeping and integrative therapies. Services are delivered monthly, for 3 months for curative patients and 6 months for metastatic (mets) patients. Impact data are gathered via voluntary surveys administered before and after services delivery, and tracking fundamental metrics contributing to patients' health and well being. Surveys are composed of the Perceived Stress Scale (PSS), the most widely used public health tool for measuring personal stress. PSS-14 is used (14 standard questions), and each response is assigned a numeric value which is compiled into a final PSS score. Twelve additional survey questions assess QOL changes using a 4-point Likert Scale.
Survey 1 (S1) is mailed to each patient upon entering the service program to establish a baseline stress level. Survey 2 (S2) is mailed upon services completion. S1 and S2 data are recorded, tabulated and compared to determine changes by metric. For this study, analysis was limited to surveys returned from patients who began services in 2022 or 2023 and returned both surveys. Differences between S1 and S2 were evaluated using the 2-sided Chi-Square test.
Results: In 2022 and 2023, 2061 patients received services. 806 recipients returned S1, and 567 recipients returned S2. Survey questions were evaluated based on a 4-point Likert Scale ranging from "a lot" to "none." The largest changes were: patients feeling "a lot" of emotional stress decreased 10.8% (54.1%→43.3%; S1 n=389, S2 n=284, p=0.006); patients feeling "a lot" of disruption of daily life decreased 7.6% (44.0%→36.4%; S1 n=387, S2 n=286, p=0.048); patients feeling "a lot" of ability to focus on family increased 13.7% (31.5%→45.2%; S1 n=321, S2 n=321, p=0.0003); patients feeling "a lot" of ability to access food increased 18% (55.3%→68.3%; S1 n=324, S2 n=325, p=0.0002); and patients feeling "a lot" of ability to care for themselves increased 9.8% (19.9%→29.7%; S1 n=326, S2 n=320, p=0.0018). All 13 measures showed QOL improvement. On the PSS assessment, patients reporting high perceived stress decreased by 6.2% (56.2%→50.0%; S1 n=333, S2 n=336, p>0.05).

PSS scores from 2019 through present were also analyzed. For both mets and curative patients who returned both surveys, patients reporting high perceived stress decreased 11.2% (59.3%→48.1%; S1 n=754, S2 n=755, p<0.00001). Mets patients reporting high perceived stress decreased 18.4% (62.8%→44.4%; S1 n=129, S2 n=124, p=0.0032), and curative patients reporting high perceived stress decreased 9.8% (58.6%→48.8%; S1 n=625, S2 n=631, p=0.0005).
Conclusion: Ellie Fund's non-medical services reduce stress and improve QOL for breast cancer patients during treatment, which other research shows to positively impact health outcomes. Mets patients experienced greater relief from high perceived stress than did curative patients, suggesting a need for supportive services over a longer time period. Data by race and income level need further examination. The data can also inform service effectiveness and program improvement; e.g., a metric showing surprisingly low impact was "Ability to Get To/From Appointments" which increased just 1.3%, suggesting Ellie Fund's transportation service requires improvement.
Limitations
Study strengths: high survey response rate, meeting a broad need for QOL data for cancer patients. Limitations: sampling bias; not every question is answered per returned survey.

P2-03-13: Quality of life in young Hispanic women with breast cancer: Long-term results from a large prospective cohort

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Background: Young women with breast cancer (YWBC) are a unique group of patients facing diverse challenges, including tumors with aggressive pathological features, intensive treatment strategies, career concerns, and family and motherhood issues. These factors can have a significant impact on their quality of life (QoL). While most studies have focused on postmenopausal women, there is limited information on how QoL evolves in young patients. Here we report longitudinal variations in QoL and the associated predictors in YWBC.
Methods: We included patients aged ≤40 with non-metastatic BC accrued in the Joven & Fuerte cohort. YWBC completed sociodemographic information and the EORTC QLQ-BR23 questionnaire at diagnosis and during four follow-up visits (approximately at month 6 and years 1, 2-3, and 4-5 post-enrollment). We also collected clinical and treatment information. To analyze the main patterns of QoL, we used group-based multivariate trajectory modeling, which identified two groups of patients that we labeled 'good' and 'poor' based on their functional and symptom scores. We used the Chi-squared, Fisher's exact, Kruskal-Wallis tests, and binomial logistic models to explore the relationship between baseline characteristics and trajectory group classification.
Results: In total, 477 women with a median age of 36 years were included in the analysis. The majority had public health insurance (87%) and stages II (49%) or III (39%) BC. Among them, 52% had HR-positive HER2-negative BC, 22% had HER2-positive BC, and 26% had

TNBC. Most patients received anthracycline-based chemotherapy (82%). Up to 62% of YWBC were clustered in the poor trajectory group, while 38% were in the good trajectory group. Both groups had similar sociodemographic, clinical, and treatment characteristics. From baseline to year 5, the mean body image score declined from 92.8 to 89.7 in the good trajectory group and from 75.4 to 64.7 in the poor trajectory group. Sexual functioning scores also deteriorated from baseline to year 5 in the good (29.3 vs. 27.8) and poor trajectory groups (30.6 vs. 25.6). Sexual enjoyment mean scores remained unchanged from baseline values in the good trajectory group (51.4), while they deteriorated at year 5 in the poor trajectory group (51 vs. 37.3). Regarding YWBC's future perspective, the good (56.4 vs. 79) and poor trajectory group (39.3 vs. 57.2) showed improvement in mean values from diagnosis to year 5 of follow-up. Compared to initial scores, the good trajectory group reported better systemic therapy side effects at year 5 (16.4 vs. 14.3), while the poor trajectory group had worse scores (25.7 vs. 27.9). Breast symptoms improved from baseline to year 5 in both the good (16.9 vs. 10.4) and poor trajectory groups (27.3 vs. 14). Compared to baseline values, arm symptoms in the good trajectory group improved at year 5 (8.2 vs. 7.1), whereas they worsened in the poor trajectory group (17.6 vs. 20.1). Regarding being upset by hair loss, the good (36.3 vs. 27) and poor trajectory groups (43.4 vs. 31.2) showed improvement in mean values from baseline to year 5 of follow-up. In a multivariable logistic model, patients with HER2-positive BC (aOR=0.57 95%CI 0.35-0.94; p=0.028) and those with public health insurance (aOR=0.41 95%CI 0.16-0.90; p=0.035) were less likely to belong to the poor trajectory group.
Conclusions: Most of our patients had a poor QoL over time. Key areas that are of concern for YWBC include body image, sexuality, and their outlook on future health. Additionally, oncologists should prioritize using available strategies to minimize treatment-related side effects and enhance patients' QoL. Future studies should identify which therapies impact the QoL in YWBC and establish effective approaches to improving their QoL.

P2-03-14: PATIENT REPORTED OUTCOMES AND LYMPHEDEMA IN NODE-POSITIVE BREAST CANCER IN THE EARLY POSTOPERATIVE PERIOD AFTER NEOADJUVANT CHEMOTHERAPY IN THE PROSPECTIVE NEOSENTITURK-trial MF18-03

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Abstract: The Neosenti-Türk/MF-18-03 is a prospective study that evaluates the outcome and whether breast cancer-related lymphedema (BCRL). patient-reported arm and shoulder morbidity and quality of life are affected by axillary surgery type in patients with node-positive breast cancer who underwent surgery following neoadjuvant chemotherapy (NAC). The present study investigated the risk factors associated with lymphedema, and how

quality of life is affected by axillary surgery type in the early postoperative period.
Methods: Patients were evaluated by the SF-12 quality of life and QUICK-DASH hand, arm, and shoulder range of motion questionnaires. Circumferential tape measurements of the arm width were performed to evaluate the postoperative lymphedema.The volume difference of 10% or more in the operated arm compared to the healthy arm was considered as lymphedema. Patients were treated either with sentinel lymph node biopsy /targeted axillary dissection (SLNB/TAD, n=243) or axillary lymph node dissection (ALND, n=130).
Results: Of 373 patients from 15 centers, breast cancer related lymphedema (BCRL) was detected in 41 (11%) patients at the 12-month follow-up. Treating with SLNB/TAD-alone (5.8% vs 20.8, p<0.001), removal <6 lymph nodes (5.6% vs 18.1, p<0.001), or cT1-2 (8.5% vs 19.1%, p=0.005), were found as significant factors associated with decreased lymphedema risk. Even though decreased lymphedema has been observed in those aged <50 (8.6% vs 14.9%, p=0.06), with cN1 (9.8% vs 18.8%, p=0.066) and breast conserving surgery (8.7% vs 13.9%, p= 0.113), these differences did not reach the statistical significance.
In the assessment of SF-12 quality of life questionnaire. patients with SLNB/TAD were more likely to have improved bodily pain scores (35.68±12.37 vs 32.35±14.76, p=0.045) compared to those with ALND. Furthermore, patients with ALNB/TAD were more likely to have a better arm and shoulder function as assessed with the QUICK-DASH questionnaire with decreasing scores at the 12th month (29.22±18.47 vs 34.85±20.35, p=0.011). Moreover. patients with SLNB/TAD were found to have an improved SF-12 bodily pain scores. Even though, there were improvements in role physical and role emotional scores in the SLNB/TAD group compared to the ALND-group, these changes did not reach the statistical significance. However, no significant differences could be found in other parameters including general health, physical functioning, vitality, social functioning and mental health of SF-12 test scores between patients with SLNB or ALND in terms of axillary surgery type.
Conclusion: These findings suggest that a more conservative axillary surgery with omitting axillary dissection and with axillary lymph node excision not more than 6 lymph nodes, are factors associated with decreased risk of BCRL in the present cohort. Patients with ALND were more likely to have impaired arm and shoulder function compared to those without ALND, and presence of lymphedema was found to be associated with decreased quality of life. Therefore, precautions such as early postoperative exercise and early diagnosis of preclinical lymphedema should be considered to prevent the BCRL in the first year after surgery.

P2-03-15: Identifying pre-habilitation targets for the mitigation of long-term side effects of chemotherapy in patients with early breast cancer

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Background: There is a need to identify pre-treatment characteristics of women with early breast cancer that are associated with persistent fatigue or suboptimal health-related quality of life (HRQOL) post-chemotherapy as potential targets for pre-habilitation interventions: Patients and Methods: Ancillary analysis of previously collected data from patients with newly diagnosed Stage I-III breast cancer scheduled to receive chemotherapy with curative intent. The objective was to identify baseline (pre-chemotherapy) variables associated with meaningful deteriorations in fatigue and other measures of HRQOL from pre-treatment to 6 months after chemotherapy completion. Percentages are reported along with unadjusted and adjusted relative risks.
Results: In a sample of 249 women post-chemotherapy, 32% reported worsening fatigue (FACIT-F), 35% worsening Physical Well-Being (PWB), 16% worsening Functional Well-Being (FWB), 8% worsening Emotional Well-Being (EWB), and 30% worsening Social Well-Being (SWB). In multivariable (MV) analysis, variables that were significant in univariate analysis – Black race, high BMI, and baseline poorer EWB – remained significant for worsening post-chemotherapy fatigue (FACIT-F). In MV analysis that included race, education, falls and baseline EWB, Black race and a positive falls history remained significant for worsening PWB. In MV analysis inclusive of race, SPPB and FWB, lower SPPB and FWB remained significant predictors of worsening FWB. In MV analysis that included baseline Mental Health Index-Anxiety, EWB and SWB, a higher SWB and lower EWB remained significant for worsening SWB.
Conclusion: Pre-chemotherapy characteristics in women with early-stage breast cancer that are associated with increased fatigue and reduced HRQOL post-treatment could be used to identify patients who may benefit from pre-habilitation interventions.
Keywords: breast cancer, pre-habilitation, cancer-related fatigue, chemotherapy, long-term toxicity,

P2-03-16: Prevalence of PIK3CA/AKT1/PTEN and other genomic alterations in primary and recurrent tumor tissue: exploratory analysis from the Phase 3 CAPItello-291 clinical trial

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Background: Based on the positive results from the CAPItello-291 trial, capivasertib plus fulvestrant is a treatment option for adult patients with HR+/HER2− locally advanced or metastatic breast cancer who have progressed on an endocrine-based regimen and whose tumors harbor one or more alterations in PIK3CA, AKT1, or PTEN. In CAPItello-291, participants were requested to provide a formalin-fixed, paraffin-embedded tumor sample from the most recently collected tumor tissue for next-generation sequencing (NGS) testing at the stage of either primary or recurrent disease. Overall, 48% of samples with a valid NGS

test result had a PIK3CA/AKT1/PTEN alteration. In this exploratory analysis, we assessed the prevalence of PIK3CA/AKT1/PTEN alterations in tissue samples from primary versus recurrent disease and explored the broader molecular profiles between cohorts.
Methods: A retrospective review of molecular profiles of non-matched primary and recurrent breast cancer samples from CAPItello-291 was performed to assess the prevalence of PIK3CA/AKT1/PTEN alterations in tissue from the primary tumor versus recurrent disease. Based on the tissue requisition forms, samples were classified as primary if the type of tissue provided was reported as 'primary' and had breast/lymph node as the anatomic site, while samples were classified as 'recurrent' if the tissue provided was reported as 'recurrence' and the anatomic site was not breast. Analysis for pathogenic/likely pathogenic genes with alteration prevalence of ≥2% captured by FoundationOne®CDx was carried out by primary/recurrent and PIK3CA/AKT1/PTEN-altered/non-altered status. Statistical analyses were performed using chi-square or Fisher exact test. All analyses were exploratory.
Results: A total of 381/594 (64.1%) of the analyzed samples were defined as primary, 156/594 (26.3%) were defined as recurrent, and 57/594 (9.6%) were defined as unknown/not valid and excluded from further analysis. Prevalence of PIK3CA/AKT1/PTEN alterations was similar in primary and recurrent cohorts, overall (50.7% primary vs 46.2% recurrent, p=0.39) and by gene (PIK3CA: 36.2% primary vs 30.8% recurrent, p=0.27; AKT1: 5.5% primary vs 6.4% recurrent, p=0.84; PTEN: 5.7% primary vs 6.4% recurrent, p=0.94). Compared with primary samples, recurrent samples were enriched for alterations in ESR1 (5% vs 17%, p≤0.001), BRCA2 (5% vs 11%, p=0.02), GATA3 (17% vs 24%, p=0.04), CDKN2A (2% vs 6%, p=0.04), and SMAD4 (1% vs 4%, p=0.04). Notably, ESR1 mutations were enriched at recurrence in both the PIK3CA/AKT1/PTEN-altered and non-altered cohorts, whereas GATA3 alterations were enriched in the PIK3CA/AKT1/PTEN non-altered cohort and CDKN2A alterations in the PIK3CA/AKT1/PTEN-altered cohort at recurrence. When comparing the overall PIK3CA/AKT1/PTEN-altered versus the non-altered cohorts, the altered cohort had more alterations in CDH1, MAP3K1, TBX3, CBFB, and BCL6, whereas the non-altered cohort had more alterations in genes associated with cell cycle, such as GATA3, CCND1, and MYC.
Conclusions: Overall, the prevalence of PIK3CA/AKT1/PTEN alterations was comparable between non-matched tumor tissue samples collected at primary or recurrent disease used for NGS testing in CAPItello-291, in line with their role as oncogenic drivers in HR+/HER2− breast cancer. Consistent with the literature, recurrent tissues were enriched for ESR1, BRCA2, GATA3, CDKN2A, and SMAD4 alterations, indicating a potential role for these gene alterations in driving disease recurrence. The PIK3CA/AKT1/PTEN-altered cohort was enriched for CDH1 mutations while the non-altered cohort was enriched for alterations in cell cycle-related genes previously associated with endocrine and cyclin-dependent kinase 4/6 inhibitor resistance.

P2-03-17: Targeting NR2F2 overcomes multiple forms of endocrine resistance

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Endocrine resistance is frequently encountered in the clinic through a variety of mechanisms such as NF1 loss that induce alternative survival pathways and suppress estrogen responsiveness. While targeting the induced pathways such as the RAS-MAPK pathway can have antitumor effects, it can also incur toxicities. To identify novel and potentially more specific therapeutic vulnerabilities in this context, we performed CRISPR/Cas9 screens in wildtype and NF1 knockout isogenic ER+ models and identified NR2F2, an orphan nuclear receptor, to be essential specifically in NF1 loss cells. Our in vitro and in vivo results revealed that NR2F2 is a critical regulator of the estrogen signaling pathway, largely playing a repressive role. We found that NF1 loss could upregulate NR2F2 expression level in ER+ breast cancer cells through the activation of the MAPK pathway. NR2F2 overexpression by itself conferred endocrine resistance, while genetic knockout and pharmacologic inhibition of NR2F2 sensitized ER+ breast cancer cells to endocrine therapies and attenuated resistance across multiple endocrine refractory tumor models including those driven by NF1 loss, ARID1A loss, and PTEN loss. These results reveal upregulation of NR2F2 to be a recurrent mechanism whereby hormone responsiveness is suppressed. At a mechanistic level, investigation into chromatin accessibility and transcription revealed that altered NR2F2 expression modulates genome-wide chromatin accessibility including ER-regulated loci and modulates ER transcriptional activity through its direct interactions with ER and its transcriptional coregulators. Collectively, our data unveil a critical mechanism whereby endocrine resistance occurs through the repression of estrogen responsiveness and highlight a new therapeutic approach to restore endocrine response through pharmacologic inhibition of NR2F2.

P2-03-18: Lymph node metastasis of breast cancer: subclonal selection for high lipid metabolism from the primary tumor

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Background: Lymph node metastasis is the most prevalent site of breast cancer dissemination and significantly influences patient prognosis and survival. Accurate prediction of axillary lymph node metastasis (ALNM) is crucial for tailoring surgical and pharmacological treatments. Understanding the underlying characteristics of lymph node metastasis can improve risk prediction and treatment strategies. Methods: We integrated single-cell transcriptomic data from 13 pairs of primary breast tumors and their matched lymph node metastases, sourced from our institution and public databases. Epithelial cells were clustered to explore the characteristics, and inferCNV was applied to trace the

evolutionary lineage of metastatic cells. We validated the association between LNM-signature and lymph node metastasis using TCGA breast cancer data. Results: Single-cell transcriptomics identified lymph node metastatic cells with heightened lipid metabolism, emphasizing fatty acid synthesis and membrane related metabolism. Immunohistochemistry of expanded clinical cohorts and a murine model corroborated the findings. Analysis of the inferCNV evolutionary tree revealed that the main ancestors of metastases in the primary tumor were clones exhibiting elevated lipid metabolism compared to others. This suggested that nodal metastasis was driven by subclonal selection rather than emerging adaptation. This association between primary tumors and lymph node metastases was further reinforced by bulk RNA-Seq data from breast cancer patients in the TCGA dataset. We generated a LNM-signature from the nodal metastasis associated cluster. The signature score increased with higher N stage and the area under the curve (AUC) for the signature score reached 0.65 (95%IC 0.61~0.71) within patients with the same T stage. Conclusions: Our findings indicate that lymph node metastasis arises from subclonal selection for high lipid metabolism from primary tumors. The corresponding signature score is a valuable predictor of nodal metastasis risk.

P2-03-19: Capivasertib-fulvestrant for patients w/ HR-pos/HER2-negative advanced breast cancer who had relapsed or progressed during or after aromatase inhibitor treatment: exploratory analysis of PTEN deficiency by IHC from phase III CAPItello-291 trial

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Background: In the phase III CAPItello-291 trial in patients with HR-positive/HER2-negative advanced breast cancer (ABC) who had relapsed or progressed during or after aromatase inhibitor (AI) treatment, the addition of capivasertib (a potent, selective pan-AKT inhibitor) to fulvestrant significantly improved progression-free survival (PFS) compared with placebo-fulvestrant in the overall population (hazard ratio: 0.60; 95% confidence interval [CI]: 0.51–0.71; p<0.001) and in patients with PIK3CA/AKT1/PTEN-altered tumors detected by next-generation sequencing (NGS; hazard ratio: 0.50; 95% CI: 0.38–0.65; p<0.001). Previous exploratory analysis also showed consistent PFS benefit across each alteration detected, including in patients with PTEN-altered tumors (n=50; hazard ratio: 0.45; 95% CI: 0.24–0.84). Here, we report a prespecified exploratory analysis of alteration prevalence and PFS in patients with deficient PTEN expression as detected by immunohistochemistry (IHC). Methods: In CAPItello-291, eligible pre-/peri- or postmenopausal women or men with HR-positive/HER2-negative ABC that had recurred or progressed on or after AI treatment with or without a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) were randomized 1:1 to receive fulvestrant (500 mg intramuscularly on days 1 and 15 of cycle 1, and day 1 of each subsequent 28-day cycle) with either placebo or

capivasertib (400 mg twice daily; 4 days on, 3 days off). PIK3CA/AKT1/PTEN alteration status was determined post-randomization using NGS in tumor tissue collected prior to study enrollment. Available samples were processed centrally to determine deficient PTEN protein expression by IHC using the VENTANA® PTEN SP218 antibody and a prespecified cutoff for this study of <10% staining of tumor cells. Hazard ratios for PFS were calculated using Cox proportional hazards models stratified by prior use of CDK4/6i. Data cutoff: August 15, 2022. Results: In total, PTEN results by IHC were obtained from 373/708 (53%) patient tumor samples. Baseline characteristics were broadly balanced between those with and without PTEN testing results. 71/373 (19%) patient tumor samples were identified as PTEN deficient by IHC; of these, 55% (n=39) had PIK3CA/AKT1/PTEN-altered tumors by NGS (26% PIK3CA [PIK3CA only n=15; PIK3CA and PTEN n=4]; 1% AKT1 [AKT only n=1]; 32% PTEN [PTEN only n=19; PIK3CA and PTEN n=4]), 30% (n=21) had PIK3CA/AKT1/PTEN-non-altered tumors by NGS, and 15% (n=11) had unknown NGS results. For tumors PTEN proficient by IHC 302/373 (81%), 164 (55%) were non-altered and 128 (42%) were altered by NGS (107/302, 35% PIK3CA, 6% AKT1, 3% PTEN) and 10 (3%) had unknown NGS result. Within samples with both NGS and IHC data, all samples with homozygous deletions or large rearrangements of PTEN by NGS were PTEN deficient by IHC. In patients with PTEN-deficient tumors by IHC, 34/71 (48%) received capivasertib-fulvestrant and 37/71 (52%) received placebo-fulvestrant. In this group, PFS benefit was observed with capivasertib-fulvestrant versus placebo-fulvestrant: median PFS: 9.3 months versus 3.7 months; hazard ratio: 0.52 (95% CI: 0.28–0.93). Conclusions: In this CAPItello-291 exploratory analysis, 19% of patient tumor samples that were available for central IHC testing were PTEN deficient by IHC. Within this subgroup of PTEN-deficient tumors, over half also had PIK3CA/AKT1/PTEN alterations detected by NGS. In the PTEN-deficient by IHC cohort, PFS benefit was noted with capivasertib-fulvestrant versus placebo-fulvestrant, although results are exploratory.

P2-03-20: Correlation of cell cycle arrest and intrinsic subtype with pathologic nodal status after neoadjuvant endocrine therapy – results from the Palbociclib and Endocrine therapy for Lobular breast cancer Preoperative Study

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Background: Biomarkers to predict response to neoadjuvant chemotherapy have been well-studied; however, for patients with estrogen receptor-positive disease receiving neoadjuvant endocrine therapy (NET), this remains an area of need. Here we examine cell cycle arrest (CCA) and intrinsic subtype (PAM50) as potential biomarkers of nodal response in the Palbociclib and Endocrine therapy for LObular breast cancer Preoperative Study

(PELOPS) trial.
Methods: PELOPS enrolled women with cT >1.5cm, N0-3, hormone receptor-positive HER2-negative breast cancer, stratifying based on histological subtype. The first 120 postmenopausal participants (60 with invasive ductal carcinoma [IDC] and 60 with invasive lobular carcinoma [ILC]) were randomized to letrozole versus tamoxifen for a 2-week window phase; all patients were then randomized 2:1 to a 24-week treatment phase of NET +/- Palbociclib (Palbo). CCA was defined as a Ki67 staining level ≤2.7% at day 15. For most, CCA measurement was after 2 weeks of NET; the only patients with CCA measured after Palbo were enrolled directly to the treatment phase with NET + Palbo. PAM50 was performed at baseline using RNASeq and the Genefu algorithm. ypN rates were compared by CCA and by intrinsic subtype using chi-square tests.
Results: 188 patients were included. 110 (58.5%) patients had T2 tumors, 82 (43.6%) had lobular histology and 96 (51.1%) were moderately differentiated. 99 (52.7%) patients were cN0, 84 (44.7%) patients were cN+, and 5 (2.7%) unknown. ypN0 and ypN+ rates were similar among patients with IDC and ILC histology. Among IDC patients who underwent axillary surgery, 37 (40.2%) were ypN0 and 55 (59.8%) were ypN+. Among ILC patients who underwent axillary surgery, 28 (34.6%) were ypN0 and 53 (65.4%) were ypN+.
Of 161 patients with complete Ki67 staining and ypN information, 93 (57.8%) had evidence of CCA and 68 (42.2%) did not, 62 (38.5%) were ypN0 and 99 (61.5%) were ypN+. CCA was not correlated with ypN status among patients with IDC (OR 0.84 [0.24-2.88], p=1, and 0.59 [0.13-2.60], p=0.708, for patients enrolled to window+treatment or directly to treatment phase, respectively) or ILC (OR 0.89 [0.26-3.17], p=1, and 1.23 [0.17-10.91], p=1, respectively). There was also no correlation between CCA and ypN status in each treatment arm: window+treatment (NET + Palbo, CCA measured after NET-only in window phase) OR 0.68 (0.24-1.87), p=0.458; window+treatment (NET) 0.86 (0.20-3.55), p=0.832; treatment (NET + Palbo), 0.27 (0.04-1.40), p=0.140; and treatment (NET) 2.25 (0.26-23.97) p=0.468.
Of 93 patients with available PAM50 and ypN data, 32 (34.4%) were luminal A, 57 (61.3%) luminal B, 3 (3.2%) HER2, and 1 (1.1%) normal. Luminal A versus B subtype did not correlate with ypN status overall (OR 0.63 [0.25-1.52], p=0.372), among patients with IDC (OR 0.5 [0.13-1.79], p=0.345) or ILC (1.15 [0.29-4.55], p=1), or among patients treated with NET + Palbo (OR 0.83 [0.27-2.44], p=0.786) or NET alone (0.37 [0.07-1.69], p=0.274).
Conclusions: Among patients treated with NET +/- Palbo, 43.6% of whom had ILC, 57.8% exhibited CCA. Most patients with available PAM50 results had luminal B subtype (61.3%). Cell cycle arrest after NET and baseline intrinsic subtype did not correlate with ypN status among patients treated with NET +/- Palbo. Alternative biomarkers of nodal status after NET need to be investigated.

P2-03-21: Longitudinal Monitoring of ctDNA for Disease Surveillance in Older Women with ER+ Breast Cancer on Primary Endocrine Therapy to Facilitate Surgical De-Escalation: A Prospective, Pragmatic, Hybrid-Decentralized Trial with Correlative Analyses

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Background: Older patients (pts) with ER+ breast cancer (BC) are at risk for both over- and under-treatment due to competing comorbidities. Those who opt to forego surgery in favor of primary endocrine therapy (pET) have approximate risks of 3% for BC-related mortality and 20% for progressive disease. Circulating tumor DNA (ctDNA) is associated with recurrence and progression across multiple solid tumors and may be particularly useful for disease surveillance in these pts.
Methods: The primary objective of this prospective, pragmatic, hybrid-decentralized clinical trial (NCT05914792) was to evaluate the rate of ctDNA positivity in pts ≥ 70 years with early-stage ER+ BC receiving pET and to investigate the clinical significance of ctDNA through longitudinal ctDNA analysis. Pts with ER- and/or HER2+ BC or M1 disease were excluded. This study used a clinically validated, tumor-informed multiplex PCR-NGS assay (SignateraTM, Natera, Inc.) to longitudinally monitor changes in ctDNA levels in pts on pET. Core needle biopsies at diagnosis served as the source of tumor DNA to generate the bespoke ctDNA assay. Blood samples were collected every 3-6mo during follow up. To make the study more accessible to the older population, pts were offered Natera's mobile phlebotomy service to complete blood draws at home. Progressive disease was defined using RECIST 1.1 criteria based on serial ultrasonography imaging. Pt- and caregiver-reported outcomes (PRO/CRO) were collected at each time point. Correlative studies utilizing spatial gene and protein expression assays, multispectral IHC (mIHC), and bulk RNA-seq were completed on a unique set of matched diagnostic core biopsy and surgical specimens treated with long-term endocrine monotherapy to investigate phenotypes of acquired aromatase inhibitor (AI) resistance.
Results: Of 39 pts enrolled to date (median age: 86 yrs, range: 75-94), 64% had stage II/III disease, 28% had regional nodal involvement, and 92% received AI alone for pET. Median follow up was 14mo (range: 2-29mo), with 7 pts (18%) deceased from a non-breast cancer related cause within 16mo of diagnosis, reflecting the advanced age and comorbidities of the cohort. PROs indicated that 75% pts were at least a little bit concerned about the possibility of tumor progression, and 80% felt that ctDNA testing helped inform their treatment plan. Nearly 80% of caregivers indicated that their daily activities often centered around caring for the pt, and they thought ctDNA testing could alleviate some follow up visits. Of 30 pts who had a pre-treatment blood draw, 33% were ctDNA-positive. By the second ctDNA draw (median time to second draw: 6mo), all pts who cleared ctDNA had

sustained responses to ET (stable disease or partial response), whereas all pts who did not clear ctDNA experienced tumor progression (cumulative risk of progression was 50% over 1.5 years, $p < 0.0001$ compared to those who had ctDNA-negativity or cleared at 6mo). Of 20 pts with negative pre-treatment ctDNA, all remained ctDNA-negative on subsequent blood draws without evidence of progression, and ultrasound imaging responses were concordant with ctDNA results (Cohen's kappa=1). Surgery was completed for progression on imaging (n=2) and for patient choice (responding to AI, n=4). Correlative studies on these specimens revealed immunosuppressive macrophage-fibroblast interactions that mediate tumor progression, with enrichment for ARG1+ macrophages and decreased effector T cells in progressing tumors.
Conclusions: This study enrolled older pts, in whom "right-sizing" therapies for ER+ BC is critical. ctDNA results were highly associated with disease outcome, and persistent ctDNA positivity conferred significant risk for progression. ctDNA monitoring may help identify those pts with indolent disease who may be safely followed on pET and those who may be at risk for AI resistance. Both patients and caregivers thought ctDNA was helpful in informing the treatment plan.

P2-03-22: Shallow whole genome sequencing of cell free DNA to predict response to CDK4/6 inhibitor treatment in ER+/HER2- metastatic breast cancer

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Background: Cell-free plasma DNA (cfDNA) from cancer patients contains tumour-derived DNA fragments (ctDNA), allowing a non-invasive method for analysing tumour molecular information in the blood of cancer patients. This allows genetic tracking of tumours and the identification of patients that may not respond or stop responding to treatments.
CDK4/6 inhibitors (CDK4/6i) in combination with endocrine therapy are the standard of care first-line treatment in ER+/HER2-negative advanced breast cancer. Clinical trials showed improvement of PFS and OS with the use of CDK4/6i. Currently there are no robust biomarkers of response or resistance to CDK4/6i and a rapid disease progression is often seen following failure of response to CDK4/6i. Early identification of response is crucial and has multiple clinical and financial implications. Here, we use shallow whole genome sequencing (sWGS) to detect copy number alterations (CNAs) and calculate a Genome Instability (GI) score with the aim to investigate correlations of GI with CDK4/6i treatment and clinical outcomes.
Methods: Blood samples were collected from a small cohort of metastatic ER+/HER2 negative breast cancer patients (n=36), including patients on CDK4/6i and patients on chemotherapy following progression on treatment with aromatase inhibitor. Bloods were processed to collect the plasma containing the cfDNA which was extracted, quantified and analysed using Agilent Bioanalysis. Samples demonstrating a clean cfDNA profile with minimal contaminating larger sized leukocyte DNA were taken forward for library preparation and sequencing. The resulting genomic data were normalised following binning

and background correction resulting in genome wide copy number patterns. GI scores were calculated as a read out of genomic disruption with respect to control samples. Analysis of PFS and OS were carried out with respect to treatment type and GI score thresholds.
Results: Patients receiving chemotherapy demonstrated significantly higher GI scores than those treated with CDK4/6i (median chemotherapy = 319.5, CDK4/6i = 263.7, T-test P-value = 0.02), evident particularly over chromosomes 3,4,5 & 21. Additionally, patients on CDK4/6i for <1 year demonstrated significantly higher GI scores than patients on CDK4/6i for >1 year (Poor response with <1year on CDKI = 328.7, good response and remain on CDKI > 1 year = 231.7). Survival analysis showed that patients on CDK4/6i for <1 year also demonstrated worse progression free survival (PFS) and overall survival (OS) then those who remained on CDK4/6i for >1 year (median difference PFS = 630 days; OS = 377 days).
Conclusion: Preliminary results examining the GIN scores from our sWGS of cfDNA suggest that high GI scores were associated with shorter progression free survival on CDK4/6i plus endocrine treatment. Patients with higher GI scores at the time of diagnosis are less likely to respond well to CDK4/6i predicting poor clinical outcomes.

P2-03-23: Charting the longitudinal mutational landscape of triple negative breast cancer

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Background: Triple Negative Breast Cancer (TNBC) is characterised by extensive intra-tumour heterogeneity (ITH) where clonal lineages diverge from distinct subpopulations over time, impacting treatment resistance and influencing metastasis. However, the mutational dynamics underlying these lineages are poorly studied. Most TNBC patients with early or locally advanced disease receive neoadjuvant chemotherapy (NACT). Pathological complete response (pCR) to NACT is considered as a surrogate for good prognosis but patient response differs greatly. Both SNVs (short nucleotide variants) and copy number variants (CNVs) have been implicated as drivers of the adaptive response to chemotherapy and metastasis in TNBC. Here, we comprehensively investigated the mutational landscapes in a longitudinally sampled TNBC cohort, relating SNV and CNV patterns during the course of the disease to patient outcomes.
Methods: All TNBC patients selected for the study were undergoing NACT. Samples included preNACT treatment (PreT), surgical postNACT treatment (PostT) and recurrence samples. Residual Cancer Burden (RCB) scores and tumour infiltrating lymphocytes (TILs) were reported by a breast cancer pathologist. DNA was extracted from FFPE tumour samples where homogenous tumour regions had been identified by the pathologist. Multiple regions of the PostT recurrence samples were selected for DNA extraction, resulting in 96 samples in total. Following whole exome sequencing, the raw sequencing reads were processed to identify germline and somatic variants. In addition, we optimised an affordable approach to profile genome wide CNVs in longitudinal samples. Bioinformatic analysis of SNV and CNV data then studied the dominant mutational patterns over time, and identified likely driver

variants and disrupted pathways.
Results: Our results demonstrate that low RCB score correlates with higher levels of TILs in the PreT samples. Tumour mutational burden (TMB) varied from 1-27 per Mb and was lowest in 2 patients with known germline mutations. SNV analysis identified variants in 69 genes previously reported to play roles in the progression of TNBC, the most frequently mutated being TP53. We also found suggestive evidence for novel driver variants under positive selection in the MICA gene. Overall, we identified an enrichment of mutations in genes associated with nucleic acid metabolic processes. The longitudinal sampling also allowed the detection of relatively early PreT mutations, showing enrichment in pathways involved in double-strand break repair and apoptotic signaling. Subsequently many of these early variants were found to be 'conserved' as mutations retained from PreT to PostT in samples from the same patient. In addition, we identified a novel class of SNVs that appear to arise only at later stages of the disease. In the CNV landscape, we identified regions that were commonly amplified or deleted in PreT samples, particularly a recurrent amplification of 8q. For some patients, the CNV landscape changed dramatically over the course of TNBC disease progression, with frequently altered genes differing markedly from those impacted by SNVs.
Conclusions: We have developed relatively inexpensive profiling techniques to identify disease-associated variants in TNBC patients, allowing us to study their dynamics over the course of the disease. We present mutational profiles from before and after NACT from the same patient, which can be compared to reveal the variants that are gained or lost genome-wide during the evolution of a tumour. Ultimately, specific SNVs and CNVs associated with survival, recurrence and metastasis can be identified. These data will direct larger scale follow-up studies for biomarker validation and treatment stratification.

P2-03-24: Cost-Effectiveness of CYP2D6 Genotyping in the Management of Tamoxifen Therapy for Breast Cancer Patients: A Focus on Adverse Events

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Background: Tamoxifen, an antiestrogen prodrug, is widely used in treating hormone receptor-positive breast cancer. Its efficacy largely depends on its conversion to the active metabolite endoxifen by the CYP2D6 enzyme, which exhibits varying levels of activity based on genetic polymorphisms. Current clinical practice guidelines recommend an initial gynecological examination with transvaginal ultrasound before starting tamoxifen. If no abnormalities are detected, the patient begins tamoxifen without additional gynecological follow-up unless symptomatic (e.g., spotting or vaginal bleeding). This study explores the cost-effectiveness of CYP2D6 genotyping to predict patients at higher risk of toxicity.
Methods: This analysis utilized data from our dose escalation trial (EudraCT: 2007-002942-40), where poor metabolizers (PM) received increased doses of tamoxifen. All women were treated with 20 mg/day of tamoxifen for 5 years, except PM, who received 20 mg/day for 4 months, 40 mg/day for the next 4 months, and 60 mg/day for another 4 months before

returning to 20 mg/day for the remainder of the 5 years. The costs for managing adverse events, updated in May 2024, were obtained from the Andalusian Health Service. These costs provide a detailed financial perspective based on public data, including costs for consultations, diagnostic procedures, follow-up treatments, and hospitalization, reflecting Grade 2 complexity. Additionally, we calculated the cost of CYP2D6 genotyping, covering expenses for DNA extraction, analysis using KASP assays, and proration of personnel and equipment costs. We analyzed the incidence of specific side effects: osteoarticular pain, hot flashes, asthenia, and gynecological alterations.
Results: Only gynecological alterations showed significant differences between CYP2D6 phenotypes. The incidence of gynecological adverse events was significantly higher in slow metabolizer (SM) patients (44.8%) compared to rapid metabolizer (RM) patients (15.2%, p=0.001). Adverse events quantified as average cost per patient include endometrial hyperplasia (€8544,81), endometrial polyps (€1834,56), adenocarcinoma (€9467,96), and endometrial thickening (€1132,27). Although costs related to medications, secondary events, extended hospital stays, or patient evolution were not included, our study recorded an average of 0.75 emergency visits per patient (range: 0-4), each costing €208.47 without hospital admission or observation. The cost of CYP2D6 genotyping is minimal (€10.98) compared to managing gynecological adverse events.
Conclusion: Considering the significant impact of CYP2D6 polymorphisms on the occurrence of gynecological adverse events and the substantial cost of managing these events, our results advocate for the implementation of CYP2D6 genotyping. Given its demonstrated cost-effectiveness, we propose CYP2D6 genotyping for all patients scheduled to receive tamoxifen. For those with a predicted SM phenotype, we recommend protocolized gynecological follow-up at least once a year, along with thorough clinical monitoring. Any emerging symptoms should prompt immediate referral to gynecology for evaluation and examination. This approach not only enhances the quality of care but also aligns with healthcare efficiency objectives.

P2-03-25: Characterization of patritumab deruxtecan activity in breast cancer (BC) patient-derived xenograft (PDX) models

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Background: HER3 is overexpressed in 30-50% of breast cancers and is associated with poor prognosis. Patritumab deruxtecan (HER3-DXd; MK-1022) is a HER3-directed ADC with a potent topoisomerase I (TOP1) inhibitor payload. Clinical trials have demonstrated promising antitumor activity of HER3-DXd in metastatic and treatment-naïve breast cancer in all major subtypes (NCT02980341, NCT04610528), showing clinical activity across baseline levels of HER3 protein or mRNA expression. The identification of relevant biomarkers of response for this treatment remains an unmet clinical need. Here, we aimed to study the activity of HER3-DXd in breast cancer PDX models to explore potential

biomarkers of response.
Methods: The antitumor activity of HER3-DXd (10 mg/kg or 3 mg/kg dosed once weekly, 4 doses in total; Q1W x 4) was assessed in 30 BC PDX models (21 ER+/HER2- and 9 triple negative [TNBC]) and compared to the antitumor activity of the clinically approved TOP1 inhibitor irinotecan (50 mg/kg dosed once weekly; Q1W). PDX models that achieved a complete response to HER3-DXd that lasted longer than 120 days were classified as non-relapsed models. Genetic alterations harbored by PDX models were determined using the MSK-IMPACTTM targeted exome panel that also includes selected intronic regions. nCounter platform was used to assess PAM50 subtype classification and the proliferation score. HER3 expression was assessed by immunohistochemistry (IHC). Tumor samples from PDXs were collected 24h or 7 days (7d) after a single dose of HER3-DXd for pharmacodynamic (PD) experiments. DNA damage induction and replication stress were evaluated in untreated/treated PD samples by quantification of γH2AX and phospho-RPA32 (S4/S8) nuclear foci formation by immunofluorescence (IF), respectively. Chi-squared test was used to evaluate the association between HER3-DXd response and different variables.
Results: 13 out of 30 (43%) PDXs exhibited a profound and sustained response to HER3-DXd 10 mg/kg. Antitumor activity of HER3-DXd was observed across baseline levels of HER3/ERBB3 expression. Basal-like PAM50 intrinsic subtype (p=0.001) and PTEN (p=0.035), RB1 (p=0.014) and TP53 (p=0.008) alterations were associated with long-term response (non-relapse). High concordance was observed between HER3-DXd and irinotecan response (p=0.019). Mechanistically, HER3-DXd induced higher transient HER3 membrane downmodulation in non-relapsed BC PDXs models (p=0.037). In addition, treatment with HER3-DXd resulted in a mild transient antiproliferative effect based on proliferation score accompanied by a sustained higher S-phase DNA damage measured as γH2AX nuclear foci in non-relapsed models versus the relapsed ones (p<0.001). Low-dose HER3-DXd (3mg/kg) showed high efficacy in BRCA1/2-altered PARPi-resistant PDX models (p=0.035), sustained higher S-phase DNA damage and induction of replication stress marker p-RPA32 (S4/S8) in non-relapsed models.
Conclusions: HER3-DXd exerts a potent antitumor response in BC PDXs across baseline levels of HER3/ERBB3 expression in ER+/HER2- and TNBC models. Based on our data, basal-like PDX models were more likely to show long-term responses to HER3-DXd than luminal B PDX models. Results also suggest that BRCA1/2-altered PARPi-resistant tumors will show high benefit with HER3-DXd treatment.

P2-03-26: Real-world prevalence of PD-L1 positivity in early-stage/metastatic triple-negative breast cancer (eTNBC/mTNBC): primary results and pathology insights from the global retrospective observational VANESSA study

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Background: Most recent phase 3 trials in eTNBC/mTNBC have included central laboratory testing to determine PD-L1 status using protocol-defined assays and scoring methods, varying according to the investigational agent. Applicability to routine clinical practice is poorly understood. In the VANESSA study, conducted in 39 sites across 19 countries, we assessed the global prevalence of PD-L1+ status in eTNBC/mTNBC using the Ventana PD-L1 (SP142) assay retrospectively in real-world clinical practice, and explored diagnostic factors and patient/disease characteristics potentially affecting detection of PD-L1+ status.
Patients and methods: The multicenter retrospective observational VANESSA study consecutively and uniformly enrolled patients treated with systemic therapy for eTNBC or mTNBC (assessed locally per ASCO/CAP guidelines) newly diagnosed between Jan 1, 2014, and Dec 31, 2017. An archival formalin-fixed paraffin-embedded tumor tissue sample was required from all patients. PD-L1 status was retrospectively assessed locally and at a central laboratory by pathologists certified specifically for TNBC on the Ventana platform. The primary objective was to determine the prevalence of PD-L1 positivity (PD-L1 expression on tumor-infiltrating immune cells [ICs] covering ≥1% of the tumor area using the Ventana PD-L1 [SP142] immunohistochemistry assay per local assessment) on primary or metastatic tumor tissue from patients with eTNBC or mTNBC. The secondary objective was to evaluate inter-observer concordance between local and central PD-L1 testing. Prespecified exploratory objectives included comparison of PD-L1+ status in subgroups according to sample size (<5 vs >5 mm), scoring method (digital slides vs light microscopy), and (in mTNBC only) sample origin (primary vs metastatic).
Results: Among 2054 eligible patients, the PD-L1+ prevalence by local assessment was 38% (95% CI 36–41%) in eTNBC (n=1902) and 20% (95% CI 14–27%) in mTNBC (n=152). The prevalence of PD-L1+ status was higher by central than local assessment (eTNBC: 55% [1007/1822] vs 38%; mTNBC: 26% [38/145] vs 20%), and comprised 35% IC1, 13% IC2, and 7% IC3 in the eTNBC cohort and 20% IC1, 6% IC2, and 0% IC3 in the mTNBC cohort. There was 75% overall percentage agreement (PA) between local and central laboratories (62% positive PA, 91% negative PA; Cohen's κ coefficient 0.52, 95% CI 0.48–0.55). Concordance was similar regardless of cohort (eTNBC vs mTNBC), sample type (biopsy vs resection), or sample origin (primary vs metastatic), but lower in 116 samples scored from digital slides. According to local assessment, PD-L1 positivity was more common in larger (>5 mm) than smaller (<5 mm) samples (eTNBC: 43% vs 16%; mTNBC 24% vs 13%). In the mTNBC cohort, the PD-L1+ prevalence was 19% (95% CI 12–27%) in 116 primary tissue samples and 23% (95% CI 10–40%) in 35 metastatic samples. Centrally assessed positive status for PD-L1 ICs and stromal tumor-infiltrating lymphocytes (TILs) was broadly overlapping (Spearman correlation 0.36 for eTNBC, 0.31 for mTNBC).
Conclusion: The PD-L1+ prevalence in real-world samples by SP142 was lower in mTNBC vs eTNBC, by local vs central assessment, and in smaller vs larger samples. We observed lower PD-L1+ prevalence in the VANESSA real-world study than in reported prospective clinical trials assessing PD-L1 status centrally. These findings underline the importance of robust PD-L1 assessment to ensure optimal selection for therapies targeting PD-1/PD-L1 in patients with mTNBC.

P2-03-27: Prevalence of actionable genomic alterations (GA) and predictive value of tumor mutational burden (TMB) for immune checkpoint inhibitor (ICI) effectiveness in HR(+)HER2(-) metastatic breast cancer (MBC)

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Background: Pembrolizumab, an anti-PD1 antibody ICI is an FDA-approved option for patients with HR(+)HER2(-) MBC who have exhausted standard of care options and have high TMB (≥10 mut/mb, FoundationOne®CDx). No clinical trials or comparative effectiveness analyses have been performed to compare ICI vs. chemotherapy (chemo) using the FDA-approved TMB biomarker. We sought to perform subgroup analyses of ICI and chemo by TMB level and characterize the prevalence of established actionable GA in tissue (TBx) and liquid (LBx) biopsies of HR(+)HER2(-) MBC patients (pts).
Methods: MBC pts who underwent genomic testing using Foundation Medicine tissue or liquid comprehensive genomic profiling (CGP) assays were included. Patient clinical data was obtained by the US-wide-de-identified Flatiron Health and Foundation Medicine real-world clinicogenomic breast database (CGDB) originated from ~280 US cancer clinics (~800 sites of care) between 01/2011 and 12/2023. The prevalence of mutations (mut) in ESR1, PIK3CA, AKT1, PTEN, BRCA1/2, and PALB2, homozygous copy loss (loss) in PTEN and BRCA1/2, and fusions in NTRK and RET was determined in TBx and LBx. TMB, microsatellite instability-high (MSI-H) and homologous recombination deficiency signature (HRDsig) were determined in TBx. Progression-free survival (PFS), and real-world overall survival (rwOS) were compared in pts receiving single-agent ICI between TMB <10 mut/Mb vs. TMB >=10 mut/Mb (TMB<10 vs. TMB10+) by Cox models. Multivariable analyses were performed adjusted for prognostic features. PFS and rwOS were also compared between pts receiving single-agent ICI vs. chemo, adjusted for propensity scores accounting for metastatic site, number of metastatic sites, ECOG performance status, use of opioid pre-therapy, and line of therapy.
Results: A total of 5,825 TBx and 1,801 LBx were evaluated. At least one actionable GA was detected in 70.6% of TBx and 58.4% of LBx [84.8% in samples with circulating tumor (ct)DNA tumor fraction (TF)>=1% and 34.6% in TF <1%]. The most prevalent GA was PIK3CAmut (42.3% TBx / 33.6% LBx: 51.9% in TF>=1% and 17.2% in TF <1%), followed by ESR1mut (16.7% TBx / 29.7% LBx: 48.4% in TF>=1% and 12.8% in TF<1%) and PTENmut (6.4% TBx / 7.8% LBx: 12.9% in TF>=1% and 3.3% in TF<1%). GA losses were more prevalent in TBx, with PTENloss detected in 4.2% of TBx and 0.7% of LBx and BRCA1/2loss in 0.9% of TBx and 0.2% of LBx. HRDsig was detected in 10.8%, TMB10+ in 8.5% and MSI-H in 0.4% of TBx. Of the 5,825 pts with TBx, 89 received ICI (49 TMB<10 and 40 TMB10+) and 1,835 received chemo (1,711 TMB<10 and 1,835 TMB10+). Pts receiving ICI TMB10+ vs. TMB<10 had more favorable PFS [median 2.9 vs. 1.7 months (mo), hazard ratio (HR) 0.61, 95% confidence interval (CI) 0.39-0.95, p=.029] and rwOS (median 12.9 vs. 3.2 mo, HR 0.53, 95% CI 0.32-0.88, p=.014). Pts receiving chemo TMB10+ vs. TMB<10 had similar PFS (median 4.6 vs. 4.6 mo, HR 0.96, 95% CI 0.8-1.16, p=.677) and rwOS (median 8.9 vs. 12.6

mo, HR 1.04, 95% CI 0.85-1.28, p=0.695). The multivariable analyses confirmed the independent association of TMB and PFS and rwOS in pts receiving ICI. Pts TMB10+ receiving ICI vs. chemo had numerically less favorable PFS (median 2.9 vs. 4.3 mo, HR 1.07, 95% CI 0.65-1.75, p=.797) and more favorable rwOS (median 12.9 vs. 8.5 mo, 95% CI 0.41-1.28, p=.268). Pts TMB <10 receiving ICI vs. chemo had less favorable PFS (median 1.8 vs. 4.1 mo, HR 1.56, 95% CI 1.09-2.25, p=.016) and rwOS (median 3.1 vs. 12 mo, HR 1.73, 95% CI 1.19-2.51, p=.004).
Conclusion: About 70% of TBx harbor at least one GA with established clinical utility. Pts with TMB10+ vs. TMB<10 had more favorable PFS and rwOS on ICI. This study supports ICI use in pts with TMB10+ determined by FDA-approved CGP test and highlights CGP role in identifying a wide range of actionable GA.

P2-03-28: Immune-based gene expression signature determines clinical efficacy of CDK4/6i in HR+HER2- breast cancer

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Background: Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) have emerged as effective treatments for patients with hormone receptor–positive (HR+)/human epidermal growth factor receptor 2–negative (HER2–) advanced breast cancer and more recently in adjuvant setting. Trials in neoadjuvant scenario are ongoing. Dedicated research efforts have been undertaken to find predictive biomarkers of response or resistance to these therapies although no molecular biomarkers have reached the clinic so far. Methods. Here, we performed a single-center prospective study to provide insights into the clinical and tumor characteristics determining the efficacy of CDK4/6i in metastatic breast cancer (n=100). Gene expression in tumors was performed using nCounter Breast 360 (BC360TM) panel. Clinical and biological data were correlated using logistic cox regression analysis. Transcriptomic data from NeoPalAna trial (GSE93204) was used as a validation dataset. Results. Taking into account clinical characteristics determining CDK4/6i efficacy, we developed a clinical stratification algorithm that allowed the dichotomic classification of the cohort based on CDK4/6i efficacy. The two resulting efficacy groups were used to identify and characterize the molecular pathways involved in CDK4/6i efficacy through the standardized tissue-based transcriptomic analysis in pre-treatment biopsies. When considering expression signatures implemented into the BC360TM panel, we identified 19 that were differentially expressed, presenting a significant enrichment of overexpressed immune-related signatures in the bad efficacy group (13/19). Multivariate Cox survival analysis indicated that elevated expression levels of the Treg, IFNGamma, PD1 and TIS signatures were independently associated to PFS only in first-line patients, overall suggesting that immune function might be a key factor determining CDK4/6i efficacy. To

further delineate the immunologic features determining CDK4/6i efficacy, we evaluated differences in single gene expression in first-line patients, resulting in the identification of a 14-gene group exhibiting at least a 50% significant expression difference between good and bad efficacy groups. Unsupervised consensus clustering, allowed us to further define a 9 immune-gene signature that successfully clustered together 90% of patients from the good efficacy group (19/21). Indeed, mean expression of the 9-gene signature was significantly different between good and bad efficacy groups, both considering first-line patients (p=0,004) or the entire cohort (p=0,003), being always upregulated in the bad efficacy patients. More importantly, multivariate Cox survival analysis indicated that elevated expression levels of the 9-gene signature were associated to poorer PFS and OS independent of treatment line (p=0,033 and p=0,034, respectively), improving predictive capacity compared to the BC360TM signatures. The predictive value of our 9-gene signatures was further confirmed using the data from NeoPalAna clinical trial, where a significant difference in the 9-gene signature expression was also observed between sensitive and resistant tumors (p=0.026). Conclusions: Our results identify a novel 9-gene immune-based signature that predicts CDK4/6i efficacy in breast cancer patients, with easy implementation in daily clinical practice. Overall, our data further strengths the key role of immune response as a valuable predictive factor for CDK4/6i efficacy in breast cancer.

P2-03-29: Upregulation of senescence signatures and interferon-signalling after short-term pre-operative CDK4/6 inhibitor – gene expression analysis from the POP and ABC-POP trials

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Background: CDK4/6 inhibitors (CDK4/6i) have been shown to induce senescence in pre-clinical models, however the extent to which this occurs in patients, and its clinical relevance, is unknown. Senescence is known to modulate adaptive anti-tumour immunity and may be implicated in CDK4/6i induced immune response. We evaluated expression of senescence-associated transcriptomic signatures in patients with early stage hormone-receptor positive, HER-2 negative (HR+/HER2-) breast cancer treated with 14 days of CDK4/6i monotherapy and assessed differential gene expression according to anti-proliferative response.
Methods: Pre- and on-treatment transcriptomic profiles of HR+/HER2- tumours from patients treated with palbociclib on the POP trial (NCT02008734) and abemaciclib on the ABC-POP trial (NCT02831530) were evaluated for on-treatment expression of senescence-associated gene signatures, compared to pre-treatment expression. In both trials, patients with previously untreated, operable, early stage breast cancer were randomised 3:1 to receive 14 days of a CDK4/6 inhibitor or no treatment, and underwent biopsy prior to treatment and at surgery (day 15). Response was defined as the natural log of on-treatment Ki67 (at day 15) < 1, previously shown to be significantly associated with recurrence free survival and breast cancer specific survival in HR+/HER2- breast cancer. Microarray

(Affymetrix Human Gene Chip 2.1ST) of RNA extracted from fresh-frozen tissue obtained from pre-treatment core biopsy and on-treatment surgical specimens was used to generate transcriptomic profiles. Differential gene expression and gene set enrichment analysis were performed.
Results: RNA data was available for 57 pre-treatment and 50 on-treatment samples from patients treated with palbociclib, and for 62 pre-treatment and 57 on-treatment samples from patients treated with abemaciclib. Expression of four senescence-associated gene signatures (SenMAYO, Fridman_UP, Purcell, Purcell RED) was significantly upregulated following 14 days of treatment with palbociclib (SenMAYO normalised enrichment score [NES] 2.41, adj. p-value < 0.01; Fridman NES 2.13, p < 0.01, Purcell_RED NES 1.90, p < 0.01, Purcell NES 1,85, p < 0.01) and abemaciclib (SenMAYO NES 2.43, p <0.01, Purcell_RED NES 2.01, p < 0.001, Purcell NES 1.88, p < 0.01, Fridman NES 1.71, p < 0.01). Both palbociclib and abemaciclib treatment were associated with upregulation of immune hallmarks including TNF-alpha signalling via NFKB, interferon (IFN)-gamma response, IFN-alpha response, and inflammatory response, as well as down-regulation of genes and pathways associated with cell cycle (G2M checkpoints, E2F targets, mitotic spindle hallmarks). Paradoxically, expression of IFN-alpha and IFN-gamma response pathways was suppressed in responders compared to non-responders, at baseline and after treatment.
Conclusion: This data provides evidence that CDK4/6i induce senescence and upregulate interferon signalling in patients with early ER+/HER2 negative breast cancer. Consistent with previous studies, high baseline and on-treatment interferon signalling appears to be related to treatment resistance. The relationship between senescence and interferon signalling in this context, and the potential therapeutic implications of this require further investigation.

P2-03-30: Association of ctDNA in patients with long-term outcome of breast cancer patients undergoing neoadjuvant treatment in the randomized ABCSG 34 clinical trial

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Introduction: Circulating tumor DNA (ctDNA) represents a minimally invasive assessment of tumor response based on longitudinal ctDNA analyses. In the neoadjuvant prospective ABCSG 34 trial, we have previously shown that lacking ctDNA clearance correlated with poor response to neoadjuvant systemic treatment (NST) assessed as pathological response (pCR) and residual cancer burden (RCB). In a follow-up analysis, we investigate the impact of ctDNA detection and clearance (or lack thereof) on long-term outcome invasive disease free survival (iDFS), distant recurrence free survival (DRFS), and overall survival (OS).
Experimental design: Previously, tumor-informed assays based on analyses of 93 genes in tissue were designed for 145 patients with sufficient plasma cell free DNA, and ctDNA

presence/absence/clearance in plasma was evaluated before (BL, baseline) , during (MT, mid-therapy), and at the end of neoadjuvant treatment (EOT). In the present analyses, follow up data on invasive disease free survival (IDFS), distant recurrence free survival (DRFS) and overall survival (OS) were available from 109 patients. Association of [DH1] BL, MT and EOT ctDNA status with outcomes was analyzed. Median FU starting from surgery was 7.1 years.
Results: Twenty of 49 patients (40.8%) with detectable ctDNA at BL had an IDFS event, while out of 60 patients who were ctDNA negative at baseline, 19 (31.7%) experienced an IDFS event (HR 1.53 (0.81 - 2.86, p=0.187). IDFS rate at 7 years was 58.5% vs. 70.8%. DRFS rate was 60.9% in patients with detectable baseline ctDNA vs. 77.9% in ctDNA negative patients (HR 1.98, 95% CI 0.99 - 3.95, p=0.053). OS rate at 7 years was 62.7% for ctDNA+ compared with 81.2% in ctDNA- patients (HR 2.12, 95% CI 1.02 - 4.41, p=0.043), respectively. In contrast to BL there was no significant association of ctDNA status with IDFS, DRFS and OS at MT or EOT, even though the HR are comparable, due to smaller numbers significance could not be reached. [DH2] [DE3]
Conclusions: Overall, our results demonstrate that in our cohort the detection of ctDNA at BL was associated with IDFS, DRFS and OS. [DH4] [DE5] Limited numbers of available MT and EOT samples reduced the power of analyses of ctDNA dynamic changes under treatment and their association with long term outcome, therefore results didn't reach significance. In summary, BL ctDNA assessment is useful to assess long-term prognosis in patients receiving neoadjuvant NST.

P2-04-01: MUC1-C integrates aerobic glycolysis with suppression of oxidative phosphorylation in triple-negative breast cancer stem cells

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Triple-negative breast cancer (TNBC) is a recalcitrant malignancy largely unresponsive to cytotoxic, targeted and immunotherapeutic agents. The Mucin1 (MUC1) transmembrane heterodimeric protein, which is aberrantly expressed in TNBCs, evolved in mammals to provide protection of epithelia from the external environment. With loss of homeostasis, the (i) MUC1 N-terminal (MUC1-N) subunit is shed and released into serum as detected by the CA15-3 assay, and (ii) MUC1 C-terminal (MUC1-C) subunit is activated and thereby promotes lineage plasticity, epigenetic reprogramming, and the cancer stem cell (CSC) state. However, little is known in regard to the regulation of TNBC CSC metabolism.

Studies of TNBC CSCs have been hampered by challenges using cell surface markers in purifying this cell population. Accordingly, we enriched TNBC CSC populations by serial passage of mammospheres. The present studies performed on enriched populations of TNBC CSCs demonstrate that MUC1-C is essential for integrating activation of glycolytic pathway genes with self-renewal and tumorigenicity. Based on the importance of GLUT1 and HK2 in driving glycolysis and tumorigenicity, we confirmed their upregulation in

passaged mammosphere cells. Here, we found that silencing MUC1-C in passaged mammosphere cells decreases MYC occupancy of the GLUT1 PLS and HK2 PLS, in support of MUC1-C dependency in the activation of GLUT1 and HK2 expression. Moreover, treatment with the 2-deoxy-D-glucose (2DG) , HK2 inhibitor, markedly decreased mammosphere formation in concordance with dependence of CSCs on MUC1-C-induced activation of the glycolytic pathway.

Futhermore, we found that suppression by targeting MUC1-C with silencing and GO-203 treatment MUC1-C further integrates the glycolytic pathway with suppression of mitochondrial DNA (mtDNA) genes encoding components of mitochondrial Complexes I–V. The repression of mtDNA genes is explained by MUC1-C-mediated downregulation of the mitochondrial transcription factor A (TFAM) required for mtDNA transcription and induction of the mitochondrial transcription termination factor 3 (mTERF3). In support of pathogenesis that suppresses mitochondrial ROS production, targeting MUC1-C increases (i) mtDNA gene transcription, (ii) mitochondrial ROS levels, and (iii) loss of self-renewal capacity.

In summary, our findings in TNBC cells indicate that MUC1-C drives CSC self-renewal by integrating the activation of glycolysis in certain subpopulations with suppression of oxidative phospholylation.

P2-04-02: TRIB3 regulates lipophagy and drives the metastasis of triple negative breast cancer by activation of PI3K/AKT/mTOR pathway

Zirong iang, Yushuai Yu, Qing Wang, Mingyao Huang, Shiping Luo, Xin Yu, Xiewei Huang, Chuangui Song, Jun Liu

Background: The balance of lipophagy is critical for maintaining oncogenic signaling pathways. However, the mechanisms by which lipophagy promotes progression and metastasis in triple-negative breast cancer (TNBC) are currently unknown. In this study, we investigated the clinical significance and biological involvement of the pseudokinase protein TRIB3 in TNBC.
Methods: Target genes associated with lipophagy were identified by GSEA database screening and tested in tissues and cell lines. The biological functions of TRIB3 in TNBC were investigated using gain-of-function/loss-of-function assays. Western blotting, laser confocal microscopy, transmission electron microscopy and immunoprecipitation were used to detect and analyze lipophagy. ChIP and luciferase reporter gene assays were used to evaluate the relationship between TRIB3 and FOXA1. Next-generation sequencing technology was applied to screen out the possible downstream molecule MSI2 of TRIB3 and verify the regulatory relationship between the two.
Results: Increased expression of TRIB3 was found in TNBC compared with paired peritumoral tissues. It was more signifcant in TNBC with metastasis and correlated with worse overall survival. Upregulation of TRIB3 accelerated TNBC cells colony formation,

migration and invasion. Mechanically, RNAseq-based analysis showed enrichment of autophagy-related pathways in TRIB3 lowly-expressed TNBC. Next, TRIB3 was demonstrated to interact and stabilize MSI2, amplify PI3K/AKT/mTOR-mediated lipophagy and then promote TNBC growth and metastasis. Moreover, FOXA1 was upregulated and transcriptionally increased TRIB3 expression in TNBC cells. Furthermore, These phenotypes could be reversed by the lipophagy inhibitor chloroquine.
Conclusions: Our fndings suggested that TRIB3 is a key pseudokinase molecule for tumor progression and metastasis and may be an effective target for TNBC patients.

P2-04-03: Progesterone Receptor-Stimulated MicroRNAs Regulate Breast Cancer Proliferation

Motoki Takaku, Annika Price, Jill Goodman, Edward Looker

Background: Breast cancer development is closely linked to the activity of hormone receptors, including estrogen receptors (ER) and progesterone receptors (PR). While the role of ER in breast cancer and its targeted therapies are well-understood and established in clinical practice, the function of PR remains underexplored. This is despite its presence in approximately 70% of breast cancers and the promising inhibitory effects of progesterone treatment. This knowledge gap, along with the dual role of progesterone in both promoting and inhibiting breast cancer, underscores the urgent need for more detailed investigations into PR mechanisms.
Methods: We conducted a microRNA (miRNA) expression analysis and identified 41 differentially expressed miRNAs upon progesterone stimulation. Many upregulated miRNAs are predicted to target cell cycle regulatory genes, suggesting that PR may regulate breast cancer proliferation through miRNAs. Consequently, we employed a CRISPR knockout screening to explore the roles of these progesterone-stimulated miRNAs. In this screening, T47D luminal breast cancer cells were treated with progesterone for one month, and resistant clones were isolated for downstream analysis.
Results: The CRISPR screening indicated enrichment of gRNAs targeting four miRNAs post-progesterone exposure. PR binding was observed near these miRNAs, suggesting direct regulation by PR. The depletion of these miRNAs in T47D cells led to resistance to progesterone treatment. Notably, among these, the expression of miR-30a correlates with PR expression and patient prognosis in breast cancer clinical data.
Conclusion: This study identifies PR-stimulated miRNAs that are crucial for inhibiting growth in luminal breast cancer cells. These findings shed light on how progesterone-activated PR may inhibit tumor growth in breast cancer, providing new insights for potential therapeutic targets.

P2-04-04: Integrated stress response-upregulated mitochondrial SLC1A5var enhances glucose dependency of human breast cancer cells

Sheng-Fan Wang, Yu-Chieh Ho, Pei-Chen Wu, Chian-Ying Chou, Yuh-Lih Chang, Hsin-Chen Lee, Ling-Ming Tseng

Breast cancer ranks as the most frequently diagnosed cancer among women. The proliferation of triple-negative breast cancer (TNBC) cells relies on glucose. The integrated stress response (ISR) represents a cellular reaction to glucose deficiency or other stress. Under glucose scarcity, the ISR-solute carrier family 7 member 11 pathway is activated, contributing to glucose dependency by reducing intracellular glutamate levels. Solute carrier family 1 member 5 (SLC1A5) and mitochondrial solute carrier family 1 member 5 variant (SLC1A5var) are glutamine transporters that play crucial roles in reprogramming cancer metabolism. However, the regulation of mitochondrial SLC1A5var by ISR and its impact on glucose dependency remains to be fully elucidated.
Our investigation indicated that activating transcription factor-4 (ATF4), triggered by glucose depletion, oligomycin (an inhibitor of mitochondrial ATP synthase), and salubrinal (an ISR activator) stimulation, induces the expression of SLC1A5var. The role of ATF4 in SLC1A5var regulation is pivotal, as it binds to specific regulatory elements in the promoter. Knockdown of SLC1A5var diminishes glucose depletion-induced cell death, while overexpression of SLC1A5var enhances it in TNBC cells. Additionally, SLC1A5var knockdown curtails cancer cell proliferation, colony formation, and migration, while SLC1A5var overexpression enhances cell proliferation and migration in TNBC cells. Furthermore, SLC1A5var knockdown reduces the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) and heightens the maximal OCR and ECAR under glucose depletion. These findings suggest that ISR-activated upregulation of SLC1A5var potentially modulates mitochondrial oxidative phosphorylation and glycolysis characteristics to augment glucose depletion-induced cell death.
In conclusion, SLC1A5var plays a pivotal role in the metabolic reprogramming process and may represent a promising target for breast cancer therapy.

P2-04-05: The dynein regulator LIS1 maintains cell cycle progression and DNA integrity in TNBC, and can be targeted to improve paclitaxel response

Parth Majmudar, Ruth Keri

Triple-negative breast cancer (TNBC) is a highly aggressive disease that typically exhibits faster growth and higher metastatic spread than other subtypes. There is a paucity of treatments available for TNBC, further contributing to poor patient prognosis. The rapid growth of TNBC cells makes mitosis a compelling target; indeed, a current mainstay of treatment is Paclitaxel (Ptx), a taxane that stabilizes microtubules during mitotic progression. Despite being initially effective, acquired resistance to Ptx is common. Moreover, additional antimitotic therapies can be similarly rendered ineffective due to

resistance, toxicity, or a lack of efficacy, underscoring the need for new therapeutic approaches. One understudied class of potential targets is the motor protein dynein and its associated regulatory proteins. Of these, LIS1 was highly ranked in several essentiality screens for breast cancer cell growth. LIS1 regulates mitotic spindle orientation and chromosome movement during neurodevelopment, yet little is known regarding its function in breast cancer. We found that suppressing LIS1 expression in TNBC cells, but not in non-transformed cells, reduces cell number. Live cell imaging indicates that reducing LIS1 alters cell cycle dynamics, increasing mitotic duration due to an accumulation of cells blocked in the G2/M phase. Such accumulation is commonly caused by activation of the spindle assembly checkpoint (SAC) or increased levels of DNA damage. Indeed, LIS1-silenced cells exhibit higher levels of Cyclin B1, a marker of SAC activation. Moreover, loss of LIS1 increases DNA double-strand breaks, highlighting a novel role for LIS1 in TNBC. Lastly, we found that the loss of LIS1 substantially enhances these defects in cells that are resistant to Ptx and sensitizes both parental and Ptx-resistant TNBC cells to Ptx treatment. As such, LIS1 represents a novel anti-mitotic target for treating TNBC, particularly in the context of paclitaxel resistance.

P2-04-06: Enhancement of TGF-β Receptor Inhibitor Efficacy through CD44 Suppression in Claudin-low Breast Cancer

Ryoichi Matsunuma, Sae Imada, Shoko Sato, Ryosuke Hayami, Michiko Tsuneizumi

Introduction: CD44 expression is implicated in various signaling pathways and has been reported to influence cancer proliferation and invasion. Claudin-low breast cancer is characterized by the properties of cancer stem cells, specifically CD44+/CD24- phenotype, and exhibits high expression of EMT markers. We focused on the involvement of CD44 and TGF-β receptors in EMT, investigating the combined effect of CD44 suppression and TGF-β receptor inhibitors in Claudin-low breast cancer. Our aim was to evaluate the potential of this dual approach as a novel therapeutic strategy for Claudin-low breast cancer.
Materials and Methods: We utilized the claudin-low breast cancer cell lines SUM159 and MDA-MB-231 to establish CD44 knockdown cells. The effects of CD44 knockdown and TGF-β receptor inhibitors on proliferation, invasion, and downstream signaling were investigated. Additionally, the potential synergistic effects of CD44 suppression combined with TGF-β receptor inhibitors were examined.
Results: CD44 was found to interact with TGF-β receptor I, but not with TGF-β receptor II. Additionally, CD44 knockdown did not affect the expression levels of TGF-β receptor I/II. In both SUM159 and MDA-MB-231 cells, CD44 knockdown resulted in suppressed proliferation, yet invasion was not inhibited, and phosphorylation of Smad2 was enhanced. However, when combined with TGF-β receptor inhibitors, CD44 knockdown led to synergistic suppression of proliferation, inhibition of invasion, and complete control of Smad2 phosphorylation.
Conclusion:
In CD44-positive Claudin-low breast cancer cells, CD44 knockdown exhibited a

proliferation-suppressive effect. The addition of TGF-β receptor inhibitors produced a synergistic effect in proliferation suppression and controlled Smad2 phosphorylation, thereby inhibiting invasion. This suggests that targeting CD44 in combination with TGF-β receptor inhibitors could provide a novel therapeutic strategy for Claudin-low breast cancer.

P2-04-07: Dual function of PRAK in TP53-associated breast cancer metastasis

Jingjing Liu, Xu Liu, Shaorong Zhao, Jin Zhang

The p53 mutation is the most common mutation in breast cancer, characterized by rapid proliferation, easy metastasis and poor prognosis. MAPK activated protein kinase 5, also named as PRAK, has been reported to function as either an oncogen or a tumor suppressor in different types of human cancers. The role of PRAK in breast cancer carcinogenesis and progression is still unknown. Our previous research found that PRAK function as a tumor suppressor in p53 wild breast cancer and function as an oncogene in p53 mutated breast cancer. In addition, PRAK influenced extracellular matrix (ECM) degradation and metastasis in breast by regulation of MMP9 expression. However, the molecular mechanism is still unknown. ChIP-seq combined with RNA-seq and protein profiling revealed that PRAK could bind to transcriptional factor CREB1 and wild p53. Moreover, we also observed the binding of PRAK to the MMP9 enhancer region. GST-pull down and IP were verified to bind from in vitro and in vivo; Transwell, scratch test and collagen contraction assay were used to evaluate the malignant phenotype of cells in vitro; inoculated SCID mice were observed to grow and metastasize in vivo by in vivo imaging; IHC was used to detect the protein expression of breast cancer tissues with different p53 statuses; and the correlation of its expression and its relationship to clinicopathological factors and prognosis were analyzed in the light of clinical data. In this study, we has found that in p53 wild-type breast cancer, PRAK binds to p53, and the activated p53 binds to the MMP9 promoter, which transcriptionally inhibits the expression of MMP9, prevents the degradation of extracellular matrix (ECM), and inhibits the metastasis of breast cancer; in p53 mutant breast cancer, PRAK binds to the MMP9 enhancer, CREB1 binds to the MMP9 promoter, and the combination of PRAK and CREB1 alters the conformation of the MMP9 genome, transcriptionally activates the expression of MMP9, induces the degradation of ECM, and promotes breast cancer metastasis. Ultimately, we elucidated the mechanism of PRAK in breast cancer with different p53 status and provide a theoretical basis for the development of therapeutic drugs in p53 mutated breast cancer.

P2-04-08: Functions of KLF5 in the cell cycle and breast cancer cell proliferation

Xiaoyun Mao, Jian Sun, Ceshi Chen

As a transcription factor associated with the regulation of growth, development, and maintenance of stemness, the expression and transcriptional activity of the KLF5 protein are influenced by the Hippo signaling pathway. This study aims to investigate the spatiotemporal expression characteristics of KLF5 during the development of breast cancer and further explore the molecular mechanisms by which KLF5, in coordination with the TAZ factor in the Hippo signaling pathway, controls cell growth and proliferation, potentially elucidating mechanisms underlying breast cancer initiation and progression. Results showed that the KLF5/AP1/TAZ/TEAD4 (KATT complex) is highly expressed in triple-negative breast cancer (TNBC), particularly in the basal subtype, regulating the transcription of downstream genes associated with stem cells and lipid metabolism, thus promoting lipid synthesis. Gel filtration chromatography and immunofluorescence staining confirmed the presence of the KATT complex in TNBC. Using knockdown experiments of the KATT complex and transcriptome sequencing analysis, we found that the KATT complex can collectively regulate the expression of lipid metabolism and stem cell-related genes. ChIP-seq analysis revealed mutual regulation among KATT complex members through super-enhancers, forming a core regulatory loop in TNBC. Furthermore, we found that the KATT complex promotes the synthesis of lipid metabolism products (lipidomics), significantly enhancing the synthesis of medium- and long-chain fatty acids (GC-MS). Knockdown of the KATT complex inhibited lipid droplet formation (Nile red staining) and suppressed cholesterol formation and distribution (cholesterol staining). Under cell culture conditions of human breast cells during the metaphase of mitosis, eGFP-KLF5 and endogenous TAZ form phase-separated granules with excellent co-localization characteristics. Similar to reported TAZ behavior, purified KLF5 protein exhibited phase separation capability in vitro. Upon formation of complexes with related transcription factors such as AP1 and TEAD4, it can collectively regulate the transcriptional expression of downstream target genes. Furthermore, we discovered that KLF5 protein undergoes liquid-liquid phase separation during the G2/M phase of the cell cycle and preliminarily found that the transcription factor KLF5 forms phase-separated granules with TAZ and participates in cell proliferation regulation. These data collectively demonstrate that KLF5 protein plays a crucial role in promoting cell cycle progression during the cell cycle process, thereby exerting significant effects on the rapid proliferation of triple-negative breast cancer cells.

P2-04-09: A Novel Role of IP6K2 in Regulating Host Cell Death through p21-mediated cell cycle arrest during HSV-1 oncolytic virus Therapy

Zhijian Huang, Xiaoting Qiu, Cuifeng Sun, Zirong Jiang

Introduction: Oncolytic virus therapy (OVT), specifically Herpes Simplex Virus type 1 (HSV-1)-based therapies, represents a promising frontier in cancer treatment. Despite its efficacy in clinical trials, variability in patient response, particularly resistance development, highlights the need for tailored therapeutic strategies. This study focuses on the role of Inositol Hexakisphosphate Kinase 2 (IP6K2) in modulating HSV-1 oncolytic virus infection, with an emphasis on understanding its influence on viral replication and host cell apoptosis.

Methods: The virus titer was measured on Vero cells using classical fluorescent plaque assay. The gene knock out was all carried by CRISPR/Cas9 system. qPCR and WB were conducted to evaluate biomarkers of apoptosis and relevant pathway. Attachment assay was conducted to verify the binding ability of virus to the host cells. Cell proliferation was assessed using CCK8 and apoptosis was assessed using flow cytometry. Subcutaneous xenograft model was used to evaluate IP6K2 knock out influence in vivo. cBioPortal and TCGA database were applied to analyze genomic alterations in pan-cancer. Results: The results revealed that HSV-1 infection induced significant apoptosis in targeted cells, characterized by enhanced apoptosis markers, p53 stabilization, and activation of the apoptotic signaling cascade including NOXA and PUMA upregulation. Knocking out IP6K2 resulted in attenuated HSV-1-induced apoptosis cell death and lower viral proliferation, but not binding ability of virus to the host cells. We further elucidated IP6K2 prevented apoptosis through p21 - mediated cell cycle G1 phase arrest by both rescue experiment and p21 CRISPR/Cas9 deletion. In vivo experiments also showed marked reduction in the efficacy of HSV-1 oncolytic virus-induced cell death, with decreased tumor regression and viral replication. Analysis of cBioPortal and TCGA databases corroborated the potential resistance stemming from IP6K2 mutations across various cancer types, underscoring the necessity for pre-treatment IP6K2 status assessment. Conclusion: This study underscores the role of IP6K2 as potential markers of resistance opens avenues for precision medicine approaches in OVT. Pre-treatment screening for IP6K2 mutations could significantly enhance patient selection processes, ensuring that individuals most likely to benefit from therapy are accurately identified.

P2-04-10: ARL3 Promotes Hormone Receptor Positive Breast Cancer Progression and Tamoxifen Resistance through ERα Stablization

Han Li, Yang Liu, Zehao Cai, Dan Shu, Yang Peng, Kang Li, Shengchun Liu

Background: Hormone Receptor Positive (HR+) breast cancer is predominantly managed with endocrine therapies that target the Estrogen Receptor Alpha (ERα), a key mediator of estrogen's role in promoting tumor growth. However, the emergence of endocrine resistance poses a significant clinical challenge, limiting the efficacy of these treatments and impeding optimal patient outcomes. Understanding the mechanisms behind ERα's importance and the development of resistance is crucial for advancing therapeutic strategies against HR+ breast cancer.
Methods: In our study, we utilized the GEO database to identify ADP-Ribosylation Factor-Like GTPase 3 (ARL3) as a gene associated with tamoxifen resistance in HR+ breast cancer. We constructed cell lines with ARL3 knockout and overexpression to assess the impact of ARL3 on cell proliferation, migration, and tamoxifen sensitivity. RNA sequencing (RNA-seq) and liquid chromatography-mass spectrometry (LC/MS) were employed to demonstrate the influence of ARL3 knockout on downstream ERα pathways. Furthermore, co-immunoprecipitation (CO-IP), immunofluorescence and Western blot (WB) assays confirmed the role of ARL3 in the ubiquitination and degradation pathway of ERα.

Results: In our analysis of TCGA breast cancer subtypes, we also observed that ARL3 is highly expressed in luminal breast cancer. Additionally, our collection of breast cancer tissue mRNA and immunohistochemistry data revealed that ARL3 expression is elevated in HR+ breast cancer, indicating a correlation with estrogen receptors. We found that ARL3 promotes the proliferation and migration of HR+ breast cancer. Furthermore, overexpression of ARL3 was associated with reduced sensitivity to tamoxifen, and in vivo tumorigenesis in nude mice showed that tumor volume and weight were significantly decreased compared to the control group. To investigate the underlying mechanisms, RNA-seq analysis of ARL3 knockout and control groups revealed a pronounced inhibition of the MYC pathway. Western blot (WB) validation confirmed that ARL3 knockout suppresses the expression of downstream pathways of ERα. Our data suggest that the association between ARL3 and ERα primarily occurs in the cytoplasm, mainly binding to the ligand-binding domain (LBD) of ERα, which is consistent with ARL3 not being recruited to the promoters occupied by ERα. We also discovered that the ARL3/ERα cascade promotes mitochondrial autophagy to enhance mitochondrial oxidative phosphorylation. In summary, our study establishes a non-genomic mechanism whereby ARL3 controls the transcription of estrogen-dependent genes associated with breast cancer cell proliferation by stabilizing ERα levels.
Conclusion: In our study, we posit that ADP-ribosylation factor-like GTPase 3 (ARL3) exerts a pivotal influence on the proliferative capacity, metastatic potential, and responsiveness to endocrine therapies in hormone receptor-positive (HR+) breast cancer. ARL3 modulates the stability of estrogen receptor alpha (ERα), thereby regulating the activation of downstream signaling cascades and the mitochondrial functionality within neoplastic cells.
Our findings suggest that ARL3 represents a promising therapeutic target for HR+ breast cancer. We envision that in-depth investigation into the molecular underpinnings of ARL3's role could pave the way for the development of innovative pharmacological agents capable of overcoming endocrine resistance in patients, ultimately enhancing their clinical outcomes and survival rates.

P2-04-11: Selective Pre-clinical Targeting of CD44+ ADAR1+ Triple Negative Breast Cancer

Wenxue Ma, Jessica Pham, Emma Klacking, Inge van der Werf, Neha Katragadda, Jenna Sneifer, Peggy Wentworth, Kendale Wirtjes, Ethan Lam, Sheldon Morris, James La Clair, Michael Burkart, Catriona Jamieson

Background: Triple-negative breast cancer (TNBC) represents a challenging subtype in breast cancer with limited therapeutic options. Both CD44 and ADAR1 are biomarkers associated with tumor progression, metastasis, and therapeutic resistance in TNBC. This study evaluates the efficacy of Rebecsinib, an experimental small-molecule anti-cancer drug designed to inhibit the splicing-mediated activation of the enzyme ADAR1 (adenosine deaminase acting on RNA 1), in targeting CD44+ and ADAR1+ cells in TNBC preclinical humanized mouse models.

Methods: Triple-negative breast cancer MDA-MB-231 cell line-derived xenograft (CDX) models were established in Rag2-/-gc-/- and NSG-SGM3 mice. First, MDA-MB-231 cells were lentivirally transduced with an ADAR-nanoluciferase-GFP reporter. Following in vivo imaging (IVIS, Caliper) to detect ADAR1-nanoluciferase reporter activity, engrafted mice were treated with DMSO vehicle control, Rebecsinib at a dose of 10 mg/kg by intravenous (IV), or Rebecsinib at a dose of 15 mg/kg by oral (PO) gavage, twice a week for two weeks. Tumor burden was assessed using in vivo imaging system (IVIS) 200. Single-cell suspensions from peripheral blood, lung, liver, spleen, and bone marrow were analyzed by flow cytometry (FACS) to determine the percentages of CD44+ and ADAR1+ cells.
Results: Both IV and oral administration of Rebecsinib significantly reduced CD44+ cells in peripheral blood ($p < 0.05$), lung ($p < 0.01$), liver ($p < 0.01$), and spleen ($p < 0.05$) in Rag2-/-gc-/- models (Student t test). A similar reduction in ADAR1+ cells was also observed in the lung ($p < 0.05$), liver ($p < 0.01$), and spleen ($p < 0.01$) following Rebecsinib treatment in NSG-SGM3 models (Student t test). IVIS imaging revealed a substantial decrease in tumor signals in Rebecsinib-treated groups compared to the controls ($p = 0.02$, student t test). Combination therapy of Rebecsinib with Fedratinib demonstrated enhanced inhibition of tumor cell proliferation, suggesting a synergistic effect ($p < 0.05$, student t test).
Conclusion: Rebecsinib effectively targets CD44+ and ADAR1+ breast cancer cells in preclinical TNBC models, demonstrating potential as a therapeutic option. These findings support further investigation of Rebecsinib in combination with other agents for treating TNBC.

P2-04-12: JAM A expression and its clinical/prognostic value in breast cancer

Yufei Lou, Johannes Benedikt, Wen G. Jiang, Tracey A. Martin

Background: Junctional adhesion molecules (JAMs) are a family of transmembrane proteins that localize in tight junctions at the apical and lateral membrane. JAM-A was the first family member to be described and functions in the cell as both a cell-cell adhesion protein and in the regulation of, barrier function contributing to the establishment of tight junction formation and epithelial polarity. Tight Junction proteins have been shown to be an important factor in the progression and metastatic progression. This study aimed to determine the role of JAM A in human breast cancer,

Method: The gene transcript expression levels of Jam A in human breast tumour and normal tissues were quantitatively determined in a Cardiff cohort of human breast cancer tissues (151 samples), supported by clinical, pathological information. Expression was analysed against the clinical and pathological parameters, together with clinical outcome of the patients including recurrence, metastasis and breast cancer related death. Statistical methods used were Mann Whitney U test for comparisons, logistic regression and Kaplan-Meier's methods for survival analyses.

Result: JAM A demonstrated an aberrant pattern of high expression in breast tumours compared with normal breast control tissues both in our own samples (p=0.06) and when analyses using the TCGA (The Cancer Genome Atlas) public database (P<0.001). Considering that our clinical sample size is not large, it is reasonable to consider both results as consistent. Our own clinical samples and TCGA samples both indicate that low levels of JAM A are related to longer overall survival (P=0.038 and P=0.003 respectively). In addition, our own samples show that for NPI (Nottingham Prognostic Index), JAM A has high expression in NPI3 group compared with NPI1 and NPI2 groups, the expression value for NPI3 group is 0.01 and the expression value for NPI1 and NPI2 group are 0.0052 and 0.0053 respectively. Though the P-value is greater than 0.05, it's still shows the expression trend. Our data also suggest that in the disease-free group, the expression of JAM A declines with ER positive status. GEPIA (Gene Expression Profiling Interactive Analysis) website analysis that JAM A correlates with ESR1(Estrogen Receptor 1) (R=0.22, P<0.001) and ESR2 (Estrogen Receptor 2) (R= -0.31, P<0.001). These results indicate that JAM A may be related to the regulation of ER expression. In addition, JAM A works as an activity regulator in TGF-beta receptor signalling in EMT (epithelial to mesenchymal transition) as assessed using the Pathcards website analysis. When considering co-expression in breast cancer, the results obtained via cBioPortal indicated that Nectin4 and DEDD were significantly correlated with JAM A (R=0.66, P<0.001 and R=0.65, P<0.001). Nectin4 is also located within the tight junction complex and DEDD (Death Effector Domain Containing) is a scaffold protein that directs CASP3 to certain substrates and facilitates their ordered degradation during apoptosis.

Conclusion: JAM A is aberrantly expressed in clinical breast cancer and is significantly correlated with predicting progression of breast cancer and outcome for patients. It can be suggested that JAM A, together with ER status could be a prognostic indicator in breast cancer and that JAM A could potentially be a future target for breast cancer treatment.

P2-04-13: DIFFERENTIAL SUSCEPTIBILITY OFMESENCHYMAL-LIKE AND BASAL-LIKE TRIPLE NEGATIVE BREAST CANCER CELLS TO STANDARD OF CARE TREATMENT

Ngoc Bao Vuong, Olga Korolkova, Michael Izban, Amos Sakwe

Triple-negative breast cancer (TNBC) is a subtype of breast cancer with negative expression of estrogen, progesterone, and HER-2 receptors. Clinically established chemotherapy is the main systemic treatment option; however, tumor heterogeneity and lack of biomarkers represent the significant challenges in TNBC therapeutic resistance and relapse. Therefore, it is important to identify the molecular mechanisms which underlie drug resistance in TNBC treatment. Our study focuses on the hypothesis that the breast tumors consist of mesenchymal-like (MSL) cells which show strong resistance to chemotherapies and basal-like (BSL) cells, which are sensitive to chemotherapy treatment. MSL tumor cells are

identified by the expression of high Vimentin and the Ca2+-dependent membrane binding Annexin A6 (AnxA6) while BSL TNBC cells are identified by low expression of AnxA6 and high Ki67 index. In this study, we develop in vitro models of chemotherapy-resistant MSL and BSL TNBC cell lines using three chemotherapeutic agents over 6 months. We aim to demonstrate that the frequent resistance of TNBC tumors to chemotherapy is driven by specific genes and/or secreted biomarkers in MSL and BSL TNBC subpopulations. We anticipate that AnxA6-high MSL cells will become resistant to treatment while AnxA6-low BSL cells will be responsive to chemotherapy. Hence, a comprehensive treatment response will depend on the proportion of MSL cells in a given TNBC tumor.

P2-04-14: Activation of Target X by Del-1 regulates the progression of TNBC

Byeongju Kang, Seol-Hwa Jeong, Soo Jung Lee, Jeeyeon Lee, In Hee Lee, Jin Hyang Jung, Eun Ae Kim, Jieun Kang, Ho Yong Park, Yee Soo Chae

Purpose: Developmental Endothelial Locus-1 (DEL-1), also known as EGF Like Repeats and Discoidin Domains 3 or Edil-3, is widely expressed and notably enriched in extracellular vesicles circulating in the plasma of breast cancer patients. Various papers have suggested that DEL-1 might be important in Triple Negative Breast Cancer (TNBC), the subtype with the highest mortality rate, even though it only accounts for 15%-20% of all breast cancers. Despite its importance, little is known about how DEL-1 regulates TNBC. Here, we report that target X, promoted by Del-1, stimulates proliferation and invasion in TNBC cells.

Methods: We performed a western blotting assay to clarify the relationship between DEL-1 and target X. To examine the effect of DEL-1 to target X, we used CRISPR/Cas9 genome editing DEL-1 knockout cell lines and pharmacological activator of X. In addition, the effects of DEL-1 and X to TNBC cells were analyzed using MTT, MTS, BrdU and Matrigel transwell assays.

Results: In TNBC cells, the expression levels of DEL-1 and target X were significantly upregulated. Both overexpression of DEL-1 and pharmacological activation of target X stimulated proliferation and invasion, whereas CRISPR/Cas9 genome editing Del-1 knockout inhibited target X and blocked proliferation and invasion. In addition, overexpression of DEL-1 increases target X, but the X activator did not have any effect on DEL-1

Conclusions: Altogether, we suggest that DEL-1-mediated activation of target X regulates TNBC proliferation and invasion.

P2-04-15: Diagnostic potential of disseminated and circulating cancer cells: A case study of a 34-years-old patient with rapidly progressing breast cancer.

Jonas Roth, Clara Chaiban, Lukas Wöhrl, Kirsten Utpatel, Valeria Gerthofer, Julian Schornbaum, Christian Werno, Olaf Ortmann, Stephan Seitz, Zbigniew Czyz, Christoph A. Klein

Presenting the case of a 34-year-old patient diagnosed with early breast cancer NST stage cT2, cN+, G3, M0, ER IRS4, PR IRS3, Her2neu 1+, Ki67 75% in July 2022 with rapidly progressing disease, we are exploring the diagnostic potential of disseminated and circulating cancer cells. Before and during the treatment of the patient, disseminated cancer cells (DCCs) were isolated using cytokeratin staining of bone marrow aspirates. Furthermore, the number of circulating cancer cells (CTCs) in 7,5 ml of blood was measured at different timepoints using the CellSearch® CTC-Assay. Single DCCs and CTCs were isolated using a micromanipulator. By comparing the number of CTCs with clinical data, we noted a correlation between the number of CTCs with treatment response, but also disease progression. As not only enumeration of CTC but also their molecular profiling may provide important information, we analyzed the mutational landscape of CTCs using the newly developed single cell IMPACT-Assay. This panel sequencing approach captures around 470 actionably or therapeutically relevant genetic changes. Based on the sequencing results we are currently performing an in vitro drug screen using 35 single drugs and drug combinations on cancer cells isolated from a pleural effusion of the patient. We will ask whether the mutational or the functional assays allow predicting the clinical response of the patient and provide a novel rational that could support decision making of the molecular tumor board. The results will be presented on the poster.

P2-04-16: Artificial intelligence can extract important features for diagnosing axillary lymph node metastasis in early breast cancer using contrast-enhanced ultrasonography

Tomohiro Oshino, Ken Enda, Hirokazu Shimizu, Megumi Sato, Mutsumi Nishida, Fumi Kato, Mitsuchika Hosoda, Kohsuke Kudo, Norimasa Iwasaki, Masato Takahashi

Background: Axillary lymph node (ALN) metastasis in early breast cancer affects the prognosis and accurate identification of ALN metastasis in early breast cancer is important for determining the treatment. Conventional ultrasonography (cUS) of breast cancer is used worldwide because it is inexpensive, simple, and does not involve radiation exposure and is not invasive. However, cUS is insufficient to evaluate ALN status accurately. Contrast-enhanced US (CEUS) improves the performance of predicting ALN metastasis, so at our facility, CEUS is performed routinely. However, there are many diagnostic indicators, and standard imaging and image interpretation methods have not yet been established, resulting in difficulty of use. To make CEUS universal and effective, we evaluate the ability of

predicting ALN metastasis and importance features, using deep learning (DL) models or weighted decision tree (WDT) model with ALN CEUS imaging data and tabular formatted data.
Methods：In this retrospective study, 788 CEUS images of ALNs were collected from 788 patients (mean age 59.7 [range, 25–90] years) , who underwent breast surgery between January 2013 and December 2021. First, CEUS images were inputted into the DL models, EfficientNet B0, B4, and B8 and VisionTransformer Base/16, which yielded an image-based predictive covariate (iP : 0–1) for ALN metastasis. Second, the tabular formatted data of primary tumor and ALN of cUS and CEUS was inputted into light gradient boosting machine (LightGBM), one of the WDT models. This data was including diameter of primary tumor and ALN evaluated by cUS and CEUS, 10 findings of primary tumor which could be evaluated by only CEUS, 9 findings of ALN evaluated by both cUS and CEUS, and 6 findings of ALN evaluated by only CEUS. These findings were evaluated by two breast surgery specialists. We also used iP for lightGBM models. Labeling data was histopathologic diagnosis of ALN surgical specimen divided into two groups pN0 or pN1mi and more.
Results： In the analysis of DL models, the area under the receiver operating characteristic curve (AUC) was highest for the EfficientNet B8 model at 0.69, which was used as the iP. In the analysis of LightGBM, the AUC for CEUS was 0.93 (0.88-0.98), significantly higher than that for cUS alone of 0.76 (0.68-0.85) ($p < 0.001$, DeLong test). Top feature importance for predicting of ALN metastasis was “heterogeneous enhancement pattern, “diffuse cortical thickening”, and “eccentric cortical thickening >3 mm”.
Conclusion：LightGBM model, an AI model, was able to extract CEUS findings that are important in predicting ALN metastasis in early breast cancer.

P2-04-17: The MIRACCL Portal for Comparing Patient and PDX Response Using Cancer Image Features and Genomics in Co-Clinical Breast Cancer Trials

Heidi Dowst, Fei Zheng, Emel Alkim, Apollo McOwiti, Ram Rajaram Srinivasan, David Hormuth, Thomas Yankeelov, Daniel Rubin, Michael T. Lewis

Introduction: The Molecular and Imaging Response Analysis of Co-Clinical Trials (MIRACCL, https://miraccl.research.bcm.edu/) platform was developed beginning three years ago to support the co-clinical breast cancer RESPONSE trial at Baylor College of Medicine. The goal of MIRACCL is to enable parallel studies which apply the same protocol to patients in a clinical trial and patient-derived xenograft (PDX) cohorts. MIRACCL evaluates treatment response by electronically measuring the changes in serial MRI. The response assessments include the measurement of multiple imagine features such as apparent diffusion coefficient (ADC) and signal enhancement ratio (SER) at pre-treatment and post-treatment along with samples taken for assessment of the genomic changes.
Methods: To achieve this goal, we employee the use of web-technologies to enable visual and quantitative comparison of the imaging and genomics. Investigators at Baylor College of Medicine leveraged their expertise in patient derived xenograft development and model

study implementation to generate the PDX imaging dataset and collect the model samples for sequencing. The University of Texas at Austin provided centralized patient and PDX image normalization, segmentation, and analysis while the Biomedical Informatics team at Stanford University provided the image visualization tool and imaging response assessment. Due to the difference in data modalities, data sources, and temporal collection of data it was necessary to place the data within the context of the study design and provide visual and quantitative comparisons of the study outcomes for various time points. The MIRACCL features created to achieve this goal include tabular cohort annotations, a side-by-side visualization of imaging response distributions, and a comparison of the upregulated and down regulated gene expression between cohorts. One of the imaging methods deployed in MIRACCL to assess treatment response measures response by tumor longest diameter which is then categorized by RECIST. Additional imaging comparison methods include signal enhancement ratio (SER), apparent diffusion coefficient (ADC), and tumor volume. Samples for sequencing taken at pre-treatment and throughout the design of the study are used to identify gene expression changes brought about by treatment. In the Omics module of MIRACCL, the 500 most frequently upregulated and 500 down regulated genes for each cohort are displayed based on user selected time points and imaging feature of interest. A Venn diagram is generated to identify the genes which are commonly regulated in both cohorts.
Results & Conclusion: While these features have provided multiple methods of comparing the outcome of co-clinical trials, the research team desired to know the significance of these correlations and differences between the two cohorts. Consequently, the Analytic module was implemented in MIRACCL this past year. The analytics module focuses on two hypotheses: 1) PDX models of similar subtyping will respond in a similar manner to the patients enrolled in the trial and 2) Response can be predicted while on-treatment to determine if changes to treatment are warranted. The change in tumor quantifications from on-treatment to baseline were statistically correlated to changes in tumor quantifications from post-treatment to baseline to determine if response could be determined while currently on-treatment. The p value and r value of the spearman correlation are provided to determine the significance and clustering of the cohort. The enhancements afforded by MIRACCL's Analytics module summarize the treatment response of the patient and PDX cohorts and effectively address the hypothesis of the REPONSE trial. MIRACCL is now available for expansion as a tool for other trials co-clinical trials.

P2-04-18: Unlocking the Complete Blood Count as a Risk Stratification Tool for Breast Cancer Using Machine Learning: A Large Scale Retrospective Study

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Optimizing early breast cancer (BC) detection requires effective risk assessment tools. This retrospective study from Brazil showcases the efficacy of machine learning in discerning complex patterns within routine blood tests, presenting a globally accessible and cost-effective approach for risk evaluation. We analyzed complete blood count (CBC) tests from 396,848 women aged 40-70, who underwent breast imaging or biopsies within six months after their CBC test. Of these, 2,861 (0.72%) were identified as cases: 1,882 with BC confirmed by anatomopathological tests, and 979 with highly suspicious imaging (BI-RADS 5). The remaining 393,987 participants (99.28%) were classified as controls, with BI-RADS 1 or 2 results.

The database was divided into modeling (including training and validation) and testing sets based on diagnostic certainty. The testing set comprised cases confirmed by anatomopathology and controls cancer-free for 4.5 to 6.5 years post-CBC. While our dataset lacked specific indicators for identifying metastasis and lymph node status required for TNM staging, the BC study population in the testing set primarily consisted of early-stage carcinomas, as reflected by the fact that 24% of the identified carcinomas were in situ and 95% of the recorded invasive T stages were at T1-2.

Statistical analysis revealed that age, hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), neutrophils-lymphocyte ratio (NLR), derived neutrophils-lymphocyte ratio (dNLR), platelet-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), systemic inflammation response index (SIRI), and aggregate index of systemic inflammation (AISI) were significantly elevated in women with BC, whereas lymphocytes and the lymphocyte-monocyte ratio (LMR) were markedly lower. Given the statistical significance of all CBC-derived ratios, they present a valuable opportunity as cost-effective predictive biomarkers in BC. Furthermore, by harnessing Artificial Intelligence (AI), we developed a ridge regression model incorporating age, NLR, and RBC, achieving an AUC of 0.64 (95% CI 0.64-0.65), demonstrating superior performance over more complex models like LightGBM, while offering full interpretability. The explanatory analysis indicates that higher age and NLR, along with lower RBC levels, may increase BC risk. Age correlates with BC due to cumulative exposure, genetic mutations, and cellular aging. High NLR suggests inflammation, potentially impairing immune response and promoting tumor growth, while low RBC might indicate a chronic inflammatory state conducive to cancer progression.

To emulate the use of a risk stratification tool, using the probabilistic output from this model, we divided the study population into four risk groups: high, moderate, average, and low risk, which obtained relative ratios of BC of 1.99, 1.32, 1.02, and 0.42, respectively. The aim of this stratification was to streamline prioritization, potentially improving the early detection of breast cancer, particularly in resource-limited environments. As a risk stratification tool, this model offers the potential for personalized breast cancer screening by prioritizing women based on their individual risk, thereby indicating a shift from a broad population strategy. To support its clinical application, external validation with diverse populations is of paramount importance.

P2-04-19: Identification and Validation of Novel Necroptosis-Related lncRNAs for Prognostic Prediction in Breast Cancer

Zhijian Huang, Xiaoting Qiu, Cuifeng Zheng, Yi Zeng

Background: Breast Cancer (BC) is the most common cancer in the world. The rapid development of tumor immunotherapy and necroptosis has brought new directions to the treatment of breast cancer. To improve the situation, this project aims to identify biomarkers that can help predict prognosis and determine a precise treatment.
Method: The Cancer Genome Atlas (TCGA) data was analyzed for coexpression relationships and univariate Cox regressions to identify lncRNAs associated with necroptosis. In addition, differential expression analysis, prognostic analysis, and time-dependent receiver operating characteristics (ROC) analysis were performed to determine whether Necroptosis-related lncRNA is an independent prognostic factor. Then, based on the risk model, principal component analysis (PCA), immune cell infiltration, immune functions, prediction of the half-maximal inhibitory concentration (IC50), and immune checkpoints were evaluated. Additionally, we divided the entire set into two clusters based on immunotherapy response between cold and hot tumors.
Results: We developed a signature consisting of seven necroptosis-related LncRNAs. Over 1, 3, and 5 years, the area's OS under the ROC curve (AUC) was 0.748, 0.753, and 0.714, respectively. High-risk group immune cells were infiltrated less frequently and had lower immune functions than low-risk group immune cells. In addition, using clusters as a means to make a distinction between a cold tumor and a hot tumor could provide more precise treatment options. In Cluster 2, the tumor is considered to be hot tumor, which is more sensitive to immunotherapy drugs.
Conclusion: According to our findings, necroptosis-related lncRNA can be used to predict prognosis and to distinguish cold and hot tumors in BC, thereby optimizing individual treatment.

P2-04-20: Advances and Detection Methods of Metachronous Bilateral Breast Cancer Detection Using AI-Assisted Mammography for Japanese women

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Background: Patients after unilateral breast cancer(BC) are at higher risk for bilateral BC. After unilateral BC, diagnosis by mammography (MG) is more difficult because the lack of comparison with contralateral side. In this study, we examined the diagnostic status of metachronous bilateral BC and tested whether the artificial intelligence (AI) system in MG can detect BC correctly or at an earlier than human diagnose.
Methods: The subjects were patients who underwent metachronous bilateral BC surgery between 2014 and 2022. We reviewed the medical records retrospectively. The AI system is FxMammo® (FathomX Pte Ltd, Singapore). FxMammo® is based on deep learning and has

been put into practical use in some countries. The AI was created by collecting 17,769 cases (of which 45% were malignant) from 10 institutions in Asia. Our institution was involved in the development of the system. The AI system analyzed four MG images (craniocaudal [CC], mediolateral oblique [MLO], left and right) The AI system indicated the probability of malignancy for each of imaging s as a percentage. The threshold value was set as 40.0 %, (Sensitivity was set at 91.5 %and specificity was 82.0%). In addition, region of interest was displayed in color on a heat map.
Results: Among the 1101 patients who underwent surgery, there were 36 cases of metachronous bilateral BC. Twenty cases (56%) were detected during postoperative surveillance for BC, 9 cases (25%) by subjective symptoms, 3 cases (8%) by screening, and 4 cases (11%) during follow-up for other diseases. Of the cases detected by subjective symptoms, except in one case, more than 10 years had passed since the initial surgery at the time of diagnosis of the contralateral BC. In 22 cases (61%), lesions were noted in both MG and ultrasonography (US), in 2 cases (5%) only in MG, and in 10 cases (28%) only in US. One case (3%) had no lesions in both MG and US and underwent Magnetic Resonance Imaging (MRI)-guided biopsy. One case (3%) had a preoperative diagnosis of Paget's disease with no abnormality and final pathology was ductal carcinoma in situ (DCIS). We used the AI system to examine MG in 10 patients who were undergoing surveillance at our hospital after partial resection for unilateral BC. The AI system diagnosed 6 of 10 cases (60%) as malignant. In 2 of these cases (20%), the radiologists diagnosed no malignancy. The AI system also diagnosed malignant in the year prior to the diagnosis of malignancy in these 2 cases. In both cases, small invasive carcinomas were found within extensive DCIS. Of the 4 cases that the AI system diagnosed as not possibly malignant, in 1 case the radiologists diagnosed malignant with MLO only. In 3 of the 10 cases, both the AI system and radiologists diagnosed MG with no malignancy.
Conclusions: Although postoperative unilateral BC is considered a high risk for birateral BC, some cases were detected by physical examinations. While MG is recommended for postoperative surveillance, there were cases that could be identified by only US. There was one case in which only MRI was effective. In some cases, the AI system diagnosed malignant even when the radiologists diagnosed no malignancy. This AI system has the potential to detect contralateral breast cancer earlier and accurately.

P2-04-21: Dynamic reporting of treatment related symptoms via ePROs can reversely identify the type of underlying cancer

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Background: Digital symptom reporting through cancer patients (ePRO) undergoing systemic treatment has demonstrated detection of symptoms, equivalent side effects regarding similar drugs, reduction of unplanned admissions, and machine learning (ML) may predict when patients will require emergency treatments. We examined whether dynamic reporting of treatment related symptoms via ePROs can reversely identify the type of underlying cancer.

Methods: 226 patients on treatment had self-reported on presence and severity (according to CTCAE) of more than 90 available symptoms via the medidux app (formerly consilium care). For a balanced analysis we used data from 25 patients treated for breast cancer, 19 for cancer of lung, 16 for colon, 12 for lymphoma and 7 for prostate cancer, respectively. Patients` symptoms over the entire study period were aggregated by counting the days on which a particular symptom was reported. Thus, each patient was represented by a vector of symptoms indicating how often the given symptom occurred. A human-interpretable ML logistic regression model was applied to predict the primary tumor of the patient from his/her respective symptom vector. All symptoms with positive coefficient above a certain threshold (0.1) were collected and then graphically displayed for association between symptoms and cancer type.
Results: The ML model was not able to recognize the prostate and blood-lymph patients in retrospect since their number was too small. Analysis for three remaining cancer types revealed a mean area under the curve (AUC) score of 0.72 (breast cancer AUC 0.74, CI: 0.62–0.85; gut cancer AUC 0.78, CI: 0.66–0.89; lung cancer AUC 0.63, CI: 0.50–0.77). Results indicate that ML performs "fair" and significantly better than random guessing (which would result in AUC = 0.5) for the reverse identification of the underlying cancer upon ePRO reporting from patients.
Conclusion: Cloud aggregation of patient reported symptoms and ML harbor the potential in identifying the type of cancer for which patients receive systemic treatment. Whether associations can be made from dynamic changes of reported symptoms, regarding the underlying cancer and adherence to oral medication shall be explored in prospective studies. Finally, ML and the anticipation of specific side effects might be a cost-effective tool in decentralized clinical trials and registries, enabling a more nuanced understanding of symptom associations with different cancer types.

P2-04-22: Predictive Modeling of Cancer Treatment-Related Cardiac Events in Breast Cancer Patients: Utilizing Dosiomic and Radiomic Features with Machine Learning

Sefika Dincer, Sefika Dincer, Muge Akmansu

Purpose: Conventional clinical study consists of clinical, radiological and dosimetric data and follow-up for many years. In the last decade, personalized medicine has become the main subject of scientific research with genome maps and biomarker discoveries. This study focused on predicting treatment-associated cardiac side effects in breast cancer patients, before they occur, using cardiac related biomarkers (hs-TopT, radiomics, dosiomics). This is the first study of heart-segmented dosiomics in breast cancer patients.
Methods: In this retrospective study, clinical and dosimetric data, along with radiomic and dosiomic features extracted from medical imaging, were analyzed for 42 women diagnosed with localized breast cancer (patients whose blood troponin levels were measured 2-3 weeks following the completion of radiotherapy). Patients with pre-existing cardiac comorbidities or previous troponin-T elevation were excluded from the study. The cutoff

value of heart specific troponin T levels at 2-3 weeks following radiotherapy was determined 14 ng/L, and the patients were classified into two groups. Those above this limit value were considered to be associated with a cancer treatment-related cardiac event. For the extraction of radiomic and dosiomic features, an open-source Python package PyRadiomics was utilized. In this study, we employed the TPOT (Tree-based Pipeline Optimization Tool) to select and optimize machine learning models and hyperparameters. This process led us to identify the 'Gradient Boosted Classification Algorithm' as the algorithm that would yield the best performance. Gradient boosted recursive feature elimination, a hybrid method, was used to select important features for prediction. Gradient boosted classification algorithm, an embedded method, was used to create and test the model. 5-layer cross-validation and nonparametric permutation testing were performed to evaluate model generalizability and randomness. The area under the curve (AUC) method was used to evaluate model performance.
Results: In total, 111 dosimic and 119 radiomic features were extracted for each patient. A total of 6 different models were created with different feature groups (clinical, dosimetric, radiomics, dosiomics). The highest prediction model was obtained with clinical + dosiomics + radiomics parameters (test set-AUC = 0.96). This value was much lower for the clinical + dosimetric model (test set-AUC = 0.67). These two models were found to be non-coincidence based on confirmation from nonparametric permutation tests ($p<0,05$). Other models were considered random based on permutation testing ($p>0,05$). Cross-validation analyses for the clinical+ dosiomic+ radiomics model showed that the generalizable performance of the model was relatively lower but still fair-to-good (mean AUC value 80.33 ± 21%).
Discussion: This study can demonstrates that imaging biomarkers, specifically radiomics and dosimics, exhibit superior predictive capability for treatment-related cardiac events in breast cancer patients compared to traditional clinical and dosimetric parameters (96% vs. 67%). Incorporating radiomics and dosiomics parameters into future clinical practice can serve as critical indicators. Fundamentally altering treatment approaches and follow-up strategies by achieving nearly perfect predictive success (96%) in anticipating cardiac side effects before their occurrence. This may suggests that high statistical imaging biomarkers should be integrated into personalized clinical practice. Further investigation is needed.

P2-04-23: Analytical validation of a high-definition tumor-informed MRD assay demonstrates robust detection at low tumor fractions common in breast cancer

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Background: Molecular residual disease (MRD) refers to the small number of tumor cells remaining in the body during or after cancer treatment. These tumor cells shed DNA into the bloodstream, resulting in circulating tumor DNA (ctDNA). ctDNA can be identified in

plasma-derived cell-free DNA (cfDNA) via the presence of tumor-specific somatic variants. We have developed a second-generation tumor-informed MRD assay to detect the presence and quantity of ctDNA in the plasma of breast-cancer patients with residual disease or tumor recurrence. Our assay detects the presence of ctDNA using a hybrid-capture based sequencing panel, targeting tumor-specific somatic variants. Each panel is designed with up to 1000 carefully selected somatic variant targets identified based on tumor profiling by matched tumor and normal whole genome sequencing and is optimized to provide high sensitivity and specificity at low tumor fraction, which is particularly important because many breast tumors have low shedding of ctDNA. Targeted sequence data is fit to a statistical model that incorporates panel-specific and sample-specific parameters to provide a robust estimate of the ctDNA fraction with a rigorous assessment of confidence for each test result. Here, we evaluated the performance of our assay at low ctDNA fraction.
Methods: Contrived mixtures of matched tumor and normal breast cancer cell lines and healthy donor plasma were were evaluated over a range of tumor DNA concentrations from 1 part per million (ppm) to 10,000 ppm, and were processed across multiple days, reagent lots, operators and instruments.
Results: Analysis of healthy donor samples paired with non-patient matched panels demonstrated the Limit of Blank (LoB) at a 95% specificity threshold—i.e., the threshold at which 95% of negative samples return a negative result—to be 0.3 ppm. When the LoB was used as the detection threshold, we found the Limit of Detection with 95% sensitivity (LoD95) to be <5ppm. Using a more stringent detection threshold of 1.4 ppm—corresponding to a specificity of 99.615%—we found the LoD95 to be 10 ppm. Both PPV and NPV were 100% when assessed with 56 positive samples at 50 ppm and 126 negative samples. Additionally, quantification of ctDNA fraction was linear over the entire range of tumor DNA concentrations assessed.
Conclusions: Overall, our assay demonstrated high sensitivity, specificity and measurement accuracy, which, together, will facilitate improved resolution in residual-disease detection and extend lead times in recurrence detection.

P2-04-24: CINDERELLA Clinical Trial (NCT05196269): initial Insights into Patient Engagement with an Artificial Intelligence-Based Healthcare Application for Enhancing Breast Cancer Locoregional Treatment Decisions

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Background: The CINDERELLA Project introduces an innovative artificial intelligence (AI)-based tool, the CINDERELLA APP, designed to enhance the shared decision-making process

in breast cancer treatment. This clinical trial aims to assess the Digital Health intervention's effectiveness in improving patient satisfaction with locoregional treatment aesthetic outcomes, aligning patient expectations with actual results, and evaluating its influence on overall quality of life and psychological well-being. The adoption and utilization of digital health applications in this context have not been extensively explored, making this study crucial for evaluating the app's acceptability, usability, clinical impact and patients' satisfaction. This work specifically addresses patient engagement and usage patterns of the CINDERELLA APP within the interventional arm of the trial during the first ten months of recruitment.
Methods: The CINDERELLA Trial is an international, multicenter, interventional, randomized, controlled, open-label clinical study. Patients with a diagnosis of primary breast cancer and no signs of systemic disease are enrolled and randomly assigned to the intervention or control group. The intervention arm utilizes the CINDERELLA APP, which leverages AI and digital health technologies to provide patients with comprehensive information (i.e., text, images, videos) about existing and proposed locoregional treatments, including photographs of similar cases treated with the same surgical techniques. Data collection encompassed patient demographics and app usage metrics (i.e., duration, logins, content engagement). Comparative analyses of app usage time across different patient subgroups were conducted using the Wilcoxon or Kruskal-Wallis rank-sum test. Statistical analysis was conducted using R 4.4.1 for Windows and RStudio (2024.04.2). The CANKADO platform was the primary digital platform for physicians to manage the app content and collect data, ensuring adherence to privacy, data protection, and ethical guidelines in AI applications.
Results: The clinical trial recruited 474 patients from August 8, 2023, to June 30, 2024, across seven study sites in five countries. Of these, 246 patients, with a median age of 53 years, were randomized to the interventional arm. The average app usage duration was 19.25 minutes (min) with a median of 4 logins/user, over a median follow-up period of 3.2 months. Israel recorded the lowest app usage time with an average of 10.26 min and a median of 1.5 logins/user, while Poland recorded the highest with 24.68 min and a median of 6 logins/user. There were no statistically significant differences in median app usage time based on age groups (<40 years, 6.55 min vs. 40-60 years, 11.8 min vs. >60 years, 14.17 min, p=0.136) or estimated type of surgery (11.58 min for breast-conserving surgery vs. 11.68 min for mastectomy with reconstruction, p=0.721). Noteworthy, patients expected to undergo radiotherapy used the app longer than their counterparts (12.81 min vs 9.06 min, p=0.034). The "Frequently Asked Questions" section was the most accessed part of the app, accounting for 44.61% of the total 3201 hits.
Conclusions: Initial data indicate good engagement with the CINDERELLA APP. The presence of only minor differences in usage patterns across countries, age groups, and treatment types suggests that the app may play a role in filling the informative gaps related to patients' characteristics. The CINDERELLA Trial is ongoing, with final results predicted to be published in 2026 once recruitment and follow-up have been concluded. These initial findings regarding the app's usability, together with the trial's final results will inform enhancements to design AI-based clinical decision support systems to better meet patient

needs and improve decision-making processes in breast cancer treatment.
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P2-04-25: Different Pyroptosis Phenotype within Breast Cancer Possess Distinctive Prognosis and Tumor Immune Microenvironment

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Pyroptosis, a type of programmed cell death that was involved in tumorigenesis, progression, and tumor immune microenvironment (TME). However, little is known about the pyroptosis phenotype of breast cancer (BC) and its' role in the prognosis and TME. Here, we acquired BC data and performed comprehensive analyses to dissect BC's pyroptosis patterns and corresponding characteristics. Based on transcriptomic data, we identified three types of pyroptosis patterns (type 1-3), which possessed distinctive prognosis, immunologic features, genomic alterations, and clinical characteristics. Type 3 with lowest pyroptosis activity showed worst prognosis, highest tumor purity, and coldest TME (significantly downregulation of cancer antigen presentation, infiltration of immune cells into tumors, recognition of cancer cells by T cells, killing of cancer cells, and so on). Type 2 with highest pyroptosis activity had better prognosis and hotter TME. Further analyses demonstrated the difference in anti-tumor immune among three pyroptosis patterns and identified CACNA1B, a potential crucial molecular that caused different prognosis and immune microenvironment. Taken together, our study firstly uncovered three types of pyroptosis patterns in patients with BC and their molecular features, which provided reference into further study targeting pyroptosis and its role in immune microenvironment.

P2-04-26: Predicting the prognosis and immunotherapeutic response of triple-negative breast cancer by constructing a prognostic model based on CD8T cell-related immune genes

Zhijian Huang, Yunyun Han, Xiaoting Qiu

Background: triple-negative breast cancer (TNBC) posed significant challenges in terms of treatment efficacy. CD8+ T cells, pivotal immune cells, can be effectively analyzed for gene expression differentials across diverse cell populations owing to the rapid advancements in sequencing technology. Leveraging these genes, our objective was to develop a prognostic model that accurately predicted the prognosis of TNBC patients and their responsiveness to immunotherapy.
Methods: The sample information and clinical data of triple-negative breast cancer (TNBC) were sourced from the TCGA database and METABRIC database. In the initial stage, we identified 67 differentially expressed genes associated with immune response in CD8+ T cells. Subsequently, we narrowed down our focus to three key genes: CXCL13, GBP2, and GZMB, which were utilized for constructing our prognostic model. The accuracy of the

model was assessed using validation set data and receiver operating characteristic (ROC) curves. Furthermore, we employed various methods including KEGG pathway analysis, immune infiltration analysis, and correlation analysis with CD274 to explore the model's predictive efficacy in immunotherapeutic responses. Additionally, we investigated the potential underlying biological pathways that contribute to divergent treatment responses.
Results: In the initial stage, we identified 67 differentially expressed genes associated with immune response in CD8+ T cells. Subsequently, we narrowed down our focus to three key genes: CXCL13, GBP2, and GZMB, which were utilized for constructing our prognostic model. We successfully developed a prognostic model capable of predicting the prognosis of TNBC patients. The area under the curve (AUC) values for 1, 3, and 5-year survival predictions were determined to be 0.618, 0.652, and 0.826, respectively. Employing this risk model, we stratified the samples into two distinct groups: high-risk and low-risk group. Through KEGG enrichment analysis, we observed that the high-risk group predominantly exhibited enrichment in metabolic-related pathways, such as drug metabolism and chlorophyll metabolism, whereas the low-risk group demonstrated significant enrichment in cytokine pathways. Furthermore, immune landscape analysis revealed noteworthy variations between CD274 expression and risk scores, indicating that our model effectively predicted the response to immune-based treatments in patients.
Conclusion: In conclusion, our study demonstrated the potential of CXCL13, GBP2, and GZMB as prognostic indicators for clinical outcomes and immunotherapy response in patients with triple-negative breast cancer. These findings provided valuable insights and present a novel avenue for immunotherapeutic approaches targeting triple-negative breast cancer.

P2-04-27: Development & Validation of RSC4All: an Artificial Intelligence-driven Machine Learning Nomogram Enhanced with Synthetic Data to Predict RSClin® Results & Guide Adjuvant Treatment of Node-negative HR-positive/HER2-negative Early Breast Cancer

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Background: RSClin™ is a proprietary algorithm that combines clinicopathological (CP) factors (age, tumor size and grade) with genomic risk to refine the prognosis of distant recurrence (DR) and chemotherapy (CT) benefit in patients with Node-negative (N0) Hormone Receptor Positive (HR+)/Human Epidermal Growth Factor Receptor Negative (HER2-) Early Breast Cancer (eBC). Since RSClin™ is only available in the USA, we aimed to develop an automated ML-based nomogram, RSC4All, to predict RSClin™ outcomes and provide free access to this predictive tool via a web-based link. Additionally, we generated synthetic data (SD) using Generative Adversarial Networks (GANs) to augment the initial dataset and enhance the tool's predictive accuracy.

Methods: We retrospective collect CP and genomic data from 290 patients with N0 HR+/HER2- eBC who underwent OncotypeDX from 2020 to 2022 at Italian and Belgian hospitals, with available RS and RSClin™ outputs of DR risk and CT benefit. Patients were randomly split into 2 groups, 70% allocated to the training set and 30% to the validation set. The ML-nomogram was developed using classification and regression models, including linear and logistic regression, to predict the categories (high vs mid/low for DR and yes vs no for CT) as well as the precise value for DR and CT benefit. Additionally, 177 synthetic patient records were generated through GANs and included in the training set to enhance the tool's predictive capability.
Results: Compared to RSClin™ outcomes, the classification model for DR, trained on the original dataset, achieved a ROC AUC of 0.97, while for CT benefit achieved a score of 0.99. The regression analyses for DR and CT benefit yielded significant R2 scores of 0.84 and 0.72, respectively. The inclusion of SD improved the dataset's diversity and representativeness, closely mirroring the real cohort's characteristics. Incorporating SD improved both classification and regression metrics, notably increasing the regression accuracy for predicting precise DR and CT benefit values. A web application was developed to provide easy and free access to the developed ML models (https://rsc4all.streamlit.app).
Conclusions: RSC4All accurately reproduces RSClin™ results to support treatment decisions for patients with N0 HR+/HER2- eBC. The incorporation of SD further enhanced the predictive accuracy of the ML tool, supporting the value of AI-driven data augmentation in oncology.

P2-04-28: Signal Processing Techniques for Investigating the role of Viral Infections in APOBEC3 Enzymes in Tumor Tissue Biopsies

Mohadeseh Soleimanpour, Jake Lehle, Diako Ebrahimi

The National Cancer Institute has designated several viruses such as EBV, HBV, HCV, HIV, HPV, and HTLV as agents that either cause cancer or increase the risk of developing cancer. However, extensive analyses of tumor genome and transcriptome datasets have identified numerous additional virus types, the functional implications of which remain largely unknown. Viral infection often dysregulates unique molecular processes within tumor cells and alters the composition of immune cells in the tumor-microenvironment. For instance, HPV-positive tumors have been shown to exhibit a significantly elevated expression of the DNA editing enzyme APOBEC3B compared to HPV-negative tumors. Furthermore, HPV positive tumors display and increased number of C-to-T mutations induced by this protein. The genome and transcriptome of tumor tissue biopsies contain a wealth of information about these virus-associated signatures. However, advanced signal processing approaches are needed to extract these viral signatures from complex tumor datasets, and elucidate their specific roles in cancer. Here, we developed a quantitative approach to investigate the link between viral infection and dysregulation of a seven-membered A3 enzyme family. The primary role of these enzymes is to restrict viral infections by inducing C-to-U mutations in viral genomes. However, at least three members of this enzyme family (A3A/B/H) can also

induce mutations in cellular DNA, and drive tumor formation and evolution. We used NMF (Nonnegative Matrix Factorization) to deconvolute the expression profiles of A3 enzymes in >10,000 bulk RNAseq datasets from diverse TCGA tumors. This analysis revealed the expression profiles of A3 enzymes within tumor and tumor microenvironment cells including various immune cells. Next, we conducted an association analysis to identify the link between the presence of viral infection and the proportion of each of these tumor and tumor microenvironment expression profiles. As expected, our analyses revealed strong associations between tumor-specific A3 expression profiles and infections by oncolytic viruses such as HPV. Notably, and somewhat unexpectedly, our analyses also revealed associations with viruses about which little is known in the field of cancer. Taken together, our analyses provide evidence for the link between A3dysregulation and infection by viruses including bacteriophages.

P2-04-29: Predictive model of prognosis index for invasive micropapillary carcinoma of the breast based on machine learning: A SEER population-based study

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Background: Invasive micropapillary carcinoma (IMPC) is a rare subtype of breast cancer. Its epidemiological features, treatment principles, and prognostic factors remain controversial.
Objective: This study aimed to develop an improved machine learning-based model to predict the prognosis of patients with invasive micropapillary carcinoma.
Methods: A total of 1123 patients diagnosed with IMPC after surgery between 1998 and 2019 were identified from the Surveillance, Epidemiology, and End Results (SEER) database for survival analysis. Univariate and multivariate analyses were performed to explore independent prognostic factors for the overall and disease-specific survival of patients with IMPC. Five machine learning algorithms were developed to predict the 5-year survival of these patients.
Results: Cox regression analysis indicated that patients aged >65 years had a significantly worse prognosis than those younger in age, while unmarried patients had a better prognosis than married patients. Patients diagnosed between 2001 and 2005 had a significant risk reduction of mortality compared with other periods. The XGBoost model outperformed the other models with a precision of 0.818 and an area under the curve of 0.863.
Conclusions: A machine learning model for IMPC in patients with breast cancer was developed to estimate the 5-year OS. The XGBoost model had a promising performance and can help clinicians determine the early prognosis of patients with IMPC; therefore, the model can improve clinical outcomes by influencing management strategies and patient health care decisions.

P2-04-30: Harnessing Technology: A Comparative Study of AI versus Manual Scoring in HER2 Ultra-low Breast Cancer

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Background: The advent of targeted therapies using the HER2 receptor as a delivery mechanism in breast cancer, introduced a need for re-evaluation HER2 scoring in previously classified negative cases (2+ ISH -ve; and 1+), as patients with these tumours may benefit from these innovative treatments. At The Christie Hospital. Manchester, U.K., our specialised unit (BTRU) has been exclusively reporting ER, PR, and HER2 status in breast cancers for the past 25 years with a dedicated staff, adhering to consistent technical guidelines and stringent UKAS accreditation standards, with an annual assessment of approximately 1,600 cases. This study aimed to evaluate the performance of an AI solution in the identification of HER2 ultra-low compared to a cohort of reported HER1+ and 0 cases that were manually scored by the BTRU.
Methods: We retrieved a cohort of 288 cases reported as HER1+ or 0 during 2023 (IHC assay: VENTANA Pathway anti-HER2/neu (4B5) rabbit monoclonal primary antibody). The original slides were digitised, scanned at x40, and submitted anonymised to Visiopharm for analysis. No re-scoring of the cases was conducted. The AI system not only provided HER2 scoring but also detailed the total number of tumour cells evaluated, the completeness of membrane staining (complete/incomplete/negative), and staining intensity (weak/moderate/strong).
Results: A total concordance of 85.7% (247 cases) was observed between the AI and previously reported results. The AI upgraded the manual scores in 6 cases from 1+ to 2+ and in 24 cases from 0 to 1+. Conversely, 11 cases originally scored as 1+ were re-evaluated by AI and scored as 0. No significant differences in tumour cell content were noted between concordant and discordant cases.
Conclusions: Overall, there was strong agreement between the two methodologies; however, the AI solution outperformed manual scoring in 24 cases (8.3%), which would otherwise be deemed unsuitable for treatment. Notably, the membrane staining in the 11 cases re-scored by AI as 0, was just bellow the 10% threshold currently established by UK and ASCO/CAP guidelines, underscoring the challenges in achieving precision in borderline cases through manual scoring. Given the unique nature of our unit, it is anticipated that a greater number of discordant cases may be identified in different clinical settings. These findings suggest that incorporating AI tools into pathology practice could enhance patient selection for new therapies, as well as optimising cut-offs for identifying potential responders in future clinical trials.

P2-05-01: Telemedicine in physical therapy after axillary surgery for early breast cancer

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Background: Reduction of long-term side effects of axillary surgery for early breast cancer is still an unmet medical need. Although axillary surgery is increasingly perceived as a diagnostic rather than a therapeutic tool there will always be patients for whom the removal of axillary lymph nodes is a necessary intervention. The onset of physical exercise for the prevention of shoulder-arm morbidity immediately after axillary surgery is recommended in national and international guidelines and is usually part of the routine work-up during the inpatient stay after breast cancer surgery. However, in times when breast cancer surgery becomes an outpatient procedure, limited access to qualified physiotherapy could lead to a higher risk of shoulder-arm morbidity. Approaches including telemedicine solutions as part of the physiotherapy concept could be an option when physical therapy in person is not possible. Here we are presenting the results of a non-randomized two-arm proof-of-concept trial comparing standard physiotherapy and a telemedicine approach.
Methods: Patients scheduled for axillary surgery for early breast cancer were prospectively recruited. All received a brief introduction to 4 physical exercises for the prevention of shoulder arm-morbidity and were handed tablets with the pre-installed EvoCare®-app. During 4 weeks, they exercised at home and took videos of themselves. Physiotherapists watched the videos and gave feedback via the app. After 4 weeks, all patients answered questionnaires with items from the BREAST Q® and the Quick Dash® instruments. The questions covered social activities, sleep, arm function and lymphedema. For the control group patients who had received the standard physiotherapy after axillary surgery including the same 4 exercises were invited to take part in the retrospective part of the study and to answer the same questionnaires. Results were compared using the t-test. A two-sided p of </= 0.05 was considered statistically significant.
Results: 41 patients were included in the analysis, 21 in the telemedicine group and 20 in the standard physiotherapy group. The groups were well balanced with no statistically significant difference regarding age (p=0.24), breast conservation (p=0.74), mastectomy (p=0.95), sentinel node biopsy (p=0.09), axillary dissection (p=0.28), targeted axillary dissection (p=0.16), ER (p=0.68), PR (p=0.78), HER2neu status (0.68), neoadjuvant chemotherapy (p=0.24) and number of involved lymph nodes (p=0.39). There was a statistically significant difference regarding the number of removed lymph nodes (p=0.03) with 3.7 (SD 4.02) in the telemedicine group and 7.1 (SD 5.39) in the standard physiotherapy group mainly resulting from the different standards regarding the postneoadjuvant management of the axilla at the different timepoints the two groups were treated.
The results of the two groups demonstrated neither statistically significant differences in the BREAST Q®items “sleeping disorders” (p=0.135), “lymphedema” (p=0.07) and “difficulties lifting something” (p=0.58) nor in the Quick Dash® items “carrying a bag” (p=0.31), “social activity” (p=0.15) and “physical limitations at work” (p=0.21).
Conclusion: Standard physiotherapy and the telemedicine approach yielded comparable results regarding lymphedema, shoulder-arm-morbidity, sleep and social activity. Telemedicine could be an option after outpatient breast cancer surgery, in settings where patients have to travel long distances to the next physiotherapy unit or where the lack of

resources leads to the omission of physical therapy after axillary surgery for early breast cancer.

P2-05-02: IDENTIFICATION OF MOST COMMON CONCERNS IN BREAST CANCER SURVIVORS FROM 2019-2023 IN THE MRW LIFE SURVIVORSHIP PROGRAM

Carol A Rosenberg, Anisha Patel, Poornima Saha

Background: The number of breast cancer survivors continues to increase. Unfortunately, many survivors experience late or long-term physical and/or psychosocial effects of cancer treatments. The National Comprehensive Cancer Network (NCCN) has established guidelines on survivorship care to help healthcare professionals address the complex and varied needs of cancer survivors and has recommended screening for common survivorship concerns. The MRW Living in the Future (LIFE) Cancer Survivorship Program at Endeavor Health offers a comprehensive educational program that includes a risk-adapted visit (RAV) during which an individualized survivorship care plan (SCP) is provided and discussed. In this report, we evaluate the pre-visit assessment as an effective way to help survivors self-identify their most meaningful concerns during their cancer journey.
MethodsOnce patients with breast cancer have completed their active treatment with chemotherapy, surgery, and radiation, they are offered a RAV facilitated by an advanced practice oncology nurse through the MRW LIFE Cancer Survivorship program. During this visit, an individualized SCP is provided and discussed. At the time of visit, each patient is also provided a pre-visit concerns assessment which surveys patients at the time of survivorship as to their immediate concerns. The questionnaire collects data as to the patient's type of cancer, age at diagnosis, time of treatment completion as well as a detailed checklist of potential concerns.
Results: From January 2019 to December 2023, two thousand one hundred ninety-nine (2,199) breast cancer survivors were referred to the MRW LIFE Cancer Survivorship Program and had a SCP completed. One thousand seven hundred forty four (1,744) breast cancer patients completed a pre-visit assessment. Of the respondents, all were female and ages ranged from 21-91 years old. Over 97% reported they had a primary care physician (PCP) as part of their care team. All respondents identified at least one or more concerns and > 95% endorsed two or more concerns. The most commonly identified concerns by ≥ 70% of breast cancer survivors were fear of recurrence, long term effects of treatments, cancer prevention/early detection, and nutrition/physical activity/weight management. Other concerns cited by ≥ 30% of breast cancer survivors were fatigue, memory/cognition, and anxiety/stress. From 2019 to 2023, we saw a steady increase in breast cancer patients attending a survivorship visit and the number of pre-visit assessments completed. The top 10 concerns identified by breast cancer survivors remained similar over time.
Conclusion: Completing a survivorship concerns assessment following treatment helps breast cancer survivors self-identify the most important concerns they have during their

cancer journey. Combined with an individualized SCP, a needs assessment can be a useful construct to gain better understanding of their cancer experience and promote self-management of symptoms and shared decision making with their care team. A better understanding of the most important concerns of breast cancer patients during survivorship is critical for the entire care team. Opportunities for targeted interventions such as referrals to supportive care and additional resources can be facilitated with use of a needs assessment. An ongoing educational curriculum geared towards the most identified needs of our breast cancer survivors is implemented year round through Evenings of Survivorship and include such topics as Fear of Recurrence, Nutrition Myth Busters, Coping with Chemo brain – all based on the greatest needs expressed by our breast cancer survivors.

P2-05-03: Work Reintegration After Breast Cancer Surgery in São Paulo Public Employees: a cross-sectional observational study.

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Objective: This study aims to evaluate the return-to-work (RTW) rate among state public employees in São Paulo with breast cancer who underwent surgical treatment at a single institution. Additionally, it compares RTW rates in relation to different oncological treatments and examines factors such as education, family income, perceived employer support and/or discrimination, workplace adjustments, and personal job satisfaction.
Methodology: This is an observational, descriptive, cross-sectional study conducted at a single institution, involving public employees with non-metastatic breast cancer who underwent surgical treatment at the State Public Servant Hospital of São Paulo from October 2021 to December 2022. Patients included in the study met the following criteria: diagnosed with breast cancer (either carcinoma in situ or invasive), female, over the age of 18, undergoing surgical treatment for breast cancer, employed as public servants in the State of São Paulo, and actively working at the time of diagnosis. Patients who declined to respond to the questionnaire and those with metastatic breast cancer at the time of the study or who progressed to a metastatic condition within six months of diagnosis were excluded. To assess quality of life, the following instruments were used: the European Organization for Research and Treatment of Cancer 30-Item Quality of Life Questionnaire (EORTC QLQ-30), version 3.0 in Portuguese, and its specific module for breast cancer: Quality of Life Questionnaire Breast Cancer - 23 (QLQ-BR 23). The study was submitted through Plataforma Brasil to the Research Ethics Committee (CEP) of Hospital do Servidor Público Estadual and approved (CAAE 68337823.4.0000.5463), and all patients signed the informed consent form.
Results: A total of 420 questionnaires were sent out after initial contact via telephone, with

355 responses received (response rate of 84.5%). Sixteen responses (3.8%) were excluded as they were completed by dependents of the state public servant. Among the 339 eligible patients, 300 (88.2%) were still working at the time of diagnosis. The RTW rate in this study was 74.41%. A significant majority of patients (80.6%) resumed employment within six months, while 15.1% returned between six to twelve months, and 12.8% between twelve to eighteen months. The predominant reason cited for RTW was personal satisfaction with financial necessity being a driving factor. A vast majority (93.8%) resumed their original positions. Upon their return, 62.5% of participants indicated no decline in work performance or productivity. In the assessment of global quality of life, patients who returned to work had higher scores compared to those who did not return, averaging 73.2 versus 51.5, respectively ($p < 0.001$). In the EORTC BR-23 subquestionnaire, patients who returned to work had higher scores in body image assessment, averaging 76.0 versus 52.3 for those who did not return to work ($p = 0.032$), as well as a more optimistic future outlook. It was found that the type of surgical treatment impacts the RTW rate ($p < 0.001$). Among patients who returned to work, 87.5% underwent breast-conserving surgery (BCS) compared to 9.1% of those who did not return to work. Additionally, 45.5% of those who returned underwent radical and oncoplastic surgery. Adjuvant treatment also correlated with RTW; patients undergoing adjuvant chemotherapy had a 6.25-fold increased risk of not returning to work compared to those who did not undergo adjuvant chemotherapy.
Conclusion: The RTW rate among state public employees in São Paulo was 74.41%. Oncological treatment (surgical, chemotherapy, and radiotherapy) was found to have a statistically significant association with RTW rates. Socially, employer-provided adjustments influenced the RTW decision, in addition to the patient's personal and work satisfaction. Returning to work is associated with improved quality of life for women surviving breast cancer.

P2-05-04: Associations between Hearing/Vestibular Problems and Levels of Physical Function Impairment among Breast Cancer Survivors in a Multiethnic Study Cohort

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Background: Cancer treatment such as chemotherapy and radiotherapy can cause hearing/vestibular problems including tinnitus, hearing loss, and vertigo or persistent dizziness. A recent study has revealed that hearing loss is more prevalent among cancer survivors than in the general population in the US. Further, hearing/vestibular problems can continue through survivorship, which can negatively affect cancer survivors' quality of life. However, research remains scarce concerning these symptoms and physical function specifically among breast cancer patients and survivors.
Methods: Between July and September 2023, we surveyed patients enrolled in the Chicago Multiethnic Epidemiologic Breast Cancer Cohort regarding whether they experienced tinnitus, hearing loss, and/or vertigo. Level of physical function impairment was measured

using a 10-item assessment of daily activities (i.e., moderate/vigorous activities, lifting, climbing, bending, walking, and bathing/dressing) adopted from the Nurses' Health Study. The total score ranges from 0 to 20, with higher scores indicating more physical function impairment. To examine correlations between hearing/vestibular problems and levels of physical function impairment, we fit separate multivariable linear regression models, controlling for age at breast cancer diagnosis, duration from breast cancer diagnosis to survey, race/ethnicity, insurance type, AJCC stage, histology, receptor status, the Charlson-Deyo Comorbidity Index, and treatment modality. Adjusted regression coefficients (β) and standard error (se) were calculated.
Results: Of the 1,462 participants surveyed, the mean age was 54.2 years (SD 11.4) and the median duration from diagnosis to survey was 8.2 years (IQR: 5.0-12.4); 69.8% identified as White, 22.7% as Black, 3.8% as Asian or Pacific Islander, and 3.7% as Hispanic; and 46.0% received chemotherapy. Overall, 15.1%, 13.6%, and 7.7% reported experiencing tinnitus, hearing loss, and vertigo, respectively. The overall mean score for levels of physical function was 4.0 (SD 5.0). Patients who experienced tinnitus had a higher average physical function score than those who did not (5.2 vs. 3.7, $p < 0.001$). Compared with patients who did not experience hearing loss, those who did had a higher mean score (5.3 vs. 3.6, $p < 0.001$). Similarly, the physical function score was higher among patients who experienced vertigo than those who did not (6.4 vs. 3.8, $p < 0.001$). After covariate adjustment, experiencing tinnitus was correlated with a greater level of physical function impairment ($\beta = 0.967$, se = 0.469, $p = 0.035$). Experiencing vertigo was also correlated with a greater level of physical function impairment ($\beta = 2.034$, se = 0.604, $p < 0.001$). The level of physical function impairment was only marginally significantly different between patients who experienced hearing loss and those who did not ($\beta = 1.053$, se = 0.548, $p = 0.055$). Additionally, in the same adjusted models, older age, Black race, having a Charlson-Deyo Comorbidity Index of 1 or ≥2, having stage III-IV tumors, and having Medicaid or Medicare were associated with higher levels of physical function impairment.
Conclusions: In this multiethnic cohort of breast cancer survivors, hearing/vestibular problems were prevalent, and patients who experienced these symptoms were more likely to have reduced levels of physical functioning. Our findings also highlight racial and socioeconomic disparities in physical function impairment. To improve the quality of life of breast cancer survivors, oncology programs should consider early and routine screening for hearing/vestibular problems and address disparities in physical function and unmet needs for survivorship care.

P2-05-05: Associations among BMI and patient-reported body image dissatisfaction during immediate autologous breast reconstruction: A prospective longitudinal evaluation

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Introduction: Cancer-related breast reconstruction is a process that can take months to years to complete, potentially impacting a patient's psychosocial well-being. Body habitus may influence a patient's adaptation to bodily changes resulting from breast cancer treatment, considering the association between obesity and body image dissatisfaction1. Thus, we investigated the association between body mass index (BMI) categorized as overweight or obese and patient-reported body image dissatisfaction at specific time points during immediate autologous breast reconstruction.
Material and Methods: The study sample included 40 patients who were enrolled in an IRB approved study from 2011 to 2014 at The University of Texas MD Anderson Cancer Center and had completed immediate autologous (DIEP/TRAM) reconstruction. Participants completed health-related quality of life measures including Body Image Scale (BIS), Brief Symptom Inventory (BSI-18), and BREAST-Q preoperatively (baseline) and at least once postoperatively at 3, 6, 9, 18, or 18+ months after reconstruction. Given sufficient abdominal fat tissue is a prerequisite for any abdominal-based tissue reconstruction, patients with baseline BMI over 25 Kg/m2 were included. Linear mixed models were conducted to investigate the effect of baseline BMI category on BIS scores and BREAST-Q physical well-being of the abdomen scores (PWBAS) during reconstruction. Post hoc analyses were conducted when a significant interaction effect of baseline BMI category and time effect was found. Baseline age, depression score, marital status, race, and ethnicity were included as control variables. Analyses were performed using SAS 9.4 and the level of significance was set at $p < 0.05$.
Results: There were no statistically significant changes in BMI across the study visits. Baseline age was significantly associated with BIS scores (ß = -0.31, p = 0.0072), with increased age associated with lower levels of dissatisfaction. Baseline depression was significantly associated with BIS scores (ß = 0.78, p = 0.0058), with higher baseline depression linked to greater body dissatisfaction. Marital status, race, and ethnicity were not statistically significantly associated with BIS scores. Obese patients reported statistically significant improvements in BIS postoperatively, whereas overweight patients had no statistically significant changes in BIS over time. At baseline, obese patients had significantly higher body image dissatisfaction compared to overweight patients (p = 0.0128) and continued to report higher dissatisfaction at follow-up visits, although the difference was not statistically significantly higher than overweight patients after the baseline visit.
Baseline depression, marital status, age, race, and ethnicity were not statistically significantly associated with PWBAS. Both BMI groups reported high abdominal satisfaction preoperatively. However, obese patients experienced a significant decrease in abdominal satisfaction at 9 months (p = 0.0482) compared to baseline, while overweight patients saw significant decreases at 3 and 6 months ($p < 0.0001$). No statistically significant longitudinal differences were observed between BMI groups for PWBAS, except at 6 months, when obese patients had significantly higher abdominal satisfaction (p = 0.0167). Abdominal satisfaction improved by the final visits, yet it remained below baseline levels.
Conclusion: Obese and overweight patients may benefit from additional body image counseling. Screening for depression and making appropriate referrals may be important

for postoperative body image adjustment. Further, these patients can benefit from preoperative counseling about early postoperative physical changes to the abdomen.

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P2-05-06: Contemporary media – highlighting a podcast hosted by a breast cancer survivor – to disseminate breast cancer education in a credible, patient-friendly format.

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Background: Most individuals and families facing a health-related decision will attempt to self-educate on their situation. Yet, the National Assessment of Adult Literacy found only 12% of Americans to have proficient health-based literacy. Individuals are searching for information on their diagnoses and ways to improve health, but accessible information that is both credible and contextualized to one's situation is often lost in noise. Many use internet search channels, with Google reporting 70,000 health-related queries per minute, and a January 2024 study of YouTube users reported that 87.6% of respondents watch health related content (HRC) and 84.7% make decisions based on what they watch. According to a 2019 study in Consumer Informatics and Digital Health, 9 out of 10 adults in the US use at least one online social network. In addition, the podcast channel has seen significant growth in recent years; the number of people listening to podcasts has doubled since 2013 to over 177 million listening monthly (Deal, 2022).

Methods: As a survivor who self-identifies as a "patient from hell," Samira Daswani found navigating the cancer care system challenging and frustrating. She led the development of a podcast to disseminate information important to cancer patients and families. Within the first 7 months of our Patient from Hell podcast launch in May of 2022, we ranked within the top 10% of podcasts shared globally (Spotify, 2022) and podcast followers have doubled since the beginning of 2024.

One of the primary areas the podcast has covered is breast cancer via interviews with oncologists, researchers, patients and survivors. A new episode is released bi-weekly on all podcast channels, with social media clips of the episode posted 2-3 times per week via Instagram, YouTube and TikTok for the week after the launch of an episode. The podcast was funded in May 2023 by the Patient Centered Outcomes Research Institute to disseminate PCORI-funded research projects and has featured guests such as Dr. Fumiko Chino, Dr. Karen Wernli, among others.

Findings: Of the 36 episodes published in the last twelve months (July 2023-June 2024), 33% (12 episodes) have been breast cancer specific (vs. more general cancer topics that are applicable to a wider audience of cancer patients, survivors and caregivers). Those 12 breast cancer specific episodes have garnered 81,468 impressions on Spotify, YouTube, Instagram, LinkedIn, TikTok, and Twitter and via our newsletter. We started to share one

minute clips via YouTube shorts in March of 2024 and have generated over 5,000 views of those “key highlight” short clips in just 3 months for the breast cancer focused episodes and our YouTube channel subscribers have nearly doubled since we started running the “Shorts.”
Discussion: The podcast medium is a novel and effective way of disseminating breast cancer information in a form that is both approachable and actionable. In addition to providing an opportunity to glean information by listening to full podcast episodes, we have learned that amplifying key messages and takeaways via short episode clips on social media platforms helps to further disseminate credible, science-backed information as well as key patient learnings to a wider audience of people impacted by breast cancer. The podcast is one patient and family education tool we are developing (JCO 42(16), E13064).

P2-05-07: Combination treatment to prevent and treat oxidative damage to neuronal cells

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Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) is a common dose-limiting side-effect of taxanes, platinum agents, vinca alkaloids, bortezomib and thalidomide. These regimens are first-line treatment for common cancers such as breast, colorectal, lung and lymphoma and used in both early-stage and metastatic cancer therapy. Therefore, numerous patients, both young and old, are at risk for developing CIPN. The symptoms have a negative impact on routine activities, functions, and behaviors in the domestic, work, and social lives of cancer patients, adversely affecting the quality of their survivorship.
A meta-analysis of 31 CIPN studies revealed an aggregated prevalence of 48%. In contrast to other adverse effects, such as hematological side effects, the symptoms of CIPN can be irreversible and there are currently no effective managements to prevent, alleviate or reverse these symptoms. As the number of cancer survivors increases, new research needs to focus on prevention and treatment of this long-term adverse effect to improve patients’ function and quality-of-life.
We have previously shown that personal biochemical responses to oxidative stress, induced by chemotherapeutic agents, during the early cycles of treatment are associated with development of CIPN. Therefore, we have tested a series of antioxidants and components of neuronal signaling transduction for their ability to prevent and/or reverse damage induced by oxidative stress in neuronal cell cultures.
Methods: All experiments were performed using the well-defined mouse neuronal model cell-line Neuro2A and the human peripheral nerve sheath cell-line S462. Cells were treated with retinoic acid in low serum conditions to induce differentiation prior to experiments. Oxidative stress was induced by adding hydrogen peroxide to cultures pre- or post-addition of antioxidant agents at various concentrations. Differentiation was followed by microscopy to determine neurite outgrowth and cell counts to demonstrate cell cycle exit. Cell viability

and membrane damage was tested using the MTT and LDH assays, respectively. PCR and Western blotting was performed to examine changes in KEAP-NRF2 pathway, Caspase-3, SOD, and NLRP3 using commercially available antibodies and reagents.
Results: To date, we have test the monosialoglycospingolipid, GM1, and two antioxidants, not listed by ASCO as unsuitable for prevention/treatment of CIPN, for their effect on hydrogen peroxide triggered neuronal cell damage. Damage to mouse Neuro2A cells could not be prevented or rescued by single agent treatment at doses ranging from 10μM to 50μM. However, when combining GM1 with one of the antioxidants, all doses were able to prevent damage while high dose could treat existing cell damage. Cell damage to the human S462 could be prevented and treated if using high dose GM1 as single agent. In contrast to the Neuro2A experiments, when combining with GM1 with an antioxidant all doses ranging from 10μM to 50μM had a therapeutic effect. The strongest response was observed using the combination treatment in both cell lines.
Discussion and Conclusions: We have been using GM1, a well-tolerated agent, but with reported mixed responses in clinical trials when used to prevent or treat CIPN. Based on our previous data and reports in the literature demonstrating the possible initiation of cell damage resulting in CIPN by oxidative stress, we have performed as series of test using GM1 as single agent and in combination with known antioxidants in cell culture experiment to select optimal treatment options prior to testing in animal models. Using existing therapeutic agents with low toxicity profiles could result in a rapid transition into clinical use for prevention and/or treatment of CIPN for cancer patients.

P2-05-08: QUALITY OF LIFE OF PATIENTS UNDERGOING NEOADJUVANT CHEMOTHERAPY: A LOOK AT SEXUALITY

Fabiana Makdissi, Isabela Rodrigues Neves, Silvana Soares dos Santos, Solange Moraes Sanches

Introduction: The sexual quality of life (QoL) of women with breast cancer (BC) during systemic therapy is one of the most disturbing aspects, not only because of the physical changes caused by the antineoplastic agents, such as fatigue, decreased libido and dyspareunia, but mainly because of the woman's intrapsychic experience of her own body, such as altered self-perception of attractiveness, body image and femininity, aspects that can further worsen the QoL of this population. Objective: To assess the impact on women's sexual QoL during neoadjuvant chemotherapy/NEOCT treatment. Methods: Prospective cohort study, carried out from July 2022 to July 2023 at A.C. Camargo Cancer Center. The study included 149 participants with non-metastatic breast cancer who underwent chemoradiation therapy; all of them signed an informed consent form approved by the local Research Ethics Committee. The assessment tool was the "Sexual Well-Being" scale, translated into Portuguese, from the BREAST_Q Portfolio (https://qportfolio.org/breast-q/). Data was collected at M0 (pre-NEOCT), M1 (halfway through the protocol) and M2 (end of NEOCT). For data analysis, we considered $p < 0.05$. Results: Of the women interviewed, 73.9% were aged between 45-59 and 68.5% were married or living with a partner. On the

question "sexually attractive when you have your clothes on", there was an increase in the "none or a few times" answers, from 13.8% to 32.9% at the end of the NEOCT; while there was a drop in the "most of the time or all of the time" answers, from 60.7% to 38.5%. A similar pattern was observed for the question "sexually attractive when you have your clothes off": an increase for the answers "none or a few times" (from 15.2% to 38.7%) and a drop for the answers "most of the time or all of the time" (from 66.9% to 44.3%). The question "satisfaction with your sex life" also followed the trend already observed: an increase for the answers "none or a few times" (from 21.5% to 50.8%) and a drop for the answers "most of the time or all of the time" (from 67.0% to 33.6%). Analysis of the median total scores on the "Sexual well-Being" scale showed p=0.000 between M0 and M1 and between M0 and M2; however, p=0.144 between M1 and M2.
Discussion and Conclusion: There was clearly a negative impact on sexual well-being throughout the course of the NEOCT, which can be explained by the side effects of the drugs: fatigue, central and peripheral neuropathy, loss of sensation, abnormal genital sensation, tingling, numbness, decreased libido, anorgasmia and vaginal dryness, as well as alopecia, edema and emesis. In addition to the physical effects, there is also anxiety, fear and stress, which can influence perception and coping with reality. However, it is interesting to note that even before chemotherapy began, 40% of women no longer felt sexually attractive. It is therefore important to analyze these aspects even before treatment begins and throughout it, so that women can be welcomed and the negative impacts on their quality of life and sexuality can be adequately reduced.

P2-05-09: Mindfulness as effective complementary therapy in the management of Breast Cancer (BC)

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Background: Breast cancer associates a broad variety of psychological and physical symptoms with a significant impact on quality of life. Mindfulness therapies (MBIs), such as MBSR (Mindfulness-Based Stress Reduction) and MBCT (Mindfulness-Based Cognitive Therapy) have been related with better control of this daily routine signs, achieving better emotional regulation, decreasing internal dialogue, increasing awareness of body sensations (posture, breathing...) and slowing down of mental processes. Mindfulness is a psychological technique that tries to reach a state of full consciousness. It is useful on the treatment and prevention of several diseases by different mechanisms. The brain areas linked to MBIs are: the insular cortex, related to body and emotional awareness; the hippocampus, involved in memory organization; the secondary somatosensory cortex and anterior cingulate, related to pain and the bilateral superior parietal lobe and left superior frontal gyrus, involved in attention. MBI is also related with an increase in telomeric length, increased expression of the hTERT (human telomerase reverse transcriptase) and HTR (human telomerase RNA) genes and a decrease in the level of methylation of the hTERT promoter regions (implying increased gene expression). These changes have potential

effect to delay cellular aging. There is a relationship between long-term meditation practice and a loss of methylation of CpG nucleotides and epigenetic changes in the tumor necrosis factor (TNF) and beta neurotrophic factor (NF-κß) signaling pathway.
PURPOSE:The aim of this study is to compile the effects of different mindfulness therapies on the symptoms associated with breast cancer, both in women with active disease and in survivors.
Methods: a systematic review was conducted using PubMed, PsycINFO, Cochrane library and Web of Science. Thirteen studies, published in the last seven years (between January 1st, 2017 and December 31st, 2023), were included: 10 on patients with "active disease" and 3 on "survivors".
Results: MBIs produce statistically and significant benefits in the short and medium term, on depressive symptoms, anxiety and stress. Among physical symptoms, a significant effect is seen about the reduction of fatigue with MBSR therapies. Other symptoms such as cognitive function and sleep quality also improve with these therapies. Regarding quality of life, only short-term improvements are been observed. MBCT protocols are included in few works. Its role in BC survivors is limited because the low number of researches and the lack of a homogeneous definition of "survivor".
Conclusion: Because of the benefit that mindfulness therapies (MBIs) (specially MBSR and MBCT) have on anxiety, stress, depressive symptoms, fatigue and other symptoms associated with breast cancer, it is proposed to use it as a complementary therapy in the management of these patients.

P2-05-10: Depression and anxiety in patients with breast cancer receiving radiotherapy: A prospective longitudinal study

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Purpose: To evaluate the prevalence and changes in depression and anxiety symptoms from pre-radiotherapy (RT) to 6 months post-RT in patients with breast cancer receiving RT; and to identify risk factors for depression and anxiety at baseline and for an increase of depression and anxiety over time.
Methods: This prospective, longitudinal observational study was conducted in China between August 2022 and August 2023. Depression and anxiety symptoms were measured by the 9-item Patient Health Questionnaire (PHQ-9) and the 7-item Generalized Anxiety Disorder (GAD-7) before and during RT, at the end of RT, 1 month and 6 months after RT. Uni-variable and multi-variable logistic regression analyses were performed to explore risk factors for baseline depression and anxiety. Generalized estimating equations were performed to evaluate changes in depression and anxiety over time and predictors of an increase of depression and anxiety over time.
Results: A total of 504 patients completed baseline questionnaires, and 458 (90.9%) patients completed all five-time assessments. Depression and anxiety decreased

significantly over time, with the prevalence decreasing from 37.3% and 26.0% before RT to 28.4% and 20.3% at 6 months after radiotherapy. However, daily insomnia (PHQ-9 Q3) and fatigue (PHQ-9 Q4) showed high scores from pre-RT to 6 months post-RT. In terms of GAD-7, feeling nervous (Q1), worrying too much (Q3), and becoming easily annoyed (Q6) showed high scores. Patients with a family history of cancer and receiving anti-HER2 targeted therapy were independently associated with depression before RT, and patients with premenopausal status, poor family income, and menopausal symptoms were independently associated with both depression and anxiety before RT. Patients receiving anti-HER2 targeted therapy, with a monthly personal income ≤8000 Yuan, and living in rural area were associated with an increase in depression level over time, while patients with poor family income and menopausal symptoms were associated with an increase in anxiety level over time.
Conclusions: Depression and anxiety levels were high among patients with breast cancer receiving RT and decreased over time from pre-RT to 6 months post-RT. Routine psychological assessments of these patients were necessary, especially for those with targeted therapy, poor financial status, and menopausal symptoms.

P2-05-11: A prospective cohort study of real-world patient-reported outcomes in HER2-positive early breast cancer patients receiving (neo) adjuvant anti-HER2 based therapy: the preliminary results at 2-cycle treatment

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Background: Anti-HER2-based regimens are the mainstay of treatment for patients with HER2-positive breast cancer. While these treatments significantly improve survival, there is growing concern about the quality of life (QoL) of breast cancer survivors during therapy. Here, we reported the preliminary results of 2-cycle treatments versus baseline and aimed to identify baseline characteristics associated with deterioration of QoL after 2-cycle (neo) adjuvant therapy in patients with HER2-positive early breast cancer.
Method: This prospective, longitudinal cohort study enrolling patients with stage I-III HER2-positive breast cancer was conducted in nine cancer centers across China. Patients eligible for (neo) adjuvant anti-HER2-based therapy have been enrolled in this study, and the treating physician has made treatment decisions according to CACA-CBCS (China Anti-Cancer Association, Committee of Breast Cancer Society) guidelines. The electronic patient-reported outcomes (ePRO) were collected at baseline (T0), during chemo (T1, after two cycles of chemo), 1-month after completion of chemo (T2), and at 12-month follow-up (T3). The ePRO instruments included EORTC QLQ-C30, PDQ-5, PHQ-9, GAD-7, and ISI to evaluate the quality of life, cognition, emotion, and insomnia. Changes ≥10 points from T0 to T1 were clinically meaningful in EORTC QLQ-C30. Univariate logistic regression was performed to explore risk factors of worsening.
Results: From November 2023 to June 2024, 600 patients with HER2-positive early-stage

breast cancer were enrolled (Median age: 51 years). By June 2024, questionnaire compliance rates were 98.7% (592/600) at T0, 86.2% (458/531) at T1, and 75.9% (236/311) at T2. General health status and functional QoL were high at T0, with worsening QoL in summary score, nausea and vomiting, diarrhea, fatigue, and appetite loss domains during treatment. The summary scores at T0 and T1 were significantly different, with a worsening rate of 38.4%, while the deteriorating rate in nausea and vomiting, diarrhea, fatigue, and appetite loss domains was 54.4%, 45.4%, 53.1%, and 55.5%, respectively. The scores in the above domains at T0 and T1 were significantly different (all $P < 0.001$). Over 50 years old (OR: 2.251, 95%CI: 1.508-3.361), post-menopausal (OR: 1.848, 95%CI: 1.259-2.712), hormone receptor-positive (OR: 0.677, 95%CI: 0.464-0.988), and histological grade 3 (OR: 1.665, 95%CI: 1.107-2.504) were associated with worsening QoL. The score varied without clinical meaning in PDQ-5, PHQ-9, GAD-7, and ISI.
Conclusion: For patients with HER2-positive early breast cancer, more than one-third of patients experienced a worsening QoL, mainly due to symptoms like nausea, vomiting, appetite loss, diarrhea, and fatigue. Patients in this prospective study are still in follow-up, and the results of influences of anti-HER2 treatment after chemotherapy on QoL are immature.

P2-05-12: Experience and perceptions with a phone-based weight loss intervention among survivors of breast cancer (BC) in France: a qualitative study within a randomized clinical trial (RCT).

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Introduction: The MEDEA (NCT04303924) RCT evaluated a phone-based educational and counselling intervention for weight loss (adapted from BWEL A011401) focused on calorie restriction and behavior change among overweight and obese survivors of BC in France. Patient's acceptability and engagement with weight loss interventions can be influenced by cross-country cultural differences related to dietary, social and behavioral habits. We performed a qualitative study to explore these aspects and inform future implementation.
Methods: 220 patients with stage I-III BC within 12 months of primary treatment and BMI ≥25 kg/m2 participated in the MEDEA trial and were randomized to receive the phone-based weight loss intervention delivered by dieticians through 24 semi-structured calls over 12 months vs. a standard health educational program. 20 patients in the intervention arm participated in focus groups to explore barriers and facilitators for uptake and engagement, as well as acceptability, satisfaction, and suggestions of improvement. A thematic content analysis was performed using Nvivo 12 software.
Results: Four focus groups were conducted with patients from diverse sociodemographic and economic backgrounds. Patients had mostly positive representations. Several themes emerged:

1) Patients expressed satisfaction with the objective weight loss and improvement in symptom burden:
“I'm very satisfied with the weight loss, that's for sure.”
“My self-image improved, I find that I'm much more toned, I'm more energetic. I have less pain. I sleep better.”
2) Patients referred to the MEDEA intervention as a catalyzer to sustainable behavior change:
“It helped me a lot because it made me understand that I was eating too much of some food categories and not enough of others. It allowed me to balance my meals.”
“I didn't have motivation anymore and she really coached me, she taught me again to feel like exercise, to boost myself, to walk, and now I've become an addict, I do 2.5 hours of sport per day.”
3) Most of them stressed the convenience of remote intervention:
“It's much better over the phone because it would have been a constraint to free up time to go to in-person meetings.”
Nevertheless, patients highlighted some barriers to uptake and engagement and suggestions for improvement.
1) Need for tools to sustain engagement such as a follow-up call, psychological support and enabling/facilitating self-monitoring:
“If we had a call from the dietician six months after [the end of the intervention], it would help to keep the momentum on which we started.”
“I would have liked to have a summary of my weight loss. A small conclusion on a diagram to see the evolution.”
“I don't know if a psychologist could be included in MEDEA, but in any case it would be a good thing or that the coaches refer to psychologists.”
2) Need for culturally adapted content:
“Sometimes it was more like an American-style method, in terms of food. The menu planning, it's not very French.”
3) Patients advocated for a more patient-centered research ecosystem (e.g. selection of patient-reported outcomes measures, communication and community building):
“I think it would be interesting to have some of the results of the study.”
“I would have liked to meet the women who did the same program, to talk to them.”
“The food questionnaire is horrible to fill out! […] The last one I still haven’t filled it, because it annoys me so much that I forgot.”
Conclusions: The MEDEA intervention was deemed acceptable and positively experienced suggesting that a North American weight loss intervention was scalable and adaptable to a different language and cultural context. This qualitative analysis also informed refinements to optimize weight loss interventions in a European context.

P2-05-13: QUALITY OF SEXUAL LIFE IN YOUNG WOMEN WITH BREAST CANCER IN THE MEDICAL ONCOLOGY SERVICE AT THE HOSPITAL REGIONAL ISSSTE LEON

Carmen Guadalup Bermudez Barrientos

Introduction: Breast cancer is a main public health problem nationally and worldwide. In addition to the increase in prevalence due to the effectiveness of current treatments, young women patients present multiple adverse effects to them, one of them most common are the sexual problems, severe, and persisten. Quality of life and well-being are influenced by different factors such as physical, social, and spiritual. Immediately after the diagnosis of cancer, sexuality becomes less important. Sexual dysfunction is common among women with breast cancer (occurs in 25 - 66% of cases).
Objective: Analyze the quality of sexual life of premenopausal women with diagnostic of breast cancer at the medical oncology service.
Methodology: Cross-sectional, observational, analytical, and descriptive study in patients diagnosed with breast cancer in clinical stage I, II, III or IV with an age ≤ 50 years at diagnosis and with active surveillance and/or treatment, in the medical oncology service at the ISSSTE Regional Hospital León, Gto. Two questionnaires were applied: EORTC SHQ-22 and EORTC QLQ-BR23, both containing questions related to quality of sexual life.
Results: From a total of 210 patients enrolled, a simple linear regression analysis was performed on the variables and scores, which predicted in the QLQ-BR23 questionnaire a higher level of symptomatology due to side effects of systemic therapy after having received chemotherapy (B=10 .15, 95% CI 0.40-19.91, p = 0.04) and living with their partner (B=8.41, 95% CI 2.31-14.50, p = 0.007), perception of Lower BR23 body image after having undergone a radical mastectomy (B=-11.81, 95% CI: -22.79 - -0.84, p = 0.04) and living with their partner (B=-11 .05, 95% CI -19.29 - -2.82, p=0.009), lower future perspective when living with your partner (B=-10.72, 95% CI -20.42 - -1.01, p = 0.031). The correlation of high QLQ-BR23 body image perception score significantly predicted a lower SHQ-22 vaginal dryness score (B=-0.35, 95% CI: -0.60 - -0.10, p=0.006). In the SHQ-22 questionnaire, there were greater problems in sexual activity after having undergone oophorectomy (B=-19.27, 95% CI 1.76-36.78, p=0.03), and fewer problems in sexual activity when living with their partner (95% CI -22.01--1.57, p=0.027) and treatment with adjuvant capecitabine predicted less decreased libido (B=-30.56, 95% CI: -52.16 to -8 .95, p = 0.006).
Discussion: 50% of all patients with BC experience problems with body image, which negatively affects personal relationships and sexual functioning. In our study, having undergone a radical mastectomy and living with their partner makes them feel less attractive, less feminine, it has been difficult to see themselves with their body and they have felt dissatisfied with their body. This correlates with the fact that the impact of surgery is greater when the surgery is radical and when a woman has had less involvement in treatment decisions. Women's body image limits their daily lives and psychological distress reminds them of their illness. BC survivors experience a significant decrease in quality of sexual life compared to survivors of other cancers. Age, time since diagnosis, marital status, educational level, chemotherapy, anti-hormonal therapy, type of breast surgery and body

image are all associated with the quality of sexual life of BC. This It has been previously demonstrated in different studies worldwide and is now demonstrated in this study in Mexican women, so it is imperative to acquire skills to refer to the relevant specialties, as well as have an established route and perform specific interventions for the comprehensive treatment of these patients. This project is still a taboo topic but interest in oncosexuality is growing and the need for specific treatment is increasing but with still insufficient access to specific care.
Conclusion: Premenopausal women with breast cancer present a decrease in their quality of sexual life during cytotoxic treatment and after surgical treatment, which affects their life as a couple.

P2-05-15: Challenges and Communication Gaps in Adjuvant Endocrine Therapy for HR+ Breast Cancer Patients in Russia: Insights from an online Survey

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Background: Adjuvant endocrine therapy is crucial in managing hormone receptor-positive breast cancer, significantly reducing recurrence and improving survival. Despite its benefits, adherence to endocrine therapy is often challenged by side effects, including psychological and sexual dysfunction. These issues are rarely discussed during doctor visits in Russia. Understanding patient perspectives on these issues and their communication with healthcare providers is vital for enhancing adherence and patient support.
Materials and Methods: An anonymous online survey was conducted using Google Forms to investigate adherence to adjuvant endocrine therapy, patient-reported side effects, and communication with healthcare providers among breast cancer patients. The survey, distributed via patient advocate groups and online forums, received 920 responses. The survey included questions on adherence, side effects, comfort in discussing side effects with healthcare providers, and whether necessary counseling was received. Data were analyzed to identify key trends and raise questions for further investigation.
Results: Participants: 920 women with breast cancer participated in the survey.HR+ Breast Cancer: 919 (99%) had HR+ breast cancer and were prescribed endocrine therapy.Therapy Context: 777 (84.5%) received adjuvant endocrine therapy, and 143 (15.5%) for metastatic breast cancer.Non-Starters: 40 (4.25%) did not start endocrine therapy.
Reasons for not starting:Concerned with side effects: 35%Unfavorable harm/benefit ratio: 27.5%Concern about potential secondary tumors: 30%Discomfort with taking pills: 2.5%Concerned with the cost: 5%Median Age: 48 years, with participants from various parts of Russia.Disease Stage:
In situ: 1.8%Stage I: 21.6%Stage II: 45.3%Stage III: 23.2%Stage IV: 8%Type of Endocrine Therapy: Anastrozole/Letrozole: 46.8%Tamoxifen: 51.7%Exemestane: 1.5%Medical ovarian suppression: 29.7%Duration of Treatment:
< 5 years: 87.6%5 years: 5%5 years: 5.8%10 years: 1%Not sure: 0.7%Adherence:

As prescribed: 94.3%Dose interruptions (with physician discussion): 2.7%Dose interruptions (without physician discussion): 0.7%Adverse Effects: Reported by 91.2% of participants.
Cardiovascular symptoms: 20.5%Cognitive/mood symptoms: 58.6%Ocular symptoms: 42.4%General physical changes: 58.3%Gastrointestinal symptoms: 25.7%Genitourinary symptoms: 32.4%Bone and joint issues: 67.2%Sexual issues: 47.6%Vasomotor symptoms: 66.2%Financial toxicity: 10%Discussion with Physicians:
Never discussed adverse events: 23.9%Attempted discussion: 31.3%Discussed: 44.8%Comfortable discussing issues: 35.1%Felt physician underscored side effects: 40.4%Felt doctor was rushed: 21.3%Management Suggestions:
No suggestion: 65.8%Lifestyle modification (diet and exercise): 35.8%Referral to other physicians: 23.6%Prescribed medications: 16.4%Offered to switch endocrine drug: 4.7%Therapy Discontinuation: 23% reported stopping endocrine therapy earlier than planned with the healthcare team.ConclusionsThe survey reveals significant challenges in adherence to adjuvant endocrine therapy among breast cancer patients in Russia, primarily due to side effects and insufficient communication with healthcare providers. A substantial number of patients do not discuss adverse effects with their physicians, and many feel their concerns are not adequately addressed. Improved patient-physician communication and better management strategies for side effects are essential to enhance adherence and support for patients undergoing endocrine therapy. Further studies are needed to develop interventions that can mitigate these issues and improve patient outcomes.

P2-05-16: Interrogating serum thymidine kinase activity with CDK4/6 inhibitor-based therapies: real-world experience in the metastatic and adjuvant setting

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Background: CDK4/6 inhibitors have become widely deployed in the treatment of HR+/HER2- breast cancer. In the metastatic setting, a number of different CDK4/6 inhibitors are approved in combination with endocrine therapy and lead to a general doubling of progression-free survival (PFS). The CDK4/6 inhibitor abemaciclib is also employed in the adjuvant setting in the treatment of HR+/HER2- tumors at high risk for recurrence. The duration of response to these treatments is variable between individual patients supporting the use of biomarkers to predict time to progression/recurrence. Here, we investigated the DiviTum® TKa assay relative to response to CDK4/6 inhibitor-based therapies using plasma samples from the prospective observational Roswell Park Ciclib Study (NCT04526587) of patients receiving CDK4/6 inhibitor-based therapy as standard of care.
Methods: The TKa assay was employed longitudinally on 69 metastatic and 20 high risk patients that were enrolled in the Roswell Park Ciclib Study. Samples collected at baseline, on treatment, and post-progression were analyzed using the DiviTum® TKa assay (Biovica). The TKa levels >50 DuA (DiviTum unit of Activity) were evaluated as a continuous variable

in association with different features of disease in either the metastatic or high-risk setting.
Results: The TKa levels in the baseline samples were inversely associated with the duration of PFS in the metastatic setting (R=-0.684, p=0.005). Furthermore, when comparing evaluable TKa levels in baseline samples to on treatment Cycles 1 and 2, there was a significant reduction in TKa levels (158.3 DuA vs. 47.6DuA, p=0.038) for patients that experienced a durable response to therapy ≥20 months. However, in patients with shorter duration of response to therapy ≤8 months, the average on treatment Cycles 1 and 2 TKa levels were (243.1DuA). Post progression TKa levels were considerably elevated across the cohort (925.3 DuA). Longitudinal analyses of individual cases illustrated dynamic features of TKa with treatment and emergence of resistance. In the adjuvant setting, there was significant reduction in TKa from baseline to Cycles 1 and 2 (87.8 DuA to 27.55 DuA, p=6.49e-11). While relatively few patients have recurred to date, elevated TKa levels were observed with disease progression.
Conclusions: These findings further support the utilization of the DiviTum® TKa assay to monitor response to CDK4/6 inhibitor-based therapy in the standard of care setting. Baseline and the TKa reduction early on treatment could be predictive of duration of PFS. Further study in the high-risk setting is warranted to predict associations with recurrence.

P2-05-17: The predictive and prognostic value of 18F-FES PET/CT in patients with advanced breast cancer treated with endocrine therapy with cyclin-dependent kinase 4/6 inhibitor

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Introduction: Estrogen receptor (ER) expression and its heterogeneity affect endocrine therapy efficacy. Tumor biopsy is a standard method for determining ER status but has limitations in analyzing ER heterogeneity. 18F-fluoroestradiol (18F-FES) PET/CT is an effective, non-invasive method to analyze systematic ER expression. This study aimed to analyze the predictive and prognostic value of 18F-FES PET/CT in patients treated with endocrine therapy with cyclin-dependent kinase 4/6 (CDK4/6) inhibitors.
Methods: Patients included in this study were identified from a prospective 18F-FES PET/CT cohort, comprising patients who underwent 18F-FES-PET/CT between April 2021 and April 2023 at Asan Medical Center. For the current study, patients with ER-positive, HER2-negative advanced breast cancer who received palliative endocrine therapy with CDK4/6 inhibitors and had pretreatment 18F-FES PET/CT were included.
Results: A total of 127 females were included. The median age was 57 years (range, 28-86). Endocrine therapy comprised aromatase inhibitors in 75.6% (N = 96) and fulvestrant in 24.4% (N = 31). A total of 25 (19.7%) presented “with FES-negative” lesions, of which 22 were mixed with FES-positive lesions, and 3 were entirely negative. The remaining 75.6% (N = 96) had “FES-positive” lesions only. 18F-FES status (FES-positive vs. with FES-negative) correlated with progression-free survival (PFS) to endocrine therapy with

CDK4/6 inhibitors, as well as overall survival (For with FES-negative, hazard ratio [HR] for PFS, 3.9 [95% confidence interval (CI), 2.1-7.2], p<0.001; HR for OS, 3.7 [95% CI, 1.3–10.2], p=0.008). 18F-FES status was predictive for PFS both in weak ER-positive and strong ER-positive tumors in biopsy specimens, showing the least benefit of endocrine therapy in ER weak-positive and with FES-negative lesions. 18F-FES status was consistently predictive for PFS across subgroups, including age, menopausal status, clinically determined endocrine sensitivity, and choice of endocrine therapy.
Conclusion: ER status assessed by 18F-FES PET/CT predicts the efficacy of CDK4/6i-based endocrine therapy and is prognostic for survival in advanced ER-positive, HER2-negative breast cancer. Integrating 18F-FES PET/CT with clinical variables, particularly tumor biopsy, may help further predict the benefit of endocrine therapy.

P2-05-18: Molecular effects of short pre-operative endocrine therapy in hormone receptor-positive and HER2-negative early breast cancer

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Background: The identification of biomarkers for evaluating sensitivity to endocrine therapy in early breast cancer (EBC) is critical. Assessing the dynamic biological changes in the tumor caused by brief pre-operative endocrine therapy (POET) can guide decisions on reducing or intensifying treatment. In this study, we examined the molecular changes induced by short-term POET and their correlation with the treatment's effectiveness.
Methods:This is a retrospective study of paired samples from patients (pts) with hormone receptor-positive and HER2-negative (HR+/HER2-) EBC treated at Hospital Clinic of Barcelona between 2014 and 2023 and from the letrozole arm of SOLTI-1501 VENTANA trial (Adamo et al. BCR 2019; NCT02802748). All pts received POET for 2 to 12 weeks prior to surgery, with tamoxifen or aromatase inhibitors (AI), administered according to menopausal status. RNA expression was assessed in baseline and surgery samples, including PAM50 and HER2DX signatures. Treatment response was defined as a value of Ki67≤10% at surgery. Logistic regression models explored the association between baseline gene expression and response. Gene expression changes were analyzed using paired SAM analysis and t-tests.
Results: A total 111 pts with both baseline and surgery samples available were included. Median age was 63 years-old (61.8-66.4) and most of the tumors were cT1 (68.5%) and cN0 (97.3%) at diagnosis. 19 pts (17.1%) were premenopausal and 92 (82.9%) postmenopausal. The median baseline Ki67 was 18% (14-25%). After POET, the median Ki67 value was 4% (1-10). 81 pts (73%) reached Ki67≤10% at surgery and 41 (37%) Ki67≤ 2.7% (complete cell cycle arrest). At baseline, PAM50 molecular subtype distribution was: 79 pts (71.2%) Luminal A, 23 (20.7%) Luminal B, 4 (3.6%) HER2-enriched, 3 (2.7%) Normal-like, 2 (1.8%)

Basal-like. At surgery, there were 82 (73.9%) Luminal A, 23 (20.7%) Normal-like, and 6 (5.4%) Basal-like tumors. In the univariate analysis, baseline clinicopathological variables associated with response were age (odds ratio [OR]=1.04, p=0.028), percentage of estrogen receptor (OR=1.03, p=0.025), Ki67 value (OR=0.95, p=0.003) and type of endocrine therapy (tamoxifen vs AI, OR=0.17, p<0.001). In terms of baseline gene expression, high Luminal A signature (OR=7.72, p<0.001) and luminal-related genes (e.g.: FOXA1 [OR=1.46, p=0.021] or ESR1 [OR=1.38, p=0.001]) were associated with response, while high Basal-like (OR=0.19, p=0.009), PAM50 proliferation (OR=0.30, p=0.005) and HER2DX proliferation (OR=0.24, p=0.037) signatures and proliferation-related genes (e.g.: MYBL2 [OR=0.58, p<0.003] or MKI67 [OR=0.71, p=0.004]) were associated with no response. After POET, all genes and signatures were up- or downregulated significantly. In particular, we observed a significant decrease of the PAM50 Luminal and proliferation signatures, as well as the HER2DX proliferation and luminal signatures, with an increase of the HER2DX immune (IGG) and HER2 amplicon signatures, both in responders and non-responders (FDR<5%).
Conclusion: POET is a simple and secure treatment that can be administrated before surgery in HR+/HER2- EBC. The molecular profiling and dynamic evaluation of biological changes induced by POET could offer opportunities for a better understanding of the tumor´s sensitivity to endocrine therapy and could help guide and optimize treatment strategies in HR+/HER2- EBC.

P2-05-19: An ICG-labeled Novel Trop2 Targeting Peptide for In Vivo and Ex Vivo NIR-II Fluorescence Imaging-guided Breast Cancer Precise Surgery

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Purpose: Positive surgical margins are closely associated with local recurrence in breast-conserving surgery. Meanwhile, accurately assessing sentinel lymph node (LN) metastasis can determine the scope of axillary LNs dissection and minimize unnecessary LN removal. Indocyanine green (ICG)-based near-infrared (NIR) fluorescence imaging provides real-time visualization for surgeons with the potential to reduce the positive margins rates and improve the detection rate of SLN, particularly imaging in the second near-infrared (NIR-II) region with enhanced contrast and deeper tissue penetration. Additionally, owing to the overexpression of Trop2 in breast cancer and the exceptional effectiveness of anti-Trop2 antibody-drug conjugates in breast cancer therapy, Trop2 has emerged as a promising tumor target. In this study, we screened a cyclopeptide (TTP) with a high affinity to Trop2, labeled with ICG, to enable more efficient intraoperative navigation under NIR-II fluorescence imaging.
Methods: TTP was screened via phage display technology, and ICG-NHS was labeled by NHS reaction to create a novel NIR-II probe called TTP-ICG. Its tumor-targeting efficiency were validated in vitro and in vivo. Subsequently, various mice models were utilized to evaluate the efficacy of in vivo NIR-II imaging-guided surgery. Finally, the ex vivo tumors and metastatic LNs were incubated with TTP-ICG to assess the validity of ex vivo NIR-II imaging-

guided surgery.
Results: With a high affinity of Trop2 (KD = 9.829 × 10-10 M), TTP was successfully screened and labeled with ICG-NHS, whose cellular uptake was 2.88-fold higher than control peptide (CP)-ICG in the MDA-MB-231-Luc-Trop2 cells with high Trop2 expression ($p < 0.0001$). Meanwhile, MDA-MB-231-Luc-Trop2 cells showed 6.33-fold higher mean fluorescence intensity (MFI) compared to MDA-MB-231-Luc-NC with rare Trop2-expression ($p < 0.0001$), which could be effectively blocked by free TTP (3.75 ± 0.06 vs 1.75 ± 0.03, $p < 0.0001$). Interestingly, when incubating the mixed cells MDA-MB-231-Luc-GFP (with rare Trop2 expression) and MDA-MB-231-Luc-Trop2 with TTP-ICG probes, cells with stronger ICG fluorescence showed minimal GFP fluorescence.
Then, in MDA-MB-231-Luc-Trop2 subcutaneous tumor models, the TTP-ICG group exhibited a 2.2-fold higher maximum signal-to-background ratio (SBR) compared to the CP-ICG group ($p < 0.0001$). Additionally, TTP-ICG showed a 2.3-fold higher aggregation in MDA-MB-231-Luc-Trop2 tumors than the MDA-MB-231-Luc-NC tumors ($p < 0.001$). Furthermore, NIR-II fluorescence guidance allowed for precise identification of microtumors (with a minimum diameter of less than 2 mm) in the multiple microtumors model with an accuracy rate of 100%. Moreover, it also effectively differentiated malignant and normal tissue with an AUC of 0.932 in the MMTV-PyVT transgenic model. Notably, this approach also facilitated complete tumor resection and improved overall survival compared to conventional white light surgery in intramuscular tumor-invasion models.
Subsequently, after footpad injection of different probes in dual 4T1-Luc-Trop2 SLN metastasis models, the TTP-ICG group showed significantly higher MFI than CP-ICG group (6266.3 ± 1866.7 vs 2260.0 ± 966.5, $p < 0.01$). Besides, a higher MFI was observed in metastasis LN than normal LN after footpad injection of TTP-ICG (6257.5 ± 569.6 vs 2979.3 ± 806.3, $p < 0.001$).
In addition, via co-incubation of TTP-ICG, the 4T1-Luc-Trop2 tumor exhibited a higher MFI relative to 4T1-Luc-NC and muscle with an AUC of 0.917 ($p < 0.0001$). Meanwhile, when co-incubating breast malignant and benign tumor from patients with TTP-ICG, malignant tumor also showed higher MFI (3.2 ± 0.7 vs 1.2 ± 0.3, $p < 0.05$). Finally, the MFI of metastatic LNs was 2.62-fold higher than that of normal LNs after TTP-ICG incubation ($p < 0.01$).
Conclusion: In this study, we successfully developed a novel TTP-ICG probe with efficient Trop2-targeting and excellent bio-compatibility. This probe demonstrates great potential for clinical translation as an effective tool for NIR-II fluorescence-guided breast surgery, which is expected to improve surgical outcomes and patient recovery.

P2-05-20: Tissue-free minimal residual disease testing in 2,000 consecutive patients with breast cancer: real-world data and case report

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Background: Studies have shown the prognostic impact of circulating tumor DNA (ctDNA) detection following curative-intent therapy in early-stage solid tumors, with detection often

preceding radiologic recurrence. Guardant Reveal™ is a tissue-free assay that interrogates epigenomics to detect minimal residual disease (MRD) with ctDNA and measure tumor fraction (TF). We report the clinical use of the assay in a large, unselected breast cancer (BC) cohort and describe its incorporation into a patient (pt)'s care.

Methods: 2,000 consecutive pts in the United States with stage I-III BC and at least one Guardant Reveal™ test ordered beginning July 9, 2023, were retrospectively queried and assessed through the data cutoff of May 31, 2024. Demographics and clinical variables were obtained from test requisition forms. Fisher's exact test was used for statistical differences. Pt consent was obtained for the case report.

Results: The median age of the 2,000-pt cohort was 61 (range: 26-100); 99.4% were female. 50.3% of pts had > 1 test, representing 3,488 samples. 99.9% passed quality control (QC); the mean turnaround time (TAT) was 10 days.

At the first test, 68% had stage I/II BC, and 32% had stage III BC. BC subtype was available for 97.4% of tested pts, with most being hormone receptor-positive (HR+)/HER2- (58%), followed by triple-negative (TNBC) (19.1%), HR+/HER2+ (14.7%), and HR-/HER2+ (8.2%). Pts with stage III, TNBC, and HR-/HER2+ disease were more likely to have multiple vs. single tests ($p < 0.01$).

Overall, ctDNA was detected in 326 pts (16.3%) and varied by stage. For stage I/II and III pts, the detection rate was 13.5% and 21.8%, respectively. The ctDNA detection rate at any timepoint by subtype was 19.4% in TNBC, 16.5% in HR+/HER2-, 14.9% in HR+/HER2+, and 13.9% in HR-/HER2+ BC. The median TF when ctDNA was detected was 0.12%, with 2.5% having >10% TF.

Case Report: A 42-year-old pt presented with early-stage HR-/ HER2+ BC. Four years after the initial diagnosis, she developed recurrent seizure-like activity and was found to have an isolated brain metastasis without systemic disease. The brain lesion responded well to Gamma Knife. She remained with no evidence of disease on tucatinib, trastuzumab, and capecitabine for over 19 months but developed severe hand-foot syndrome.

In an attempt to de-escalate her treatment, the Guardant Reveal™ test was used for disease monitoring as the patient did not have adequate tumor tissue for sequencing with a tumor-informed MRD assay. As the pt was clinically stable and ctDNA was not detected for the first two blood draws, her capecitabine dose was reduced further. ctDNA was then detected a year later, prompting earlier restaging scans that showed increased enhancement at the treated brain lesion site but no overt progression. Radiologic progression became evident 3 months later. The enlarging brain metastasis was resected, with pathology confirming disease recurrence. The pt's capecitabine dose was increased, and letrozole was added as her resected brain metastasis showed positive progesterone receptor expression. She has had no radiologic evidence of disease progression or ctDNA detection since resection.

Conclusions: This study describes real-world use and results of a tissue-free monitoring test in early-stage BC. The high QC pass rate (99.9%) and rapid 10-day TAT support the feasibility of a tissue-free assay for MRD detection. Results suggest a potential provider preference for monitoring for patients with stage III BC, TNBC, and HR-/HER2+ BC. The pt case shows the potential utility of tissue-free MRD testing in brain-only oligometastatic disease, where radiation necrosis can be hard to discern from true radiologic progression and biopsy can be risky. Further study is needed to explore how pt characteristics influence the use of MRD testing as assays and data continue to evolve.

P2-05-21: BRCA1/2 alterations in circulating tumor DNA: correlation with germline origin and impact on survival in breast cancer.

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Background: Circulating tumor (ct) DNA testing is a standard of care approach to evaluate patients (pts) with advanced or metastatic breast cancer (BC) for the detection of resistant and actionable somatic (s) alterations. ctDNA testing could identify incidental germline (g) mutations. While the prognostic and predictive role of known gBRCA1/2 mutation is well established, the significance of sBRCA1/2 alterations is still debated. We aimed to establish a variant allele frequency (VAF) threshold to distinguish germline from somatic mutations and to explore the impact of BRCA1/2 alterations detected by ctDNA on survival outcomes.
Methods: A retrospective multi-institutional cohort of pts with BC and at least one BRCA1/2 mutation detected by standard clinical ctDNA next-generation sequencing (Guardant 360®) for metastatic disease was included in the analysis. The incidence of BRCA1/2 mutation detected by ctDNA and germline testing was analyzed to assess VAF threshold to predict the likelihood of detecting germline mutations. Receiving operating characteristic (ROC) curves were generated to determine the VAF cut-off; differences in survival were tested using the log-rank test.
Results: 294 pts were included in the analysis. BRCA1 and BRCA2 mutations were detected in 104 (35.4%) and 166 (56.5%) pts respectively, while 24 (8.2%) had a co-mutation in BRCA1 and BRCA2 genes. The median age at diagnosis was 50 years (interquartile range [IQR] 44-62), and family history of cancer was known for 45.9% of pts. The most represented subtype was hormone receptor positive/HER2 negative (HR+/HER2-) (70.4%), followed by triple negative (TN) (15.7%) and HER2 positive (13.9%). At the time of the first BRCA1/2 detection in ctDNA, bone (67.1%) and visceral (56.2%) sites were mainly involved. The mean VAF was 10.0% (standard deviation [SD] 19.7%, range 0.02%-84.3%) for BRCA1 and 12.1% (SD 20.1%, range 0.06%-80.3%) for BRCA2 alterations. The most co-

mutated genes were TP53 (58.1%), PIK3CA (35.8%), EGFR (25.1%), ESR1 (23.7%), and ERBB2 (17.2%). The germline testing was available for 157 (53.4%) pts, detecting a gBRCA1 mutation in 16 (10.2%) pts and a gBRCA2 mutation in 37 (23.6%) pts. Comparing the ctDNA and germline testing, 13/16 pts had concordant BRCA1 mutation. In 3 discordant cases, only BRCA2 alteration with different VAF (0.30-80.1%) was detected by ctDNA. All pts with gBRCA2 mutations (37) also had a BRCA2 mutation in ctDNA. An optimal VAF cut-off of 38.4% (AUC 0.99) for BRCA1 and 16.1% (AUC 0.96) for BRCA2 was established as the threshold for the likelihood of a germline mutation detected by ctDNA analysis. For 18 pts with TNBC and 91 pts with HR+/HER2- BC, the detection of BRCA1/2 mutations was at the ctDNA baseline test, before starting a new treatment, with a median of 2 (range 0-9) previous lines of therapy. The median progression-free survival (mPFS) was 7.3 months (mos) (CI 3.9-10.7) for TNBC pts and 8.4 mos (CI 4.5-12.3) for HR+/HER2- pts; no significant survival differences were observed between BRCA1 and BRCA2 mutations. The 2-year overall survival (OS) rate was 69% and 78% in the TN and HR+/HER2- subgroups. Exploring the outcome according to therapy in the HR+/HER2- subgroup, no significant differences were assessed between chemotherapy, CDK4/6 inhibitors plus endocrine therapy, or PARP inhibitors, although a longer mPFS for the latter was observed (4.5 vs. 8.9 vs. 14 mos).
Conclusions: The VAF cut-off identified for the likelihood of a germinal mutation detected by ctDNA resulted lower than expected, underlining the importance of a larger germline BC screening, with considerable impact on therapeutic decision making and germline testing of other family members. Further analysis to explore the interplay of different co-mutations with BRCA1/2 will be performed and validation in additional dataset is needed.

P2-05-22: Plasma proteomic profiling of early recurrence of breast cancer

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Background: Almost 30% of the breast cancer patients who are free of disease after initial treatments may experience disease recurrence later on. Early detection of cancer recurrence during follow-up care improves patient survival. Even though blood-based tests are non-invasive and often considered the most accessible method for monitoring cancer, the dynamic nature of the circulatory system poses a challenge to identifying potential protein targets. In this study, we utilized a highly specific aptamer-based assay, to perform comparative plasma protein profiling of breast cancer patients with known clinical outcome (early recurrence [disease relapse within 5 years after surgery] vs non-recurrence).
Methods: The subjects were enrolled and specimens were collected through the Clinical Breast Care Project using IRB-approved protocols. We analyzed heparinized plasma samples taken at the time of diagnosis from patients who subsequently relapsed within five years after primary tumor diagnosis (n = 65, ipsilateral recurrence/distant metastasis) and those with no relapse reported (n = 65, minimum follow-up: 5 years). Both groups were

matched primarily on race, age, tumor stage, and IHC subtype. The association between sample groups and other clinical features was assessed using Fisher's exact test and logistic regression. SomaScan (7k) assay (SomaLogic, Inc.) was used to measure 6289 unique proteins from the plasma samples. Differential expression analysis of proteins was performed using limma, followed by LASSO regression to identify the top proteins associated with recurrence. Leave-one-out cross-validation (LOOCV) was used for further validation of the identified protein markers. The GSEAPreranked tool was used for gene set enrichment analysis.
Results: There were no significant differences in the distribution of race, age, tumor stage, or IHC subtype between the early recurrence and non-recurrence groups, indicating a balanced cohort. Principal component analysis of protein expression also did not reveal any distinct clusters based on these features, unlike tissue-based studies. Differential expression analysis between the two groups revealed 19 proteins to be significantly differentially expressed (p-value < 0.05, |fold change| > 1.2), with 7 upregulated and 12 downregulated. Some of these proteins like MSMB and OXT are previously-reported plasma protein biomarkers for breast cancer. LOOCV based prediction models, using the 19 proteins' expression, performed moderately (Accuracy = 0.72, Kappa = 0.45). Gene set enrichment analysis revealed key pathways differentially enriched between the two groups, including those related to immune response, embryonic stem cell differentiation, and cancer.
Conclusions: Our study indicated that protein expression in plasma is different compared to that in tumor tissue. We identified 19 proteins that could be potential biomarkers for early recurrence detection. Additional studies need to be performed on a larger sample size to validate these findings.
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P2-05-23: Inhibition of de novo fatty acid synthesis demonstrates broad synergistic effects with anti-cancer therapy, alters cellular lipid composition and induces endoplasmic reticulum stress

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Background: Metabolic rewiring is a characteristic feature of cancers. Our previous study showed metabolic isoenzyme Acetyle-CoA-carboxylase-1 (ACC1/ACACA) is overexpressed in cancer relative to normal tissues. Targeting ACC1 with small molecule inhibitor of ACC1 and ACC2, PF05175157, inhibited breast cancer cell growth and affects metabolic processes including cell membrane structural glycerophospholipids metabolism that are required for cellular membrane synthesis. Unfolded protein response (UPR) restores endoplasmic reticulum (ER) homeostasis and extreme ER stress can induce pro-apoptotic cell death. We hypothesized that PF05175157 displays synergy with anti-cancer agents, induces changes

in the lipid composition of biological membranes and perturb cellular homeostasis leading to ER stress and cell death.
Methods: High-throughput screening was performed using Approved Oncology Drugs Set X (AOD X) available from Developmental Therapeutics Program at National Cancer Institute. The plate set contains 166 FDA-approved anticancer agents (https://wiki.nci.nih.gov/display/NCIDTPdata/Compound+Sets). The compounds were diluted to the final concentrations of 0.05 mM, 0.1 mM, 1 mM and 10 mM using Beckman Biomek NXp. Cell lines tested include BT-20, BT-474, BT-549, HCC1395, HCC1500, MCF7, MDA-MB-231, MDA-MB-468, T47D and ZR-75-1. Hierarchical clustering was performed and heatmap was generated using the aheatmap function from NMF package. Mass spectrometry-based lipid analysis was performed by Lipotype GmbH (Dresden, Germany) and analyzed on a QExactive mass spectrometer (Thermo Scientific) equipped with a TriVersa NanoMate ion source (Advion Biosciences). Data were analyzed with lipid identification software based on LipidXplorer. For western blot analysis, ER Homeostasis Antibody Sampler Kit primary antibodies were purchased from Cell Signaling (#53898) and used at 1:1,000 dilution. Cell viability was assessed using CellTiter-Glo luminescent cell viability assay (Progmega).
Results: To identify active drug combinations enhancing the anticancer effect of PF-05175157, we performed a combinatorial high throughput screen with PF-05175157 (10 μg/ml) and 166 FDA-approved anticancer drugs for 72 hours. We observed broad synergy across different compounds, more prominently noticeable among tyrosine kinase inhibitors across different cell types. The top 10 chemotherapy agents with broad synergy with PF-05175157 across multiple breast cancer cell lines based on inhibit delta encompass lapatinib, vinorelbine tartrate, pazopanib hydrochloride, tamoxifen citrate, sunitinib, avapritinib, alectinib, enasidenib, gefitinib and nilotinib. To investigate the potential mechanism of PF05175157 affecting subfamily members of glycerophospholipids, MDA-MB-468 cells were treated with PF05175157 (10 μg/ml) for 24 and 72 hours. Lipid profiling revealed a decrease in phosphatidylglycerol, phosphatidylserine, phosphatidylinositol, and phosphatidylcholine levels treated with PF05175157 for 24 hours. PF05175157 also decreased the level of sphingomyelin, the most abundant sphingolipids in the plasma membrane. The levels of these fatty acids remained low at 72-hour treatment. Levels of long chain unsaturated fatty acids, triacylglycerols and cholesterol ester are increased after 72 hours treatment. UPR signaling is controlled by ER transmembrane-associated sensor proteins – IRE1α, PERK and ATF6.Treatment of MDA-MB-468 cells with PF-05175157 (10 μg/ml) for 6, 24 and 72 hours upregulated the protein levels of downstream signal transduction pathways of ER stress sensors: p-IRE1α, XBP-1S, p-EIF2 α, ATF4 and ATF6. Treating multiple breast cancer cell lines including MDA-MB-468, BT-20 and BT-474 with PF-05175157 (10 μg/ml) for 72 hours significantly inhibited cell viability.
Conclusion: PF05175157 treatment showed broad synergy with various anti-cancer therapies, alters cellular lipid composition and induces endoplasmic reticulum stress and cell death.

P2-05-24: Impact of the gut microbiome on immune-related adverse events (irAE) in HR+/HER2- locally advanced or metastatic breast cancer (MBC) patients (pts) receiving palbociclib, endocrine therapy, and pembrolizumab

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Background: Based on preclinical data demonstrating a synergistic effect of combining immune checkpoint inhibitors (ICIs) with CDK4/6 inhibitors, we conducted a phase I/II study of palbociclib, endocrine therapy, and pembrolizumab in patients (pts) with HR+/HER2- MBC. We previously reported that the gut microbiome can predict clinical response to this ICI combination. Emerging evidence also suggests that the gut microbiome is closely linked to the development of immune-related adverse events (irAE), and that the onset of irAE may be associated with improved ICI response. Here, we explore the role of the gut microbiome on the onset of severe irAE in this cohort of pts with HR+/HER2- MBC treated with palbociclib, endocrine therapy, and pembrolizumab.
Methods: Pts with stage IV HR+/HER2- MBC were enrolled and treated with palbociclib, endocrine therapy, and pembrolizumab. Baseline stool samples were collected at Day -28 to C1D1 (N=28 pts). Next on-treatment sample was used if baseline unavailable. Gut microbiota composition was assessed using deep metagenomic sequencing. Taxonomic profiling was conducted using MetaPhlAn4. Functional profiling was performed using HUMAnN3. Correlative studies assessed the association between the gut microbiome and severe (grade >3) irAE.
Results: Between March 2017 and May 2022, 40 pts (39 female/1 male) were accrued, with a median age of 51 (range 39-75). With a median follow-up of 35.8 months (mos) (95% CI: 26.1, 63.5), the median progression-free survival (PFS) was 27.8 mos (95% CI: 11.2, NR). The incidence of severe irAE was 11/40 (27.5%). There was no difference in PFS between pts with and without severe irAE (P=0.6). The median PFS was 27.8 mos (95% CI: 6.7, NR) and 27.1 mos (95% CI: 8.7, NR) in pts with and without severe irAE, respectively. ANCOM-BC (Analysis of compositions of microbiomes with bias correction) identified Dialister invisus (log fold change [LFC] 2.45 [P=0.02]), Blautia caecimuris (LFC 2.43 [P=0.01]), Flavonifractor plautii (LFC 2.40 [P=.01]), and Eisenbergiella massiliensis (LFC 2.12 [P=0.001]) in greater abundance in pts without severe irAE, while Coprococcus eutactus (LFC -3.94 [P=0.0009]) was in greater abundance in pts with severe irAE. Beta diversity using Bray-Curtis analysis showed significant differences in gut microbiome composition between irAE groups (P=0.04). Although alpha diversity using the Shannon diversity analysis showed no differences across irAE groups (P=0.3), Shannon diversity analysis showed significant differences in functional genetic pathways between irAE groups (P=0.03). ANCOM-BC identified several distinguishing metabolic pathways between irAE groups, including the CMP-pseudoaminate biosynthesis pathway which was enriched in pts without severe irAE (LFC 2.37 [P=0.0002]).

Conclusions: Certain gut bacteria can predict the development of severe irAE in a cohort of pts with HR+/HER- MBC treated with palbociclib, endocrine therapy, and pembrolizumab. Several metabolic pathways were enriched between irAE groups, which suggests differences in immune activation that can lead to the onset of irAE. This study suggests that the gut microbiome can serve as a risk factor and biomarker of severe irAE in breast cancer pts, which warrants further evaluation.

P2-05-25: Comparative Study of Droplet-digital PCR and Ultra-sensitive NGS Assay for ctDNA Profiling in Breast Cancer

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Background: The variant allele frequencies (VAF) of oncogenic mutations often correlate with drug response or resistance following treatments of tumors. However, the sensitivity of molecular diagnostic assays are limited by technological factors. For example, the lower report range of NGS-based assays for tissue FFPE and plasma samples are usually above 1% and 0.1%, respectively. In recent publications, our assays have been used to demonstrate that a more complete view of cancer development and drug resistance can be achieved if variants with VAF below the current resolution can be detected. In this study, we demonstrated the use of both PredicineCARE Ultra™ and ddPCR (droplet digital PCR) to push detection limit further lower down to 0.01%.
Methods: The NGS-based PredicineCARE Ultra™ assay sequences targeted regions with 100,000X coverage, identifying mutations in over 150 genes. ddPCR methods test specified mutation sites faster (same day) and at a lower cost. We applied both methods to multiple solid tumor studies, collecting whole blood samples from patients at baseline and follow-up time points. Cell-free DNA was extracted and tested with the PredicineCARE Ultra™ assay, analyzing over 150 genes with up to 100,000X read coverage to detect variants with VAF down to 0.01%. Tumor fraction estimation based on longitudinal samples provided higher detection sensitivity by tracing multiple mutations together. ddPCR achieved single molecule detection sensitivity, dependent on the input amount, with a limit of detection down to 0.01% for 100ng of input cfDNA.
Results: In this study, 216 plasma samples from 98 solid tumor (including 58 breast cancer) subjects were analyzed. Some samples also had baseline tissue samples sequenced separately for cross-reference. The PredicineCARE Ultra™ assay achieved 0.01% VAF sensitivity, providing significant insights into longitudinal tumor dynamics. For PIK3CA mutations, mutation frequencies were 23% (50/209) at the sample level and 28% (27/95) at the subject level at a 0.1% VAF threshold. With a VAF down to 0.01%, the frequencies increased to 29% (60/209) at the sample level and 34% (32/95) at the subject level. This allowed more detailed analysis of VAF changes in PIK3CA and other genes like ESR1, KRAS, and TP53. The ultra-sensitive assay detected 31.3% more mutations and 30.1% more CNVs compared against PredicineCARE ctDNA assays with 20,000x coverage in 46 patients. Hotspot variants were further validated using ddPCR, with over 95% concordance.
Conclusion: Using the high sensitivity of the ultra-deep cfDNA assay and the low-cost ddPCR

assay, the fraction of variants detectable in blood-based monitoring significantly increased, aiding therapy monitoring. Unlike personalized MRD assays, the PredicineCARE Ultra™ assay is tumor-agnostic and can detect novel mutations, crucial for drug resistance studies. The assay's longitudinal tumor fraction estimation offers higher sensitivity by tracing multiple mutations. While ddPCR is less efficient in exploring numerous mutations simultaneously, it provides a fast turnaround for pre-designed hotspots, complementing the PredicineCARE Ultra™ assay. Together, these methods enhance the detection of low VAF variants that are otherwise undetectable.

P2-05-26: Differential TROP2 expression patterns among inflamed tumor microenvironments in HER2-negative breast cancer

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Introduction: The combination therapy of immune check point inhibitor (ICI) and antibody-drug conjugates (ADC) targeting trophoblast cell surface antigen 2 (TROP2) have recently been progressed. This study aims to evaluate the association between the status of the inflamed tumor microenvironment, as indicated by programmed death-ligand 1 (PD-L1) expression and tumor-infiltrating lymphocytes (TILs), and TROP2 expression in HER2-negative breast cancer.
Methods: We collected 227 archival paired samples of primary tumors and metastatic sites from 105 breast cancer patients treated at our hospital from 2000 to 2018. Surgical specimens and samples from one or more recurrent sites were required for inclusion. TROP2 was stained using Anti-Human TROP-2 (Clone77220) for IHC assays and results were categorized based on the histochemical score (H-score). PD-L1 expression was evaluated using the VENTANA SP142 assay. PD-L1 positive was defined as ≥1% of tumor-infiltrating immune cells staining positive for PD-L1. The following categories were used: IC1 (PD-L1 positivity in ≥1% and <5% of cells), IC2 (PD-L1 positivity in ≥5% and <10% of cells), and IC3 (PD-L1 positivity in ≥10% of cells).Stromal TILs were assessed according to international guidelines and scored using semi-continuous (10% increment) methods and TIL-high was defined as ≧30%.
Results: Among the samples, 140 were Estrogen receptor (ER)-positive and 87 wereER-negative (triple-negative, TN). Samples from primary sites(P) accounted for 49% and those from metastatic sites(M) accounted for 51%. The median TROP2 H-score was 10, with a mean of 37.5 (range: 0-265). PD-L1 positive was observed in 15% of samples and 13% were TIL-high.
TROP2 expression was higher in metastatic site (median TROP2 H-score: 2[range 2-200]in P, 30 [range 30-265] in M, $p<0.001$). However, there was no significant difference in PD-L1 positivity and TIL-high status between primary and metastatic sites (PD-L1 positive :19% in P, 11% in M, $p=0.138$; TIL-high:16% in P, 12% in M, $p=0.337$). There were no significant

difference in TROP2 expression between PD-L1 positive and negative samples or between TIL-high and TIL-low samples (median TROP2 H-score were 55[range:10-265] in PD-L1 negative and 44 [range 15-215] in PD-L1 positive, p=0.852; 54.25[range 10-265] in TIL-low and 53.5 [range 25-215, p=0.69]). However, among TN samples, TROP2 expression was higher in PD-L1 positive and TIL-high samples (median TROP2 H-score was 59[range:5-248] in PD-L1 negative and 80 [range 36-155] in PD-L1 positive, p=0.04; 59[range 10-248] in TIL-low and 88 [range 50-175, p=0.0153])
Conclusions: This study showed in TN, TROP2 expression is notably higher in PD-L1 positive and TIL-high samples, and no significant correlation was found between TROP2 expression and inflammatory markers in the overall HER2-negative breast cancer. It provides the insight of the combination therapy of ICI and ADC targeting TROP2 in TN. Further research is warranted to explore these associations and develop personalized treatment approaches.

P2-05-27: Revealing prognostic subtypes and related model of early-stage TNBC

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Background: Triple-negative breast cancer (TNBC) is the most malignant subtype of breast cancer. Effective approach stratifying the risk of recurrence for early-stage TNBC patients is still lacking. This study aims to explore the molecular heterogeneity of early-stage TNBC yielding a practical tool evaluating the risk of disease recurrence.

Methods: This retrospective study included stage I-III TNBC patients treated at our institution between December 2013 and December 2019. RNA sequencing was performed on surgical samples, and correlations were analyzed with clinical data and prognosis.

Results: A total of 240 patients were included, with a median follow-up of over 5 years. Patients were categorized into two cohorts: recurrence-free within 3 years post-surgery (good prognosis, n=203) and recurrence within 3 years (bad prognosis, n=34). Differential gene analysis revealed 423 up-regulated and 1,818 down-regulated genes in patients with good prognosis. Consensus cluster analysis identified three clusters (C1, C2, C3). C1 had the worst prognosis for DFS (p=0.020) and OS (p=0.055). Further analysis of the three groups' characteristics showed that C1 patients exhibited significant upregulation of angiogenesis, hypoxia, and glycolysis signals. Differential gene analysis indicated that NOX1, HNF1A, CXCL8, TGFBI, and NT5E (CD73) were significantly upregulated in C1 patients. This suggests that tumors in these patients might create a hypoxic microenvironment through NOX1 upregulation, promoting glycolysis and adenosine metabolism, which in turn stimulates ATP and lactate release, angiogenesis, and immune evasion, creating a tumor growth-promoting microenvironment. Given the notably shorter DFS in this group, therapies targeting angiogenesis or CD73 may improve clinical outcome of C1 patients. C2

patients showed enrichment of canonical oncogenic pathways like WNT, NOTCH signaling. C3 patients displayed pro-inflammatory tumor microenvironment with elevated IFN-γ signatures. Additionally, potential infiltration of activated dendritic cells, activated CD8+ T cells, CD4+ T cells, and plasma cell signature (IGHV1OR15-2, IGKV1D-43, IGHV2-26) were observed in the C3 group. This deconvoluted estimation of TME indicated the presence of matured tertiary lymphoid structures (TLS) in the C3 group. Eight subtype and prognosis related genes (ABCD2, ACSM3, ADCYAP1, ADM, CXCL13, GLO1, PIGR, RRP8) were selected. A prognostic prediction model was constructed, categorizing patients into high-risk and low-risk groups. In the training set (n=166), high-risk patients had significantly lower OS than low-risk patients (P<0.0001), with an AUC of 0.874 for predicting 3-year recurrence. The validation set (n=71) showed an AUC of 0.844, with high-risk patients having significantly lower OS (P<0.0001). External validation with the GSE103191 dataset showed an AUC of 0.761 for predicting 3-year survival.
Conclusions: This study disclosed novel molecular subtypes with prognostic and therapeutic significance. A subtype-derived prognostic model was also constructed and validated to stratify recurrence risk for early-stage TNBC patients. Further validation via multi-center study was warranted.

P2-05-28: High throughput analysis in HER2 positive locally advanced breast cancer (BC): pathological complete response (pCR) and mutational status.

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Background: Pathologic complete response (pCR) after neoadjuvant treatment (NAD) is a strong prognostic biomarker associated with improved survival in breast cancer patients, especially those with HER2-positive BC and Triple negative subtypes. PIK3CA is mutated in up to 20% of HER2 positive BC patients, contributes to anti-HER-2 resistance and may be predictive of the lack of response to anti HER2 NAD. PIK3CA mutations in breast cancer occur primarily at hotspots E545K at exon 9 and H1047R at exon 20. Next generation sequencing has improved our knowledge regarding the biology of the mechanisms behind treatment resistance. The aim of this study is to evaluate the genomic landscape of tissue samples obtained from locally advanced breast cancer patients treated in a neoadjuvant setting, to correlate the genomic analysis to response and clinical outcome.
Methods: DNA extracted from archival 84 formalin-fixed paraffin-embedded (FFPE) samples obtained by core needle biopsies was subjected to deep sequencing using the TruSight Oncology (TSO) 500 panel (Illumina, San Diego, USA; 523 genes, size: 1.94 Mb), following the manufacturer's protocol, which assesses microsatellite instability (MSI) status, tumor mutation burden (TMB), recurrent somatic copy number variations (sCNV),

somatic sequence variants as single nucleotide variants (SNVs) and Insertion/Deletion (InDel). Libraries were sequenced on a NovaSeq 6000 instrument (Illumina) to reach a minimum of 500× read depth. All samples were evaluated for RNA single cell transcriptional profile. 72 of 84 patients were treated with traditional neoadjuvant chemotherapy plus trastuzumab meanwhile 12 of 84 received neoadjuvant chemotherapy plus trastuzumab and pertuzumab. Statistical analysis was performed to detect the genes whose mutation involved in the progression and / or non-response of the disease using the Statistical Package for Social Science (SPSS), release 23.0. Continuous variables were expressed as median [range], categorical variables were displayed as frequencies and the X2-test or Fisher's exact test were used to assess significance of the differences between subgroups (patients with or without pCR). Molecular and histopathological data were uploaded to local instance of cBioPortal to provide a comprehensive overview of the molecular data in association with clinical data. Actionability of the mutations was annotated with OncoKB and the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT). ClinVar significance were used to assess the germline pathogenic variants in BC susceptibility genes.

Results: In total we analyzed samples from 82 of 84 patients, for 2 samples it was not possible to carry out reliable genomic analysis due to not adequate tissue material. The median age was 48 years. 20 of 82 (24%) were HER2 enriched, 62 of 82 (76%) were triple positive, 66 (80.5%) with G3 and 29 (35.3%) with stage III. 46 of 82 patients (56%) obtained pCR.

The most common frequently altered genes were TP53 (55/82, 67%) PIK3CA (25/82, 31%), GATA3 (21/82, 26%), CCND1 (17/82, 21%) cMYC (14/82, 17%), and BRCA1 (7/82, 7%). In tab. 1 is reported main clinical characteristics of patients and genomic alterations detected through the use of the cBioPortal.

73 out of 82 samples showed an HER2 amplification, one patient harbored an HER2 mutation. 10% of samples (8/82) were not HER2 amplified with discrepancy between immunohistochemistry (IHC) and genomics (Fig.1). Among patients with a mutated PI3KCA (31%), 32% (8/25) obtained a pCR versus 67% (17/25) who did not; PIK3CA wild type patients (57/82, 69.5%) obtained pCR in 58% of cases (33/57) compared to 24 of 57 patient (42%) did not (p=0.03). It is interesting to note the position of the mutations in the PIK3CA and p53 gene sequence based on pCR versus not pCR: the lollipop plots in Fig. 2 demonstrate how missense and truncating mutations are frequent in mutated patients with not pCR compared to patients with pCR after neoadjuvant chemotherapy.

Conclusions: In this preliminary report, genetic drivers such as PIK3CA and p53 may have a clinical implication for prognosis and treatment response after NAD in locally advanced HER2 positive breast cancer. PIK3CA and p53 altered patients have a lower pCR rate than not altered patients. More samples are needed to evaluate the significance of both single mutation and co-alteration in this patient setting. We are waiting for the results of the RNA single cell transcriptional profile of these samples. Event free survival data is not yet mature at this moment. We would like to acknowledge the contribution of Multispecialistic Biobank Research Core Facilty G-STeP, Fondazione Policlinico Universitario "A. Gemelli" IRCCS (Biobank-FPG) who provided the bioresources.

P2-05-29: Orally bioavailable cyclin A/B-RxL inhibitors elicit antitumor activity in breast cancer patient-derived xenograft models

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Background: Complexes of cyclins and cyclin-dependent kinases (Cdk) regulate the activity of Rb and E2F to drive cell cycle progression. The association of some substrates and regulators of Cyclin/Cdk complexes is mediated by the interaction of their RxL motif with the highly conserved hydrophobic patch (HP) on the cyclins. Disruption of this interaction was previously shown to selectively drive lethality in cancer cells containing oncogenic alterations that drive high levels of E2F1 (Chen1999, Mendoza 2003), suggesting an opportunity for a targeted therapeutic approach. CID-078 is a novel, orally bioavailable, passively cell permeable, potent, and selective macrocycle that binds to the HP of cyclins A and B, preventing the RxL-motif mediated binding of both E2F1 with Cyclin A2-Cdk2 and Myt1 with Cyclin B1-Cdk1. This activity of CID-078 causes cell cycle arrest at G2M resulting in apoptotic tumor cell death. We have previously shown that CID-078 causes inhibition of cell cycle progression in multiple cancer cell lines. CID-078 is further associated with in vivo tumor regression in small cell lung cancer. We have previously shown in lung tumor models that phosphorylation of separase (ESPL1), a mitosis specific protein which is a direct substrate of Cyclin B1-Cdk1, at serine 1126 increases upon treatment with CID-078. Here, we explore the pharmacodynamic effect of CID-078 and show its anti-tumor activity in eight patient-derived xenograft (PDX) models from patients with triple negative breast cancer (TNBC), estrogen receptor-low (ERlow/HER2-) or ER+/HER2- breast cancer.
Methods: The models were chosen based on varying expression of E2F1, ESPL1, as well as hallmark pathway scores of E2F targets and G2M checkpoint. PDXs were treated with either vehicle or active therapy, including standard of care (cisplatin for TNBC and ERlow, and cisplatin alone or palbociclib in combination with the oral SERD elacestrant for ER+/HER2- models) or oral formulation of CID-078 at 100 mg/kg QD, BID or TID for at least 28 days. Tumor volume and body weight were measured biweekly. Biomarker analyses were conducted with or without treatment with CID-078 by mRNA and IHC for cyclin A2-Cdk2 and cyclin B1-Cdk1 and other targets including E2F1, pATM, cleaved caspase 3, cyclin B1 and phospho-separase.
Results: Treatment with CID-078 resulted in substantial tumor regression in 3/4 TNBC and ERlow models as a single agent, and in 1/4 ER+/HER2- models (either as a single agent or combined with elacestrant). All PDX models with higher E2F1 and ESPL1 expression levels (3/7) responded to CID-078 and all models with lower E2F1 expression (2/7) did not respond. The median E2F1 and ESPL1 expression trends higher in responders compared to non-responders. Oral treatment with CID-078 was well tolerated with no significant body weight changes. Pharmacodynamic evaluation of additional biomarkers including Cyclin B1 and phospho-separase by IHC and RNAseq is underway.
Conclusions: CID-078 exhibits potent anti-tumor activity in the TNBC, ERlow and ER+ preclinical models tested, consistent with the proposed mechanism of action and correlating well with E2F1 and ESPL1 expression. Given these compelling results, CID-078

may be a new treatment option for TNBC, ERlow/HER2-, or ER+/HER2- breast cancer. This hypothesis and the correlation of anti-cancer activity with biomarkers will be explored in the CID-078 first-in-human clinical trial CID-AB1-24001.

P2-05-30: Ki-67 dynamics during neoadjuvant treatment of breast cancer and their added prognostic value to the Neo-Bioscore model: a population-based cohort study

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Introduction: Ki-67 is a commonly used proliferation marker in breast cancer. Although strongly prognostic, its role following neoadjuvant chemotherapy (NACT) is still evolving. We here investigate the prognostic implications of the changes in Ki-67 pre- and post-NACT, and the value of adding Ki-67 to the Neo-Bioscore prognostic model.
Methods: Patients with invasive breast cancer treated with NACT in the Stockholm-Gotland region between 2007 and 2020, were identified through the National Breast Cancer Register (n=2533). The register data were complemented with information from the electronic patient records. Individuals who only received neoadjuvant endocrine therapy and patients with stage IV or unknown disease were excluded (n=39). The efficacy endpoints of the study were defined in accordance with the NeoSTEEP definitions. Relative change (RC) of Ki-67 was defined in 4 subgroups: decreased (High-Low) if RC < -30%, unchanged (Low-Low) if -30% ≤ RC ≤ 30% and pre-NACT Ki-67 < 40%, unchanged (High-High) if -30% ≤ RC ≤ 30% and pre-NACT Ki-67 ≥ 40% and increased (Low-High) if RC > 30%. Multivariable analysis included covariates; adjuvant chemotherapy use, age at diagnosis, clinical tumor size and node status at diagnosis, Estrogen Receptor-status, Progesteron Receptor-status, HER2-status, grade, diagnosis year. The addition of Ki-67 to Neo-Bioscore staging system was examined by fitting Cox regression models. The improvement in fit was determined using the concordance index (C-index), the Akaike information criterion (AIC), and likelihood ratio test in the multivariable analysis.
Results: A total of 2494 patients were included. The median pre-NACT Ki-67 expression was 40% in the total population. Patients with higher pre-NACT Ki-67 had higher pathologic complete response (pCR) rates in ER+/HER2- (n=1091, 5.3% vs 21.4%, p<0.0001), TNBC (n=543, 26.2% vs 37.3%, p=0.0168) and HER2+ (n=820, 41.5% vs 54.0%, p=0.0013) subtype, when categorized by a Ki-67 threshold of 50%. Pre-NACT Ki-67 was prognostic for breast cancer specific survival (BCSS) (p=0.022). The median post-NACT Ki-67 expression in patients with residual disease (RD) was 12%. Post-NACT Ki-67 was associated with BCSS (p<0.0001), which was also observed for ER+/HER2-(p=0.0001) and TNBC (p=0.0007), but not for HER2+ (p=0.8223) subtype. Among patients with RD, those who had decreased (High-Low, 65.2%) or unchanged (Low-Low, 9.7%) RC had improved 5-year BCSS (p<0.0001) compared to those with increased (Low-High, 8.1%) or unchanged (High-High, 17.0%) RC. This trend was statistically significant in ER+/HER2- (p<0.0001), and TNBC (p<0.0001), but not in HER2+ (p=0.13) subtype. Neo-Bioscore was associated with BCSS

and identified distinct prognostic groups in all patients with well-defined Neo-Bioscore (n=1730, p<0.0001) and across subtypes. The prognostic performance of Neo-Bioscore improved with the addition of post-NACT Ki-67, increasing the C-index from 0.751 to 0.797 and reducing the AIC value from 1807.59 to 1781.59. The likelihood ratio tests were significant for all models with p<0.001 for both ER+/HER2- and TNBC, and p=0.029 for HER2+ subtype. Exploratory cut-off points for post-NACT Ki-67, based on median values, were explored to improve Neo-Bioscore and were found to be 10%, 12% and 55% for ER+/HER2- (p=0.00033), HER2+ (p=0.046) and TNBC (p<0.0001) subtype, respectively. Conclusion: In this large population-based cohort study our data indicates that the prognostic value of Neo-Bioscore can be further enhanced with the addition of post-NACT Ki-67. Ki-67 should be considered in clinical trials exploring post-neoadjuvant treatment strategies.

P2-06-01: CBP/P300 bromodomain inhibition reduces neutrophil accumulation and activates antitumor immunity in TNBC

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Tumor-associated neutrophils (TANs) have been shown to promote immunosuppression and tumor progression, and a high TAN frequency predicts poor prognosis in triple-negative breast cancer (TNBC). Dysregulation of CREB binding protein (CBP)/P300 function has been observed with multiple cancer types. The bromodomain (BRD) of CBP/P300 has been shown to regulate its activity. In this study, we found that IACS-70654, a novel and selective CBP/P300 BRD inhibitor, reduced TANs and inhibited the growth of neutrophil-enriched TNBC models. In the bone marrow, CBP/P300 BRD inhibition reduced the tumor-driven abnormal differentiation and proliferation of neutrophil progenitors. Inhibition of CBP/P300 BRD also stimulated the immune response by inducing an IFN response and MHCI expression in tumor cells and increasing tumor-infiltrated cytotoxic T cells. Moreover, IACS-70654 improved the response of a neutrophil-enriched TNBC model to docetaxel and immune checkpoint blockade. This provides a rationale for combining a CBP/P300 BRD inhibitor with standard-of-care therapies in future clinical trials for neutrophil-enriched TNBC.

P2-06-02: Identification of Antigenic Determinants in SV-BR-1 derived Cellular Breast Cancer Vaccines

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Background: Identifying antigenic determinants is crucial for developing effective cancer vaccines. This study focuses on SV-BR-1 derived cellular breast cancer vaccines, aiming to delineate specific antigens that elicit an immune response. These vaccines rely on two key

concepts: tumor cells display immunogenic antigens activating T-cells via cross-presentation, and genetic engineering enhances their role as antigen-presenting cells, amplifying immune responses. Bria-IMT, the first version, is a genetically modified tumor cell line engineered to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF). This vaccine has shown encouraging clinical outcomes, demonstrating its potential in cancer immunotherapy. Bria-OTS+, an advanced version, enhances tumor cells' ability to present antigens by expressing cytokines, co-stimulatory factors, and HLA alleles.
Methods: MHC-associated Peptide Profiling of Bria-IMT: Cell pellets were lysed, centrifuged, and clarified lysates were used for immunoprecipitation of MHC class I and II molecules. Peptides were eluted, analyzed by LC-MS/MS with a 2-hour gradient, and raw files processed using PEAKS software for peptide identification, including PTM and mutation analysis. Using class-specific peptide length, abundance, and -10logP values, we shortlisted ~130 peptides (class I, and class II) for further evaluation of antigen-specific responses. Class I and II antigen/epitope mapping: CD154-expressing T cells (Class II) and CD137-expressing T cells (Class I) were enriched using magnetic beads and flow sorted with activation and memory markers. The sorted T cells were expanded as oligoclones in 96-well plates with feeder cells, PHA, and IL-2. They were then stimulated with peptide pools, and positive responses were restimulated with individual peptides to identify the specific antigenic peptide.
Results: In our study, MHC class I analysis identified 6,932 peptides at a 1% PSM FDR using forward/decoy database searching, including Post-Translational Modified (PTM) and mutated peptides. Peptide length distribution showed a predominant length centered around specific motifs. In MHC class II analysis, 5,197 peptides were detected under similar conditions, with motif analysis highlighting two distinct motifs and a portion of peptides remaining unclustered. We conducted Class I and II antigen/epitope mapping to test a subset of candidate peptides post-vaccination in one individual, No CD4+ T cell epitopes were identified, but a specific Class I peptide was recognized as a CD8+ T cell epitope. These preliminary results suggest a specific CD8+ T cell response, warranting further investigation.
Conclusions: This study identified a wide array of antigenic determinants in SV-BR-1 derived cellular breast cancer vaccines using MHC-associated peptide profiling and antigen/epitope mapping. The analysis revealed diverse peptides, including PTM and mutated variants, in MHC class I and II molecules. These findings highlight the potential of vaccines like Bria-IMT and Bria-OTS+ to elicit strong immune responses by presenting a broad range of tumor-specific antigens, paving the way for improved personalized cancer immunotherapy strategies.

P2-06-03: Age Conditions the Tumor Microenvironment of Hormone Receptor Positive Breast Cancer

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Hormone receptor positive (HR+) breast cancer is the predominant molecular subtype in postmenopausal women. Although menopause decreases the concentration of estrogen in the body, HR+ breast tumors are sustained by local production of estrogens in the surrounding adipose tissue. The aged breast is characterized by an expansion of white adipose tissue, a phenomenon with unknown implications for breast cancer progression and treatment. Current clinical trials focused on HR+ breast cancer treatment fail to accurately represent aged women, with the average age of enrollment 7.76 years younger than the average age at diagnosis. It is important to understand how age-related changes to this environment impact disease progression, as breast adipose tissue both surrounds and bidirectionally communicates with cancer cells. Considering that age is not accurately represented in clinical trials, there remains a critical need for clinically relevant models of aged HR+ tumors and breast adipose in vitro. This project set out to characterize how age-based differences in the microenvironment alter HR+ tumor growth and disease progression, using a combination of in vivo analyses of age and in vitro conditioned media studies. Our results show that tumors from aged mice have a higher growth rate and overall total tumor volume at endpoint compared to young counterparts, indicating age as a driver in vivo tumorigenesis. In vitro proteomic analysis demonstrated that HR+ cancer cells exposed to conditioned media from aged breast adipose derived stem cells (brASC) have elevated expression of markers associated with adhesion, EMT, and angiogenesis at the mRNA and protein levels. Cytokine arrays of aged tumors show decreased expression of CXCL12/SDF1 and increased expression of SERPINE1, supporting possible changes in adhesion, EMT, and extracellular matrix with age. These findings suggest that age-educated components of the tumor microenvironment alter signaling within the tumor and promote tumorigenesis.

P2-06-04: Potential Impact of the Cancer-Associated Fibroblast Secretome in the Tumor Microenvironment

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Background: Proteins secreted by cancer-associated fibroblasts (CAFs) can influence inflammation and recruit external immune cells to the tumor microenvironment. However, the cross[1]talk between CAFs and immune cells in the tumor microenvironment is not well understood. Therefore, in this study, we determined the association of proteins secreted by the CAF secretome with immune cell infiltration in patients diagnosed with breast cancer (BC) and ovarian cancer (OC).
Methods: The Human Protein Atlas was explored to identify potential gene targets associated with the CAFs secretome, including inhibin subunit beta A (INHBA), Dickkopf-related protein 3 (DKK3), and follistatin (FST). BC and OC patient data sets (n=14 samples) in the Gene Expression Omnibus were analyzed as discovery cohorts to compare CAFs and normal fibroblasts. Kaplan-Meier Plotter, Gene Expression Profiling Interactive Analysis, and Tumor Immune Estimation Resource bioinformatics databases were explored to

computationally understand relationships between gene expression, tumor prognosis, and immune infiltration. Single cell analysis datasets and opal multiple staining were used to validate the contribution of CAFs to overexpression of the gene targets in BC and OC cancer patients. Proteogenomic BC and OC data sets (n=46) from Clinical Proteomic Tumor Analysis Consortium were used for validation of the correlation between marker expression and immune cell infiltration.
Results: Increased expression of INHBA, DKK3, and FST correlated with shortened survival of patients with BC and OC. All three genes were negatively associated with multiple tumor-infiltrating immune cell types, both in RNA and protein levels. INHBA expression was positively correlated with the expression of T cell exhaustion markers. Pathway analysis demonstrates that INHBA's role in immune infiltration may be a result of its association with the Smad2 signaling pathway, which promotes the transition of fibroblasts to activated CAFs via an inflammatory response. In BC and OC, the three genes were generally positively correlated with immune cells characterized as "protumor", such as macrophages, CAFs, and neutrophils and negatively correlated with those considered as "antitumor," such as B cells.
Conclusion: INHBA, DKK3, and FST are candidate therapeutic targets for the treatment of breast, ovarian, and other cancers. Their therapeutic effect should be evaluated in the context of regulating infiltration and inflammation of the tumor microenvironment.

P2-06-05: Use of breast adipose-derived stromal cells in a 3D spheroid model to recapitulate the triple-negative breast cancer tumor microenvironment

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Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by the absence of hormone receptors and human epidermal growth factor receptor 2. This subtype is difficult to treat due to lack of known protein targets, is often refractory to chemotherapy, and has a high rate of recurrence. Stromal components, like collagens in the extracellular matrix (ECM) in the TNBC tumor microenvironment (TME) can contribute to treatment resistance by altering the immune microenvironment. Identifying the drivers of immunosuppressive ECM will yield novel predictive biomarkers in TNBC and guide effective treatment options.
In the TME, different stromal cells secrete different proteins and breast adipose-derived stromal cells (BrASCs) produce different collagens than differentiated breast adipocytes. Thus adipogenesis in the TNBC TME can alter the ECM significantly. Furthermore, adipogenesis in TNBC is associated with increased pro-tumorigenic M2 macrophages. To determine the interplay between TNBC and components of the TME on immune recruitment, we performed spatial profiling of primary patient TNBC tumors with a specific focus on the transcriptome of tumor regions with macrophage infiltration compared to tumor regions without macrophage infiltration. Results demonstrated that in M2 macrophage infiltrated areas of TNBC tumor, expression of FN1 and COL5A1 was decreased

in both the tumor and the stromal compartments. In contrast regions without macrophage infiltration had enriched FN1 and COL5A1. To determine if TME stroma composition modulated the collagen type and thus macrophage phenotype, we next developed a 3D tumor spheroid model using either BrASCs or BrASCs differentiated to an adipocyte lineage.
Model analysis revealed a phenotypic change in differentiated and non-differentiated 3D BrASC spheroids. Post induction of differentiation with supplemented media, spheroids were measured for circularity, referenced to a perfect circle value of 1; induced spheroids retained compact circularity ~0.8, while non-induced controls were eccentric and irregularly shaped ~0.5. Spheroids in both conditions were viable on day 7, as assessed by staining with calcein AM and ethidium homodimer-1. Next spheroids were evaluated for adipogenesis and collagen production. Once validated, this model will be used as a platform to understand the impact TNBC ECM on immune regulation.

P2-06-06: The Role of iNOS Inhibition in Enhancing Chemotherapy Efficacy by Modulating the Tumor Microenvironment in Obese TNBC Models

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Background: This study investigates the impact of obesity on the treatment outcomes of triple-negative breast cancer (TNBC), focusing on the modulation of the tumor microenvironment (TME) through inducible nitric oxide synthase (iNOS) inhibition. Obesity fosters an adverse TME characterized by immune suppression, metabolic alterations, and pro-tumoral cytokine release. Elevated nitric oxide (NO) levels associated with obesity can undermine vascular integrity and promote metastasis. We explore whether combining standard chemotherapy (docetaxel) with iNOS inhibitor L-NMMA can enhance antitumor efficacy in an obese mouse model of TNBC.
Methods: An orthotopic TNBC syngeneic mouse model was developed using C57BL/6 mice fed either with a normal diet (ND) or a high-fat diet (HFD) for 11 weeks before injecting the E0771 murine cancer cell line into the mammary fat pad. Once tumors reached 100 mm^3, mice were randomized into vehicle and L-NMMA treatment groups. Formalin-fixed, paraffin-embedded (FFPE) tumor tissue sections from ND, HFD, and HFD+LNMMA treated mice were analyzed for immune cell phenotypes and TME markers using a panel of 64 proteins by Digital spatial transcriptomics using NanoString™ GeoMx and nCounter platforms.
Results: Mice on a HFD exhibited significantly increased tumor growth compared to those on ND. Serum analysis revealed higher levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-17) and the granulocyte-recruiting chemokine G-CSF in HFD mice, along with a downregulation of T-cell recruiting chemokines like Eotaxin and RANTES, suggesting a shift towards a pro-tumoral inflammatory state. Immunohistochemistry (IHC) analysis showed that HFD tumors had higher iNOS expression and increased markers for cytotoxic T cells (CD8), macrophages (F4/80), neutrophils (Ly6G), and exhaustion markers (FOXP3 and PD-

1) compared to ND tumors. Combining docetaxel with L-NMMA in HFD mice showed greater potency in slowing tumor growth and reducing lung metastasis incidence compared to either treatment alone, as demonstrated by smaller tumor volumes and fewer metastatic nodules in treated mice. Heatmap analysis of the expression of an immune panel revealed that HFD tumors clustered differently than ND and HFD+LNMMA tumors, indicating a distinct protein expression profile. L-NMMA treatment reduced the expression of exhaustion markers and immunosuppressive macrophages, aligning the TME closer to that of ND tumors. Furthermore, L-NMMA treatment in HFD tumors led to decreased expression of survival (p53) and proliferation (Ki67) markers, as well as phospho-AktSer473. These changes suggest that iNOS inhibition can modulate critical pathways involved in tumor growth and survival.
Conclusion: Our findings highlight the impact of obesity on the TME in TNBC, in promoting tumor growth and creating a pro-inflammatory, immunosuppressive environment. iNOS inhibition through L-NMMA, in combination with standard chemotherapy, effectively remodels the TME, reducing tumor growth and metastasis, and modulating pathways associated with cell proliferation and survival. These results underscore the potential of targeting iNOS as a therapeutic strategy to enhance the efficacy of TNBC treatments, particularly in obese patients.

P2-06-07: Function of NR4A1 in Natural Killer Cells

Kenneth Martinez-Algarin, Isaac S. Chan

Background: Natural killer (NK) cells are critical members of the innate immune system that control breast cancer growth and metastasis. Despite these anti-tumor characteristics, our lab has shown that NK cells exhibit functional polarity and can become reprogrammed directly by breast cancer cells to promote tumor metastasis. Single cell RNA-seq analysis of human breast tumor infiltrating NK cells revealed a reprogrammed subset which was characterized by overexpression of Nuclear Receptor 4 A1 (NR4A1). NR4A1 is an orphan nuclear receptor known to regulate immunologic activity and cellular apoptosis. While NR4A1 has been shown to regulate T cell activity, the function of NR4A1 in NK cells is not well understood. In our RNA seq data, NR4A1 negatively correlated with KLRG1. Previously, we showed when Killer cell Lectin-like Receptor G1 (KLRG1), an inhibitory receptor on NK cells, is blocked with a monoclonal antibody, reprogrammed NK cells more effectively kill breast cancer cells. Previous data suggests NR4A1 can regulate NK cell function, but mechanisms for this remain elusive. We hypothesized that NR4A1 regulates NK cell function through KLRG1 and sought to further characterize the role of NR4A1 and KLRG1 in NK cells in the breast tumor microenvironment.

Methods: To genetically overexpress NR4A1 in NK cells, we developed a transposon-containing vector and used electroporation to successfully introduce and overexpress NR4A1 in NK cells. To test the functional relevance of NK cells, we utilize in vitro co-culture models and measure cytotoxicity between NK cells and target breast cancer cells. Breast cancer cell lines were selected based on receptor-ligand pairing using bioinformatic

analysis and experimental validation of their sensitivity to NK cell cytotoxicity. To assess the impact of NR4A1 on NK cell apoptosis, we assessed NK cell viability after challenge with reduced IL-2 concentrations. We then characterized the expression of NK cell surface receptors in our models using flow cytometry. Cell cycle state of cancer cells in coculture with NK cells were also measured by flow cytometry.

Results/Discussion: Using NK cells isolated from patients with breast cancer, we found that NR4A1 is more highly expressed at the protein level when compared to NK cells from healthy volunteers. Functionally, NR4A1 overexpression (OE) in NK cells decreased their cytotoxicity to K562 cancer cells by 33%. When NR4A1 OE NK cells are co-cultured with BT474 breast cancer cell line, a cell line shown to be resistant to NK cell cytotoxicity, the amount of breast cancer cells in S phase is increased compared to co-culture with WT NK cells. Notably NR4A1 OE NK cells also expressed 50% less KLRG1. NR4A1 OE in NK cells also decreased NK cell death by 50% under conditions of IL-2 depletion. The data suggest NR4A1 plays a unique role in NK cells and drives them to a state of reduced cytotoxicity and a direct or indirect tumor promoting state. NR4A1 also regulates NK cell survival and proliferation, potentially through KLRG1. Future studies will focus on understanding the mechanistic relationship between NR4A1 and KLRG1. Understanding how NK cell function is regulated could lead to improved NK cell-based therapeutics for patients with breast cancer.

P2-06-08: A Novel SERM Reinvigorates Natural Killer Cells by Uniquely Downregulating DKK1 in Breast Cancer Cells

Kristen Young, Govinda Hancock, Steven Kregel, Sean W. Fanning

Although Estrogen Receptor positive (ER+) breast cancer has treatment options that have initial success, many women often experience disease recurrence and hormone therapy resistance. A subset of these resistant patients develop a distinct tyrosine to serine missense mutation at position 537 (Y537S) in ER, encoded by the ESR1 gene, that leads to allele-specific transcriptional programs that enhance metastasis. Probing unique ER structural features can reveal new transcriptional activities critical for therapeutic efficacy. Our lab developed a structurally novel Selective Estrogen Receptor Modulator (SERM), T6I-29, to better understand these structure-transcriptional relationships. T6I-29 shows effective anti-tumoral activities in Y537S ESR1 breast cancer xenografts. To examine the anti-tumoral mechanism of T6I-29 action, we performed RNA-sequencing on Y537S ESR1 mutant breast cancer cells. Compared to other clinically relevant compounds, our drug uniquely and significantly downregulated DKK1, a tumor secreted glycoprotein. Upregulation of DKK1 correlates with metastatic burden across multiple cancers but has not been evaluated in ESR1 mutant breast cancers. Furthermore, online database information is limited on the correlation of DKK1 with ER+ breast cancer. Using patient and donor plasma samples, we find that DKK1 levels are significantly elevated in 108 ER+ breast cancer patients compared to 100 matched healthy donors. Furthermore, in breast cancer cell lines, those with Y537S ESR1 mutation show constitutive expression and secretion of

DKK1, while DKK1 expression and secretion is estrogen-dependent in WT breast cancer cells. Intriguingly, WT cells show significant surface bound DKK1, which is secreted into the media upon hormone stimulation and blocked by T6I-29. Recent findings in triple negative breast cancer and lung cancer models show DKK1 induces Natural Killer (NK) cell dormancy to enhance metastatic capabilities of tumor cells. We find in co-culture experiments with human NK cells and ER+ breast cancer cells, treatment with specifically T6I-29 enhances killing ability of NK cells. Understanding the ER-dependence and functional importance of DKK1 secretion offers a novel approach to potentially reduce breast cancer metastatic burden.

P2-06-09: Impact of lipid composition on triple-negative breast cancer progression and survival.

Khudeja Salim, Elnaz Sheikh, Bridgette M. Collins-Burow, Van T. Hoang, Elizabeth C. Martin, Matthew E. Burow, Manas R. Gartia

Triple-negative breast cancer (TNBC) is an aggressive type of breast cancer, with high resistance to chemotherapy and low survival rates, and accounts for 10-15% of all breast cancer cases. TNBC incidence has been associated with obesity and fatty acids in the tumor microenvironment (TME) derived from lipids can induce obesity-driven metabolic inflammation and affect TNBC pathology. However, the unique impact of the TNBC subtype on lipid composition in the TME, and its subsequent role as an energy source for cancer cell metabolism and progression is unknown. Understanding lipid heterogeneity in the TME will provide novel insights to target TNBC. It is, therefore, crucial to understand the impact of TNBC-driven TME remolding in the context of lipid composition. Our study aims to decipher the role of lipid composition in the progression of TNBC. Spatial lipid profiling of hormone receptor-positive (HR+) and TNBC primary tumors was performed and compared to matched normal breast adipose tissue. Raman mapping of lipids showed a heterogeneous distribution of lipids across the TNBC tissues, including five omega-3 fatty acids, two omega-6 fatty acids, and one unsaturated fatty acid were upregulated in TNBC tumors compared to normal-matched adipose. To determine the unique impact of lipids elevated in TNBC, we next demonstrated that the uptake of lipid compounds (linoleic acid, docosahexaenoic acid, and oleate acid) by TNBC cells increased cell proliferation after 72 hours of treatment. Overall, our results suggest that lipid composition plays a key role in breast TME and may represent a novel therapeutic strategy to target TNBC by alternate sensitivity to therapeutic modalities including apoptosis and ferroptosis.

P2-06-10: The transcription factor HLF (Hepatic Leukemia Factor) : a potential therapeutic target in triple negative breast cancer

Ibrahim Bouakka,Tran Dien Alicia, Sirerol, Marine, Aymeric Silvin, Rocca Anna, Fabrice Andre

Pivotal role of immune response in the evolution of triple negative breast cancer has been largely reported in the last decade. Our previous study highlighted the role of HLF gene as part of a four-gene signature and was found to be negatively correlated with tumoral immune cells infiltration. We investigated more precisely its implication in the immune response and TNBC tumor development. Bioinformatics analysis of publicly available TNBC patient datasets revealed that, in mesenchymal tumor subtypes expressing low levels of HLF, upregulation of genes associated with neutrophil attraction and activation pathways was observed in comparison with those expressing high HLF levels. The implication of neutrophils in cancer is contentious, as they can function either as active anti-tumoral partners or as pro-tumoral agents promoting immunosuppression and tumor growth. This ambiguity stems from their high plasticity, which enables them to switch roles during tumor progression, influenced by signals from tumoral microenvironment, in particular those from surrounding tumor cells. In this study, we employed CRISPR/Cas9 technology to inactivate the HLF gene in TNBC cell lines, enabling us to elucidate the downstream effects on the chemokine and protease inhibitor landscape that governs neutrophil infiltration and function within the microenvironment of TNBCs, especially in mesenchymal subtypes. In vivo experiments using immunodeficient mouse models revealed that HLF downregulation in TNBC mesenchymal subtypes correlates with increased neutrophil infiltration into the tumor, leading to inhibit tumor growth. Altogether, our results suggest that a reduction of HLF expression can lead in the early steps of tumor development to the awakening of the anti-tumoral function of neutrophils to enforce the necessary immunological tone to tumor elimination. Thus, HLF emerges as a potential therapeutic target in order to favor infiltration and function of anti-tumoral neutrophils to restore an efficient immune response in the aggressive mesenchymal TNBC subtype.

P2-06-11: TBK1 Promotes Growth of Aggressive Breast Cancer by Modulating the Tumor Microenvironment

Lan Phi, Manuel Van Gijsel-Bonnello, James Long, Naoto T. Ueno, Xiaoping Wang

Background: Triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC) are the most aggressive breast cancer subtypes. TANK-binding kinase 1 (TBK1), a member of the IKK family, regulates innate immune responses to viruses and other pathogens and functions as an immune-evasion gene. Studying TBK1 is crucial in the context of aggressive breast cancer due to its potentially significant role in tumorigenesis driven by regulation of the tumor microenvironment (TME). However, the molecular mechanisms of TBK1 signaling in regulating the tumorigenesis of TNBC and IBC remain unclear. To address this knowledge gap, we tested the hypothesis that TBK1 signaling promotes the aggressiveness of breast cancer by generating an immunosuppressive TME.
Methods: We knocked out (KO) TBK1 expression in two human cancer cell lines [one IBC (SUM149) and one TNBC (HS578T)] and tested the effect on colony formation, migration, and invasion in vitro. Additionally, we performed RNA sequencing analysis on the control (Ctrl) and TBK1 KO clones to determine the underlying mechanism by which TBK1

regulates IBC and TNBC cell growth. Last, we inoculated Ctrl and TBK1 KO murine 4T1.2 TNBC cells into BALB/c mice and examined the effects of TBK1 KO on tumor growth and TME components.
Results: TBK1 KO significantly reduced colony formation, migration, and invasion in both SUM149 and HS578T cells. RNA sequencing analysis of TBK1 KO clones revealed downregulation of pathways associated with the inflammatory response, such as IL-17 signaling and NF-kB signaling. Furthermore, cytokine-mediated signaling pathways and cytokine-stimulated cellular response were downregulated in these TBK1 KO clones. In the syngeneic murine 4T1.2 xenograft model, tumors derived from TBK1 KO cells exhibited slower growth ($P = 0.0382$) and had fewer M2 macrophages ($P = 0.0068$) than Ctrl cells. Additionally, TBK1 KO tumor samples showed a reduction in protein expression of immunosuppressive cytokines, including CCL27, CXCL1, CXCL2, and CCL2, compared to Ctrl tumors. These results collectively suggest that TBK1 can modulate the TME, promoting aggressive breast cancer tumorigenicity.
Conclusion: TBK1 plays a critical role in the aggressiveness of aggressive breast cancers through generating an immunosuppressive TME. Further understanding TBK1's mechanisms can uncover novel therapeutic targets, potentially improving breast cancer treatment and patient outcomes.

P2-06-12: MGAT1-Mediated Glycosylation Orchestrates Immune Checkpoints and Antitumor Immunity

Junlong Chi

Despite the widespread application of immunotherapy, treating immune-cold tumors remains a significant challenge in cancer therapy. Using multiomic spatial analyses and experimental validation, we have identified MGAT1, a glycosyltransferase, as a pivotal factor governing tumor immune response. Overexpression of MGAT1 leads to immune evasion due to aberrant elevation of CD73 membrane translocation, which suppresses CD8+ T cell function, especially in immune-cold triple-negative breast cancer (TNBC). Mechanistically, addition of N-acetylglucosamine to CD73 by MGAT1 enables the CD73 dimerization necessary for CD73 loading onto VAMP3, ensuring membrane fusion. We further show that THBS1 is an upstream etiological factor orchestrating the MGAT1-CD73-VAMP3-adenosine axis in suppressing CD8+ T cell antitumor activity. Spatial transcriptomic profiling reveals spatially resolved features of interacting malignant and immune cells pertaining to expression levels of MGAT1 and CD73. In preclinical models of TNBC, W-GTF01, a newly developed inhibitor, specifically blocked the MGAT1-catalyzed CD73 glycosylation, sensitizing refractory tumors to anti-PD-L1 therapy via restoring capacity to elicit a CD8+ IFNγ-producing T cell response. Collectively, our findings uncover a strategy for targeting the immunosuppressive molecule CD73 by inhibiting MGAT1.

P2-06-13: HOXB13 Expression Induces an Immunosuppressive Tumor Microenvironment in Breast Cancer

Dennis Sgroi, Yun Xia, Marinko Sremac, Shawn Demehri

Background: Multiple studies has shown that HOXB13 gene expression is associated with poor prognosis for hormone receptor positive (HR+) post-menopausal breast cancer (BC) patients. However, the biological contribution of HOXB13 in such patients is unclear. Previous gene expression- and proteomic-based analyses of HOXB13-high and -low human breast cancers and breast cancer cell lines indicate that HOXB13 expression is associated with interferon gene signatures, suggesting that HOXB13 may modulate the tumor immune microenvironment (TIME). Herein, we investigate the role of HOXB13 expression in breast cancer development in two independent mouse mammary tumor transplant models.
Methods: Murine HOXB13 was ectopically expressed the ER+ SSM3 and the ER- PyMT mouse mammary tumor cell lines, and these cells along with non-HOXB13 expressing control cell lines were transplanted into the inguinal mammary fat pad of syngeneic wild-type (WT) mice on the 129S6/SvEv and C57BL/6 backgrounds, respectively. Tumor volumes and weight were monitored every other day and mice sacrificed at day 40. Tumor single-cell suspensions were prepared, and tumor infiltrating lymphocytes (TILs) were isolated using CD45+ Microbeads on magnetic columns. Cells were stained with the following surface antibodies: CD3, CD4, CD8α. After fixation and permeabilization, intracellular stains were performed using antibodies to TNF-a and TGF-b. Stained cells were assayed using a BD LSRFortessa flow cytometer, and data were analyzed using FlowJo software. To demonstrate that mature T and/or B cells lymphocytes play an important role in the modulation of tumor growth, PyMT-HOXB13 and -control cells were transplanted into Rag1 knockout (Rag1 KO) mice on the C57BL/6 background.
Results: The SSM3-HOXB13 and PyMT-HOXB13 cells exhibited significantly increased tumor growth rate compared with their corresponding control cells after implantation into the inguinal mammary fat pad of WT 129S6/SvEv and C57BL/6 mice, respectively (Figure 2A). Analysis of tumor-infiltrating lymphocytes revealed a marked decrease in CD3+ T and CD8+ T cells in both the ER+ SSM3-HOXB13 and the ER- PyMT-HOXB13 tumors, along with a significantly reduced percentage of activated (TNF-α+ and IFN-γ+) CD8+ T cells in these tumors. Importantly, PyMT-HOXB13 and PyMT-Ctrl tumors grew at a similar rate in the inguinal mammary fat pad of Rag1 KO mice lacking T and B lymphocytes.
Conclusions: HOXB13 expression in two independent mouse mammary tumor models confers tumoral growth advantage by impeding antitumor T cell immunity.

P2-06-14: Calcium-Sensing Receptor Agonist Enhanced Anti-Tumor Activity of Adoptive NK Cell Therapy in Triple-Negative Breast Cancer

Yu Shi, Qun Lin, Zhuxi Duan, Jinpeng Luo, Jieer Luo, Xiaolin Fang, Chang Gong

Triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2), often presents a higher infiltration of lymphocytes, suggesting a favorable immune microenvironment for immunotherapy. Recently, natural killer (NK) cells have emerged as a promising option for adoptive cell transfer in cancer immunotherapy. While adoptive NK cell therapy has shown promising efficacy in hematologic malignancies, its practical application in TNBC treatment remains uncertain and variable. Therefore, strategies to enhance the anticancer immune responses of adoptive NK cell therapy in TNBC are urgently needed to improve survival outcomes for TNBC patients. In this study, we explored a novel strategy that combines a calcium-sensing receptor (CaSR) agonist with adoptive NK cell therapy to improve anti-tumor activity in low-response-rate TNBC patient-derived xenograft (PDX) models. Mechanistically, an intracellular Ca2+ imbalance prompted the integration of HLA-DP molecules and circRNA-encoded 296aa, resulting in the retention of HLA-DP intracellularly. The CaSR agonist was observed to aggregate at photodamage sites, leading to ESCRT complex aggregation and membrane repair, which facilitated the budding of HLA-DP-enriched microvesicles. These microvesicles specifically targeted the activating receptor Nkp44 on NK cells, thereby enhancing their cytotoxicity. In vivo experiments further confirmed the efficacy and safety of this combined therapy. These results indicate the feasibility of using adoptive NK cell therapy in combination with a CaSR agonist as an efficient immunotherapy approach for TNBC patients who may be resistant to conventional poly adoptive NK cell therapy.

P2-06-15: DCIS-associated myoepithelial cells drive transcriptional alterations in macrophages through up-regulation of integrin αvβ6

Michael Allen, M. Reza Roozitalab, Stephen Murtough, Eleni Maniati, J. Louise Jones

Ductal carcinoma in-situ (DCIS) is a non-obligate precursor of invasive breast cancer, however, less than 50% of DCIS will ever progress to invasive cancer. There is therefore a need to understand how we can predict those DCIS cases that will progress. A key component in promoting tumour invasion is the immune microenvironment, though its role in DCIS is unclear. We previously have shown that up-regulation of integrin αvβ6 on DCIS-associated myoepithelial cells switches the tumour-suppressor properties of myoepithelial cells to tumour-promoter properties, leading to enhanced tumour cell invasion. We found that macrophages surrounding ducts positive for αvβ6; exhibited a more M2 tumour-promoter phenotype compared to DCIS ducts with myoepithelial cells negative for αvβ6. We therefore hypothesised that the altered phenotype of myoepithelial cells in DCIS may directly influence the peri-ductal immune infiltrate which could influence disease progression. Macrophages were isolated from fresh tissue from patients with DCIS. These were confirmed as either positive or negative for myoepithelial αvβ6. The macrophages were subjected to RNA sequencing. In parallel, primary myoepithelial cells were isolated from cosmetic mammoplasty samples and used to overexpress αvβ6 using a lentiviral inducible system. Conditioned Medium (CM) was collected and this was applied to

macrophages which then underwent RNA seq. RNA-seq analysis demonstrated novel transcriptomic profiles revealing upregulation of genes, e.g. AREG and FOSL2, with potential prognostic significance in DCIS. Utilising conditioned medium generated from primary myoepithelial cells with inducible expression of αvβ6, we demonstrate induction of immune checkpoint inhibitor - CD274 (PD-L1) in monocyte-derived macrophages in vitro. We identified a novel macrophage gene signature, outside of the usual M1/M2 dichotomy, pointing towards a macrophage spectrum which is driven by an in vivo αvβ6-dependent tumour progressive ME. This work thus suggest a panel of microenvironmental markers could be used to predict progressive DCIS cases and may represent novel therapeutic targets to influence a tumour suppressive immune response.

P2-06-16: Addressing Thermal and Battery Efficiency in AI Enhanced Portable Ultrasound Screening Protocols for Breast Cancer

Nusrat Zaman Zemi, Dustin Valdez, Arianna Bunnell, John Shepherd

Background: Breast cancer remains a significant cause of mortality in women, especially in rural and underserved communities where access to mammography is limited or nonexistent. The incidence of advanced-stage breast cancer is 66% higher in Hawaii than in the mainland US, and 5 – 9 times higher in the U.S. Affiliated Pacific Islands (USAPI). AI-enhanced point-of-care ultrasound (POCUS) breast imaging may be an effective method for detecting breast cancer while still in the early stages. The hypothesis is that AI detection and classification algorithms will increase the accuracy of POCUS such that it would approach that of mammography. Further, it may reduce the training levels needed to do the early detection POCUS scanning. One such candidate device is portable, wireless, and provides an SDK for inserting AI models before presenting the image to the user. We have been exploring a suitable protocol for using this POCUS device in remote conditions where infrastructure may be limited. Further, we asked if the portable battery-operated scanner could keep up with a scanning rate of 2 patients per hour for an indefinite amount of time. In this study, we seek to identify the performance parameters that need to be considered to use this and other POCUS systems in rural, remote, and underserved communities.
Methods: The POCUS system consists of a portable handheld scanner (Clarius L7 HD3 model; Clarius Mobile Health Inc, Vancouver, Canada), a tablet computer (Samsung Galaxy Tab S9 Ultra; Samsung Electronics Co., Ltd, South Korea), and a laptop (Dell XPS 15 running Windows 11; Dell Inc., Texas, US). The scanner communicates with the tablet and laptop via an ad hoc WIFI connection. A breast phantom (Gphantom, EDM Medical Solutions, Florida, US) was scanned continuously for 10 minutes, followed by a 20-minute charging period, and repeated. Pretrained AI models were inserted into the image stream using the Cast API. Scanner temperature and battery levels were monitored to determine their time characteristics utilizing the Clarius app. Longer scan and charging periods were used as well to capture the full extent of heating, cooling, and charging cycles. Probe temperature and charging/discharging characteristics were fit to exponential functions.
Results: The system was able to operate for 24 minutes continuously before hitting a

thermal protection temperature of 48°C. The probe was able to fully recharge from 0 to 100% within 60 minutes. For the 2-patient-per-hour protocol, the probe was able to regain its starting charge and temperature for back to back phantom scans over 4 hours. Time constants were found to be 16.1 minutes for temperature increase and 49.6 minutes for battery discharge. The user found it difficult to hold the probe for temperatures above 42°C. A shorter time between scans may not be feasible but being tested.
Conclusion: The findings show that the device can be used continuously for at least two patients per hour with 10 minutes of continuous scanning followed by 20 minutes of charging. However, the operating temperature of the probe is quite high and it may be difficult to handle. Healthcare workers can optimize usage protocol to maximize functional time without frequent recharging in remote areas. Future work will focus on refining AI models for real-time applications and exploring alternative devices with better thermal performance.

P2-06-17: An Integrated Analysis of Metastatic Gene Co-expression in Triple-Negative Breast Cancer via Bulk and Single-Cell Transcriptome Data

Melody Hong, Gábor Balázsi

Background: Triple-negative breast cancer (TNBC) is clinically significant for its high invasiveness (metastasis) and poor prognosis. The molecular classification of breast cancers and characterization of TNBC have been aided by the analysis of both bulk and single-cell RNA-sequencing data. Specifically, they have facilitated the study of the roles of various regulator and effector genes associated with pro-metastatic behaviors such as invasion, and drug resistance-related epithelial-to-mesenchymal transition (EMTI). Yet, it remains unknown how informative and consistent bulk and scRNA-seq TNBC data are in regards to co-expressions of EMTI genes.
Methods: Bulk TNBC RNA-seq data was retrieved from the TCGA-BRCA cohort of the TCGA-TARGET-GTEx Recompute Project, cleaned, and normalized. Single-nucleus TNBC tumor cell data curated by Pal et al. (2021) was separated into six datasets based on patient(s) of origin/cell cycling status and preprocessed. A list of 363 EMTI genes from the EMTome was defined for all gene-gene correlation analyses. Significantly correlated gene pairs with co-expressions concordant among TNBC dataset pairs and larger groupings were found. To identify possibly TNBC-specific correlations, co-expression analysis was repeated for both bulk normal breast from the GTEx cohort of the Recompute project and snRNA-seq normal breast epithelial data from Pal et al. (2021). EMTI regulatory networks were reconstructed using co-expression data between 26 key EMT genes for both TNBC and normal. Co-expression comparisons and differential co-expression analysis were performed between the TNBC and normal samples. Differential expression analysis was also performed between the bulk TNBC and normal to investigate the relationship between differential EMTI gene expression and co-expression.
Results: EMTI gene co-expression patterns exhibited greater similarity between TNBC single-cell datasets than between any single-cell and bulk TNBC. Moreover, the datasets

most concordant with each other were single-cell datasets originating from the same patient(s), indicating that EMTI gene co-expressions do not significantly differ with cell cycling status. The SPINT1 and LSR genes were significantly positively correlated in all TNBC samples but not in normal, revealing a previously undocumented link. Additionally, we found 11 TNBC single-cell-specific gene-gene correlations (7 positive, 4 negative), relationships that would not have been identified in heterogeneous bulk TNBC. We observed known interactions between EMTI genes, including an EpCAM-keratin-claudin link well preserved across both TNBC and normal breast. Out of the three breast epithelial lineages (luminal progenitor (LP), mature luminal (ML), basal), EMTI gene co-expressions in TNBC single-cell were most similar to those in LP; however, those in the bulk TNBC were most similar to basal. With the exception of 2 gene pairs common to the bulk datasets, there were no EMTI gene pairs with consistent co-expression patterns across the different TNBC/normal groupings. Interestingly, differential EMTI gene co-expression was weakly associated with differential gene expression; this applied for genes non-differentially expressed (KRT5), upregulated in TNBC (EPCAM), or downregulated (TGFB1I1) (Spearman's ρ: 0.139, 0.145, 0.0839; all $p < 10e-07$).
Conclusions: Our integrated analysis reveals that EMTI gene pairs are moderately concordant among TNBC or normal datasets, but not between the two. Moreover, we show both known and new interactions in reconstructed networks. We identify a TNBC single-cell-specific 11 gene pair signature that may be promising for further study. Finally, although contrary to previous work we find that differential EMTI gene co-expression can be partially explained by differential gene expression, the association is weak enough where the two remain complementary approaches.

P2-06-18: Deep learning-based tumor microenvironment signature predicts progression-free survival in early-stage hormone receptor positive and HER2 negative breast cancer

Firas Khader, Omar S. M. El Nahhas, Firas Khader, Evan Paul, Pavol Cekan, Nicoleta Antone, Daniel Truhn, Jakob Nikolas Kather

Molecular risk assays, such as OncotypeDX, have emerged as a robust method for guiding adjuvant chemotherapy alongside endocrine therapy in early-stage hormone receptor positive (HR+) and HER2 negative (HER2-) breast cancer (BC). However, these assays have limitations in that they lack the capability to capture the heterogeneity and complexity of the tumor microenvironment. They do not account for morphological features in routine hematoxylin and eosin (H&E)-stained histopathology slides—features that can provide insights into how the tumor cells are behaving in situ. Additionally, molecular assays are expensive and time-consuming, limiting their widespread use in clinical practice. Consequently, there is a need for alternative or complementary prognostic tools that can analyze tumor morphology and the TME from H&E slides.
Deep learning (DL) can predict cancer biomarkers directly from H&E slides, enabling the automated and consistent quantification of the tumor microenvironment (TME) for clinical

decision-making. Our study focuses on the prediction of TME signatures on a large international and multi-centric cohort for the prognostication of BC patients. We developed a weakly-supervised, transformer-based multi-task deep regression model to predict signatures of the TME directly from routine H&E-stained histology slides. We trained the model using 5-fold cross-validation on a large pan-cancer cohort of patients (n=2,690), and validated its prognostication performance on an external cohort of early-stage HR+/HER2- female BC patients (n=470).
The model predicted signatures of tumor cell proliferation and tumor infiltrating lymphocytes (TILs) as a continuous variable, which was carried forward for survival analyses as a composite interaction score. Using longitudinal data from the multi-centric external cohort (median follow-up = 133 months), survival analysis was performed using a Cox proportional hazards model with adjustment for age, nodal status, stage, administration of adjuvant chemotherapy, and a research-based molecular risk of recurrence score. Our AI-based TME signature was significantly associated with improved 12-year progression-free survival, with a hazard ratio = 2.28, (95% confidence interval; 1.19, 4.36), p=0.01.
Our new AI-based prognostication method, which we call SAIBR (StratifAI BReast), provides a cheap and readily available algorithm for analyzing digital pathology images of H&E sections of BC to quantify tumor cell proliferation and TILs. The data show that SAIBR is an informative prognostic marker for 12-year progression-free survival and that it provides prognostic information beyond standard clinicopathological factors and validated molecular risk assessment in early-stage HR+/HER2- breast cancer.

P2-06-19: The clinical relevance of infiltrating hematopoietic stem cells in the breast cancer tumor microenvironment

Masanori Oshi, Rongrong Wu, Akimitsu Yamada, Kazutaka Narui, Takashi Ishikawa, Itaru Endo, Kazuaki Takabe

Background: There are numerous types of cells in the tumor microenvironment (TME) that are known to affect breast cancer (BC) biology. However, the roles of cells that are rare in TME are vastly understudied. Recent advances in transcriptomic analyses enabled us to quantify and study the roles and clinical relevance of the extremely rare cells in TME, including hematopoietic stem cells (HSCs).
Material and Methods: In silico analyses was conducted on total of 5,176 breast cancer (BC) patients from large independent cohorts; SCAN-B and METABRIC, and single cell sequence data including GSE169246, GSE246613, GSE 161529, GSE167036, and GSE255107 from NCT03366844clinical trial. HSC was quantified using xCell algorithm for bulk tumor, and SingleR algorithm for single cell sequence data. High HSC patients were defined as more than the median in each cohort.
Results: Utilizing 5 single cell sequencing data, HSCs were less than 0.1 % of immune cells in ER+/HER2- as well as 0.1% in triple negative BC, and inversely related with pathological grade. HSCs did not collate with any of the lineages including lymphocyte, myeloid,stromal, nor stem cells in TME. BC patients with high HSCs was significantly associated with better

overall survival (OS) compared to low, particularly in ER+/HER2- BC in both SCAN-B and METABRIC cohorts (both p<0.02). Further, high HSCs ER+/HER2- BC was significantly associated with better disease-specific survival (DSS) in the METABRIC cohort (p=0.028). High HSC ER+/HER2- BC enriched several pro-cancerous gene sets, including TGF-β, KRAS signaling, NOTCH signaling, epithelial-to-mesenchymal transition, angiogenesis, and hypoxia. On the other hand, low HSC patients enriched cell proliferation-related gene sets; E2F targets, G2M checkpoint, MYC targets-v2, and mitotic spindle. High HSC ER+/HER2- BC was significantly associated with high fraction of dendritic cells, adipocytes, endothelial cells, pericytes, and fibroblasts, and with low fraction of Th1 cells, Tregs, NK T cells, memory B cells, M1-macrophages, and eosinophils consistently in both cohorts (all p<0.001). The level of HSC infiltration showed no association with response to neoadjuvant chemotherapy consistently with low Area Under the Curve, and neither OS nor DSS after chemotherapy in the METABRIC cohort (p=0.705 and 0.643, respectively). On the other hand, high HSC was significantly associated with lower risk of lung metastasis and with better survival.
Conclusion: In silico approach detects HSCs in the BC TME and revealed that it is inversely associated with cancer cell-proliferation, and better patient survival in ER+/HER2- BC. Given that HSCs was not associated with response to chemotherapy, but with risk of lung metastasis and survival, we speculate that it may have a role in the BC biology rather than chemotherapy resistance.

P2-06-20: Use of an AI Algorithm to Determine the Prevalence of Breast Arterial Calcifications in Women Undergoing Screening Mammograms Based on Race, Age and Cancer Status

Chirag Parghi, Jennifer Pantleo, Jeff Hoffmeister, Julie Shisler, Wei Zhang, Avi Sharma, Zi Zhang

Background: Breast arterial calcifications (BAC) on mammography has been historically overlooked and underreported as an “incidental finding”. Due to the success of mammography as a screening platform and known gaps in cardiac screening for women, BAC presence and extent can potentially identify women that may benefit from enhanced cardiac screening and medical optimization.
Methods: A set of 3558 Hologic Digital Breast Tomosynthesis (DBT) screening mammograms, including 394 cancer cases and 3164 noncancer cases from October 7, 2014 to April 16, 2021 across 3 healthcare systems were analyzed using a deep learning AI algorithm trained to detect BAC on 2D images from combo DBT or 2D synthetic images from DBT. Patients ranged from 35 to 94 years of age. The dataset was weighted relative to a screening population based on Breast Cancer Surveillance Consortium based on specific clinical characteristics, namely age, race, and mammographic density with a cancer incidence of 6/1000. The study assessed overall prevalence of BAC as well as distribution among women with cancer and without cancer and by race and age.
The AI model was trained using an internal dataset of 2D/synthetic mammograms to detect

BAC based on expert annotation and provides a BAC of present or absent. The accuracy of the AI model was validated on a data set of 2D mammograms from 8,881 women. Ther was no overlap in the training, validation and the 3558 women prospective study data sets.
Results: The unweighted overall prevalence of BAC in this cancer enriched dataset of screening exams is 17.7%. When normalized by standard age, mammographic density, and racial demographic data with a cancer incidence of 6/1000, the (weighted) prevalence of BAC changed to 15.0%, which was used for subsequent analyses. BAC is present in mammogram exams in 33.9% of women with cancer and 14.8% of women without cancer. BAC prevalence per race in the screening adjusted dataset is 14.6% White, 17.7% Black, 13.9% Asian and 17.4% in other races. BAC prevalence per age group in the screening adjusted dataset is 3.7% in women <50 years old, 8.6% in 50-59, 17.3% in 60-69 and 37.4% in women 70 and older. When the age deciles were consolidated into two groups above and below age 60, the weighted BAC prevalence was 25% in patients age 60 or above and 6.5% below the age of 60.
Conclusion: The weighted prevalence and distribution of BAC increases with age as expected in a screening population. Interestingly, BAC prevalence did not vary by race suggesting it could serve as an effective cardiovascular biomarker across racial groups. AI based BAC detection on mammography demonstrates high prevalence of BAC in women with mammographically detected breast cancer. Women with increased BAC and breast cancer may benefit from cardiovascular assessment in addition to undergoing oncological treatment. In that sense, a conventional mammogram can identify cardiac needs of patients prior to or at the time of breast cancer diagnosis.

P2-06-21: Preliminary validation of a multi-modal AI for stratification of early-stage breast cancer patients utilizing a foundation model for digital pathology

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Early breast cancers are risk-stratified based on clinicopathological characteristics and refined by genomic assays that predict the risk of recurrence. Clinical features alone are often insufficient for accurate predictions, while genomic assays are limited by long processing times, tissue exhaustion, and high costs.
Digital pathology is an imaging modality that captures information about tissue morphology and the tumor microenvironment, providing valuable insights into tumor biology and patient outcomes. However, the complexity of these images make it impractical to depend on pathologists for manual assessment. Whole slide image (WSI) analysis is labor-intensive, time-consuming, and subject to interobserver variability. However, recent advances in artificial intelligence (AI), specifically in self-supervised learning, have enabled researchers to train models to extract meaningful histopathological features by learning over large unlabeled datasets of pathology slides, providing a path to overcoming the limitations

presented by traditional assays.
We developed an AI model that integrates digital pathology with clinical information to predict risk of breast cancer recurrence. We extracted pathology imaging features using an AI foundation model trained on 500 million image patches derived from 80,000 WSIs. Imaging-based features were combined with routinely collected clinical information and used in time-to-event models to predict risk of an event. These models can assess patient risk in minutes, instead of the weeks it takes for genomic assays, and with a fraction of the labor. In this analysis, we have developed and trained models to predict disease-free interval (DFI).
We evaluated our model's performance on 992 stage I-III patients from TCGA's BRCA cohort. This dataset has not been used to train or optimize the model's hyperparameters. Additionally, we performed a subgroup analysis in 365 hormone receptor-positive (HR+) node-positive (N+) cases and 102 TNBC cases. Continuous risk scores between 0 and 100 generated by the multi-modal test accurately predicted DFI, achieving a C-index of 0.71 (95% CI 0.63–0.77) in the all-TCGA group, 0.69 (95% CI 0.59–0.76) in the HR+ N+ cohort, and 0.81 (95% CI 0.71–0.90) in the TNBC cohort. When categorized into low- and high-risk groups with a naive, non-optimized 90/10 threshold, we observed a statistically significant difference in DFI between high- and low-risk groups (log-rank tests $p<0.01$). The hazard ratio between high- and low-risk patients was 3.40 (95% CI 2.15–5.40) in the all-TCGA group, 3.28 (95% CI 1.59–6.79) in the HR+ N+ cohort, and 4.86 (95% CI 1.37–17.32) in the TNBC cohort.
Additionally, we conducted an analysis showing that the features generated by our AI foundation model provide independent information beyond the clinical data. In a Cox proportional hazards model, after adjusting for the clinical-only score, the digital pathology imaging-based score remained statistically significant with the log-rank test ($p<0.001$). Even accounting for the clinical score, the hazard ratio per 20-unit increase in our digital pathology score corresponds to a hazard ratio of 2.99 (95% CI 1.54–5.80).
Our model is prognostic in early-stage breast cancer, including in clinically meaningful groups such as HR+ N+ and TNBC patients. As the model is fully digital, it can significantly expedite the risk assessment process compared to molecular tests and can provide access to a wider population at a fraction of the cost. Finally, AI-derived imaging features provide novel prognostic information not captured through traditional clinicopathological features.

P2-06-22: PreciseBreast, an AI-enabled digital test predicting breast cancer recurrence is equivalent with Oncotype in an observational, retrospective study of patients from Baptist Health Miami Cancer Institute in partnership with COTA, Inc.

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Background: PreciseBreast (PDxBR) is an AI-enabled clinical grade digital test combining demographic and pathology data e.g. age, tumor size, anatomic stage and lymph node status with outcome-curated image features (biomarkers) from an H&E image of early-stage invasive breast cancer (IBC) to provide prognostic information regarding risk of recurrence within 6 yrs. PDxBR was previously evaluated on a cohort of patients (pts) with Oncotype (ODX) results from the Mount Sinai Health Care System, NY, NY and demonstrated improved risk discrimination when compared to ODX. We sought to extend these initial observations on a cohort from the Miami Cancer Institute and Baptist Health South Florida in partnership with COTA, Inc.

Methods: An early-stage retrospective cohort of IBC pts with ODX results and 6-yrs outcome data from Miami Cancer Institute were identified utilizing COTA's real-world database. H&E glass slides were scanned, and images processed with the PDxBR platform to generate an algorithm validated low (</=58) vs high (>58) risk score (scale 0-100) for predicting disease recurrence. Risk stratification, adjuvant treatment choice and event classification between PDxBR and ODX were evaluated using c-index/AUC and KM curves with hazards ratio, NPV, PPV, sensitivity (SE), specificity (SP). Adjuvant treatment decisions based on ODX were compared with PDxBR risk stratification and summarized descriptively. Subgroup analysis stratified by age and positive lymph node status was also performed.

Results: This cohort included 425pts with stage I-II, HR+, HER2- IBC, with a median age of 59 years (75%>50), 91% were White, and 60% Hispanic. 90% tumors <2.5cm, 50% clinical grade 2, 81% LN negative, with median 6-years of follow-up. 20% of patients had received adjuvant chemotherapy, 99% endocrine therapy and 56% radiation. There were 40 events (9.4% event rate: 11 deaths, 19 distant recurrences and 10 local-regional recurrences). ODX classified 87% (n=371) as low/intermediate risk with a RS </=25 vs PDxBR 79% (n=337) low risk score </=58 ; ODX AUC was 0.69 vs. PDxBR AUC 0.66 (Delong test p-value=0.3). The NPV for ODX vs PDxBR was 0.93,0.93; PPV 0.26, 0.17 and HR 4.26, 2.41 (p-value=0.22) with a SP of 0.90, 0.81, and SE 0.35, 0.38. The avoidance of adjuvant chemotherapy was comparable between both ODX (81%) low/intermediate and PDxBR (79%) low risk pts, respectively. Of the pts who developed an event, ODX correctly classified 14/40 (35%) as high risk (RS>25) vs PDxBR 15/40 (38%) as high risk (risk score >58). For the combined 29 distant metastasis and loco-regional events, the ODX identified 14/29 (48%) vs PDxBR 13/29 (45%). Similar results were obtained by conducting a subgroup analysis stratified by age >50 years. Due to the limited sample size, we were unable to conduct a comprehensive analysis on patients <50 years and positive lymph nodes.

Conclusion: The results support the equivalence of PDxBR vs. ODX in providing prognostic information regarding the risk of recurrence for pts with early stage IBC. The NPV of 93 along with comparable specificity, event classification and use of risk directed adjuvant therapy supports the use of this assay to provide a standardized AI-enabled grade and

phenotype of IBC. This information may enhance overall risk discrimination when used in conjunction with genomic assays which also provide important predictive results that aid in decisions regarding the use of adjuvant chemotherapy. Additional studies are underway to further confirm these proposed clinical applications.

P2-06-23: Using a Machine Learning Model for Prediction of Palbociclib Response in a Single Institution, Real-World Analysis of Patients with ER+ HER2- Advanced Breast Cancer

Xiaojie Zhang, Akshat Singhal, Safia Hassan, Kelly Gu, Trey Ideker, Kay T. Yeung

Introduction: CDK4 and 6 inhibitors (CDK4/6i) have transformed the treatment landscape of ER+ HER2- advanced breast cancer (ABC), significantly improving progression free survival in several phase III trials. Despite this, overall response rates hover around 50% in the 1st line setting, and development of resistance is nearly universal. To date, no established biomarkers predict sensitivity to CDK4/6i, emphasizing the need for a predictive tool for optimized CD4K/6i use. We recently published work (Park et al., Nature Cancer 2024) on an interpretable, 'visible' neural network model (VNN) based on a hierarchical proteomics map of 718 genes assessed by one or more clinical cancer gene panels. This model, validated in the metastatic breast cancer cohort from the AACR Project GENIE registry, predicts response to palbociclib (palbo). In this study, we successfully applied the palbo-VNN model in identifying patients with ER+ HER2- ABC who have palbo sensitive vs resistant disease in a single institution, retrospective analysis.
Methods: We conducted a retrospective study of patients with biopsy-proven ER+ HER2- ABC and available tissue-based genomic sequencing data treated with palbo between February 2016 and September 2023 at UC San Diego. Clinical data gathered included patient demographics, disease characteristics (de novo vs recurrent metastatic breast cancer), therapy line, treatment duration, and overall survival (from palbo start date to death). Tissue-based genomic sequencing data from commercially available vendors were used as input for the palbo-VNN model. Palbo-VNN is an ensemble of five models; tumors with a predicted score <0.645 (previously validated and described in our published work) in at least two models were categorized as palbociclib sensitive (palbo-VNNsen), with the rest categorized as palbociclib resistant (palbo-VNNres).
Results: A total of 139 patients met inclusion criteria. The median age was 63 (range 33-91) with a median ECOG performance status of 1 (range 0-3). Among the patients, 34% had de novo metastatic disease, 17% had prior localized disease with metastatic relapse within 2 years of adjuvant endocrine therapy (ET) initiation, and 41% had metastatic relapse after 2 years of adjuvant ET. Bone metastases were present in 71% of patients, visceral involvement in 66%, and central nervous system involvement in 9%. 75 out of 139 patients (54%) received palbo in the 1st line setting. In the 1st line setting, median therapy duration was 12.3 months (0.26-72). At a median follow-up of 72.5 months since palbo start, patients with palbo-VNNsen tumors (n=38) had a median treatment duration of 49.6 months compared to 15.6 months for those with palbo-VNNres tumors (n=37) (HR=0.63, p=0.01).

Patients with palbo-VNNsen and palbo-VNNres disease had a median overall survival of 79.3 vs 45.7 months (HR=0.68, p=0.03), respectively. Further subgroup analyses, such as stratification for de novo vs early relapsed disease, visceral vs bone only metastatic disease, and additional resistance pathways analysis, will be presented at the meeting.
Conclusion: This is one of the first real-world retrospective analysis using a machine-learning model to predict CDK4/6i therapy response in ER+ HER2- ABC. The palbo-VNN model effectively predicted sensitivity to palbo, correlating with longer therapy duration and overall survival in palbo sensitive disease. This highlights the clinical utility of the palbo-VNN model in optimizing patient selection for CDK4/6i therapy in the first line setting. Future research could explore the model's application in selecting patients for sequential CDK4/6i treatment and could enhance our understanding of CDK4/6i resistance mechanisms, potentially improving outcomes and quality of life for patients with ER+ HER2- ABC.

P2-06-24: Effect of an Image-Derived Short Term Breast Cancer Risk Score in the Analysis of Breast Cancer Prevalence in Screening Population by Race and Breast Density

Chirag Parghi, Jennifer Pantleo, Julie Shisler, Jeff Hoffmeister, Wei Zhang, Avi Sharma, Zi Zhang

Background: Screening with digital breast tomosynthesis (DBT) improves breast cancer detection and reduces false positives. Adding a short term breast cancer risk assessment model using image-derived artificial intelligence (AI) has shown higher performance than traditional lifestyle and family-based models in cohort studies. Although the image-based risk is reported as a discrete number across all patients, the predictive accuracy may vary across patients by race and/or amounts of breast density. Contextualizing image-based risk categories by race and density can potentially enhance clinical use during interpretation of subsequent screening mammograms.
Methods: A set of 3558 Hologic (DBT) mammograms, including 394 cancer cases and 3164 noncancer cases from October 7, 2014 to April 16, 2021 across 3 healthcare systems were analyzed with a deep learning AI Risk model. Patients ranged from 35 to 94 years of age. An imaging only AI risk model utilizes mammographic features, density and age at exam to provide a single risk score, identifying women more likely to be diagnosed with breast cancer at the next annual screening exam. Women are considered average risk with an absolute one year risk score of <0.34%, intermediate 0.34-<0.6% and high ≥ 0.6%. The dataset was analyzed adjusting to a screening population based on Breast Cancer Surveillance Consortium clinical characteristics of age and race for screening DBT exams with a cancer incidence of 6/1000. The study assessed the distribution of overall risk score as well as sub analysis by density and race.
Results: In women with an average risk score, the prevalence of cancer is 1 in 1369 within 68.7% of adjusted screening population, intermediate is 1 in 171 (13.1% of population) and high is 1 in 38 (18.2% of population). For women with non-dense breasts the prevalence of

cancer in the average risk score group is 1 in 1427 (39.5% of adjusted screening population), intermediate is 1 in 231 (8.4% of population) and high is 1 in 41 (10.3% of population). For women with dense breasts the prevalence of cancer in the average risk score group is 1 in 1297 (29.3% in adjusted screening population), intermediate is 1 in 118 (4.7% in population) and high is 1 in 36 (7.9% in population). When analyzing by race for women with high risk scores the prevalence of cancer in White is 1 in 38 (15.5% of adjusted high risk screening population), Black is 1 in 41 (1.4% in population), Asian is 1 in 34 (0.5% of population) and for all other race is 1 in 5 (0.8% of population).
Conclusion: An image-derived AI risk model is equally affective across race and density and can provide accurate insight on short-term breast cancer risk. Based on our results, image-based risk can potentially offset known gaps in breast cancer detection by traditional mammography in patients with dense tissue and address known disparities across races.

P2-06-25: Is Mammography Artificial Intelligence consistent across race and density?

Chirag Parghi, Jennifer Pantleo, Julie Shisler, Jeff Hoffmeister, Zi Zhang, , Avi Sharma, Wei Zhang

Background: Artificial intelligence (AI) is increasingly utilized as an interpretive adjunct for radiologists reading screening mammograms. The case score is a central feature of tomosynthesis-based AI applications as the combination of images for a single patient is consolidated into a single number. An increased case score may suggest the presence of breast cancer in select mammograms and a low score would imply the converse. Although the case score is currently provided as a single number, the clinical value of this score may vary with parameters such as patient race and breast density, which are known to affect the overall incidence of breast cancer. Therefore, offering context of the case score based on screening populations with a mix of racial and density distributions can guide radiologists for more accurate breast cancer detection.
Methods: A set of 3558 Hologic Digital Breast Tomosynthesis (DBT) mammograms, including 394 cancer cases and 3164 noncancer cases from October 7, 2014 to April 16, 2021 across 3 healthcare systems were analyzed with a deep learning AI detection system. Patients ranged from 35 to 94 years of age. The AI system based on deep convolutional neural networks processes the DBT images and provides a case score of 0-100 to indicate the algorithm's confidence that a case showed malignancy. The dataset was weighted relative to a screening population based on Breast Cancer Surveillance Consortium clinical characteristics of race and density for screening DBT exams with a cancer incidence of 6/1000. Breast tissue was categorized as non-dense (BIRADS A and B) or dense (BIRADS C and D). Scores were assigned to 3 categories based on suspicion of cancer: low 0-19, intermediate 20-69 and high 70-100. Statistical analysis was performed using a Mann-Whitney U Test and Chi-Squared Test utilizing Stata (v18.0) software. A p-value at or below 0.05 was defined as the threshold for statistical significance.
Results: When sub analyzed by race across all case scores, the frequency of cancer in white

women is 1 in 161, Black 1 in 187, Asian 1 in 220 and other races is 1 in 221. The cancer rates across race were consistent across the high, intermediate, and low Case Scores with no clear difference between racial subgroups.
Breast density sub analysis:
The overall analysis of case score showed the frequency of cancer differs significantly by breast density (p= 0.0006). The frequency of cancer in women with non-dense breast tissue for low case scores is 1 in 11,363, intermediate is 1 in 304[JS1] and high is 1 in 28. For women with dense breast tissue the frequency of cancer for low case scores is 1 in 1952, intermediate is 1 in 213 and high is 1 in 22. Yet, when individually analyzing frequency of cancer by case score categories and breast density, a statistical difference only appeared for the intermediate category in dense vs. non-dense breasts (p=0.0018).
Age sub analysis:
The mean age of women with a cancer detected in the low Case Score category is 63.3 years compared to 56.2 years in the non-cancer category (p=0.053). The mean age of women with cancer and intermediate Case Scores is 65.8 years compared to 60.2 in the non-cancer group ($p<0.05$). The mean age for women with cancer in the high Case Score category is 64.9 years compared to 64.0 in the non-cancer group (p=0.296).
Conclusion: AI generated case scores effectively stratify mammograms into categories with varying frequency of cancers. The case scores did not vary significantly across racial subgroups in our dataset suggesting the accuracy of the AI software was consistent across races. In women with non-dense, or fatty breast tissue on the mammogram, a low case score corresponded to a lower frequency of cancer (1 in 11,363) compared to those women with dense breast tissue and a low case score (1 in 1952). Although finding was not statistically significant by the Mann-Whitney U test, the difference between categories is notable and the lack of statistical confirmation is likely due to the low absolute number of cancer case in the low case score, non-dense cohort. Therefore, the negative predictive value of a low case score on a screening mammogram is presumably higher in women with non-dense breast tissue across a large data set.

P2-06-26: nf-hlamajority: a Nextflow pipeline for consensus MHC class I typing and its application to neoantigen identification in breast cancer stromal cells

Kevin Ryan, Domhnall O'Connor, Barry Digby, Laura Barkley, Pilib Ó Broin

Introduction: Cancer-associated fibroblasts (CAFs) are a heterogeneous cell type found in the tumour microenvironment (TME). CAFs can support tumour growth and metastasis and contribute to therapeutic resistance. They can also impact immune infiltration and immune responses in the TME. Therefore, therapeutic targeting of CAFs is potentially a viable strategy to treat cancer. Here, we aim to identify somatic mutations in CAFs, that may give rise to neoantigens. HLA genotyping is an important step for neoantigen prediction. This can be carried out in silico using DNA sequencing data, with numerous tools available for this purpose. Claeys et al. (PMID: 37161318) benchmarked several HLA typing tools,

finding that a majority voting approach using a combination of four tools resulted in superior performance for each HLA gene. No end-to-end pipeline currently exists to apply this majority voting approach, making it difficult for non-informaticians to implement. The objectives of this work were to: 1) develop a Nextflow bioinformatics pipeline implementing majority voting for MHC class I typing from DNA sequencing data, and 2) use HLA calls from this pipeline to identify potential neoantigens in cancer-associated fibroblasts.
Materials and Methods: CAFs and corresponding tumour-associated normal fibroblasts (TANs) were cultured from tissue of 12 breast cancer patients (11 Luminal A and one triple-negative). Bulk RNA-sequencing was carried out on all samples and whole-exome sequencing (WES) was carried out on CAFs and TANs from eleven patients. The Nextflow pipeline developed, nf-hlamajority, was used to determine HLA genotypes using WES from each breast cancer patient and 12 NCI-60 Human Tumor Cell lines. nf-hlamajority takes FASTQ files and performs HLA typing using Optitype, Polysolver, HLA-LA and Kourami. For each HLA gene, it then assigns the HLA genotype called by the highest number of tools. In the case of a tie, the HLA genotype called by the tool with the highest accuracy for that HLA gene, as determined by Claeys et al., is assigned to the sample. HLA calls made by nf-hlamajority in the samples from breast cancer patients were used as input to Landscape of Effective Neoantigens Software (LENS) to identify CAF-specific neoantigens (PMID:37184881).
Results and Discussion: In the NCI-60 dataset, 68/70 (97%) nf-hlamajority HLA calls matched the ground-truth PCR genotyping call, demonstrating the high accuracy of the pipeline. nf-hlamajority was then used for the automated high-confidence HLA typing of all breast cancer patients. Using HLA alleles called using nf-hlamajority, LENS identified a number of potential neoantigens resulting from missense mutations, all of which were private to each patient. Interestingly, genes with these mutations included CAF markers and genes implicated in lipid metabolic pathways. CAFs contribute to lipid metabolism within the TME, thus impacting cancer progression and tumour immunogenicity.
In this study, we have developed an automated pipeline for consensus HLA typing which we envisage will be useful to the research community. nf-hlamajority has helped us identify candidate neoantigens in breast cancer CAFs. Future work will focus on validation using T-cell immunogenicity assays, improving our understanding of the potential of targeting CAF neoantigens to enhance the efficacy of anti-cancer therapy.

P2-06-27: Mapping APOBEC Profiles in Breast Cancer Microenvironments: Bridging Bulk and Single-Cell RNA-seq Data

Jake Lehle, Mohadeseh Soleimanpour, Diako Ebrahimi

The apolipoprotein B mRNA editing enzyme, catalytic polypeptide- like 3 (APOBEC3 or A3) family of cytosine deaminases has been implicated in some of the most prevalent mutational signatures in cancer. A3-associated mutational signatures have been identified in more than 70% of cancer types and around 50% of all cancer genomes, with prominence

in breast cancer as well as other cancer types. Many tumors are hypermutated by C-to-T/G mutations induced by A3 within TCW (W:T,A) motifs, accounting for many driver mutations in genes such as PIK3CA, ERBB2, and PPP2R1A. The genome and transcriptome of tumor tissue biopsies contain a wealth of information about these A3-associated signatures. However, quantification of A3 expression changes in tumor cells is confounded by the ubiquitous expression of these enzymes in infiltrating immune cells. To overcome this we will utilize single-cell RNA-seq analysis across a wide range of publicly available breast cancer datasets in order to follow the relative changes of A3 expression within individual cell types. Results from this analysis will give our lab insights into to changes in A3 expression in tumor cells which will be helpful in unveiling the complex relationships among breast cancer cells, stromal elements, and immune cells, facilitating the development of personalized therapies and prognostic markers, while also shedding light on breast cancer heterogeneity and potential vulnerabilities.

P2-06-28: AI-driven feature discovery enables accurate identification of breast cancer biomarkers and histology

Joseph Cappadona, Ken Zeng, Jan Witowski, Krzysztof Geras

The advent of foundation models in artificial intelligence presents a transformative opportunity for oncology. These large-scale models, trained via self-supervised learning on large repositories of unlabeled data, can learn to recognize complex patterns within images and can easily be adapted to downstream tasks with minimal additional training. Additionally, foundation models excel at downstream tasks with relatively few training data, which is especially important within oncology where obtaining large and diverse labeled datasets is challenging, time-consuming, and expensive.
We demonstrate the potential of foundation models for oncology by training a pathology foundation model and evaluating it on a diverse suite of clinically relevant downstream tasks. The model, based on a vision transformer architecture (ViT-L), was pretrained using state-of-the-art self-supervised learning method DINOv2 on 500 million image patches from 80,000 H&E-stained whole slide images. We evaluated the model on 12 slide-level and patch-level tasks identifying breast cancer tumor and patient characteristics based solely on digital pathology. The datasets comprised over 2000 patients across all tasks and entailed identifying major histological subtypes (ILC, IDC, etc.), hormone receptor statuses (ER and PR), and genomic characteristics. For all downstream evaluations, we keep the foundation model frozen and train regressors or classifiers on top of embeddings obtained by passing slide patches through the model.
The model learned to identify patients with ILC with an AUC of 0.84±0.03 in the TCGA cohort (n=1033) and 0.79±0.05 in the PLCO cohort (n=1011). For IDC, the model achieved an AUC of 0.80±0.03 in TCGA and 0.82±0.04 in PLCO. The model also learned to classify breast tumors as ER/PR-positive versus -negative. For ER classification, the model obtained an AUC of 0.80±0.04 in TCGA (n=983) and 0.83±0.05 in PLCO (n=848), and for PR classification, the model obtained an AUC of 0.70±0.04 in TCGA (n=980) and 0.80±0.04 in

PLCO (n=842).
Additionally, the model, when trained on 938 patients from TCGA and evaluated out-of-distribution on 116 patients from CPTAC, learned to identify mutations in genes associated with endocrine resistance, including TP53 (AUC=0.76±0.04) and PIK3CA (AUC=0.61±0.04), and in genes associated with lobular breast cancer, such as CDH1 (AUC=0.89±0.02).
We also evaluated our foundation model on 5 patch-level tasks identifying spatially resolved histological features. We demonstrate that the model can learn to predict tumor cellularity (Kendall's tau of 0.78±0.01), classify metastatic regions (balanced accuracy of 0.98±0.00), and identify areas with various histologies, including dysplasias, adenomas, and lobular carcinomas (AUCs of 0.74±0.03, 0.92±0.01, and 0.97±0.00 in three independent datasets).
Following these evaluations, we analyzed the effect of varying model size and pretraining dataset size. In particular, we looked at the differences in performance between ViT-S (21M parameters), ViT-B (85M parameters), and ViT-L (303M parameters) when trained on 130 million patches from 11,000 slides versus 500 million patches from 80,000 slides. We find that larger model sizes and pretraining dataset sizes yield significantly better downstream models, yielding up to 8% relative improvement in performance depending on the task.
In this study, we have demonstrated that our AI foundation model can be used to infer a wide range of breast cancer characteristics from a single H&E-stained slide, including histological subtypes and clinically relevant gene mutations. The unique strengths of our approach—leveraging small amounts of supervised data along with large-scale unsupervised pretraining—create significant opportunities for advancements in pathology research and clinical applications.

P2-06-29: Automated Breast Density Assessment on Chest CT with a Deep-Learned 3D Ordinal Regression Model

Artur Wysoczanski, Elsa D. Angelini, Sachin S. Jambawalikar, Andrew F. Laine, Mary M. Salvatore

Introduction: Breast cancer is the most common malignancy and the second-leading cause of cancer mortality in women. Breast density, defined as the extent of fibroglandular tissue within the breast, is assessed semi-quantitatively on a four-grade scale, from mostly fatty (grade 1) to extremely dense (grade 4), with a severalfold increase in prospective cancer risk between the lowest and highest grades. Millions of chest CT studies are performed annually in patients eligible for routine mammographic screening, representing a significant opportunity for incidental density reporting and risk stratification. While automated density grading on mammography is robust and widely deployed, assessment on CT remains limited by comparison. To facilitate the efficient and standardized assessment of breast density on chest CT, we train and evaluate a 3D convolutional neural network (CNN) model to predict breast density grade from chest CT volumes using a retrospective dataset of patients at Columbia University Irving Medical Center.
Methods: A retrospective, IRB-approved chart review identified female patients at CUIMC

who underwent both mammography and chest CT acquired less than one year apart from January 2010 through March 2019. All cases were reviewed by a faculty radiologist, recording both CT breast density and whether the entire breast was present within the scanner field of view (FOV). All CT scans with or without IV contrast, with axial soft-tissue reconstruction and no breast malignancy, prosthesis or prior mastectomy were included. CT volumes were downsampled to isotropic 2.5 mm resolution and 80x80x80 voxel volumes of interest (VOIs) were extracted containing each imaged breast. Scans were randomly allocated to training, validation, and test sets in a 3:1:1 ratio with stratification by density grade.
Our 3D CNN model consists of five blocks containing 3D convolutional layers with ReLU activation, convolutional block attention modules (CBAM), and batch normalization, interleaved with pooling operations. Rank-consistent ordinal regression (CORN) was applied to the final fully-connected layer to predict the density grade for each image. The model was trained to minimize ordinal cross-entropy with L2 regularization, using training batches balanced across density grades and train-time augmentation, over 60,000 training steps with early stopping conditioned on validation ordinal cross-entropy. Patient-level predictions were generated on the test cases by averaging predictions over the right- and left-breast VOIs.
Results: Of 503 scans which met the inclusion criteria, 86 (17.1%) were assigned breast density grade 1, 244 (48.5%) were grade 2, 131 (26.0%) were grade 3, and 42 (8.3%) were grade 4. One or both breasts were partially outside the FOV in 134 scans (26.6%). On prediction of high (grades 3-4) versus low breast density, the optimized model achieved test-set accuracy of 90.1%, receiver-operating characteristic AUC of 0.935, and Cohen kappa of 0.787 with respect to radiologist-assessed ground truth, comparable to reported values for inter-radiologist agreement. One-vs-all accuracy for breast density grade was 67.3%, with 98.0% of predicted grades at most one level off from the ground truth. ROC AUC and Cohen kappa improved to 0.955 and 0.873, respectively, on the subset of test cases (N=80) where both breasts were contained within the FOV.
Conclusions: We have introduced a 3-D CNN model for automated ordinal regression of breast density on chest CT using a retrospective clinical CT dataset, with performance relative to ground-truth comparable to published inter-reader agreement among trained radiologists. Estimating breast density grade independently of threshold-based volumetric percent density is a direct precursor to deep-learning on chest CT to improve on image-based breast cancer risk stratification.

P2-06-30: A Machine Learning Platform for In Silico Design of DNA Targeting Cancer Therapeutics Using SwRI's Rhodium® Docking Software

Tristan Adamson, Dillon Cao, Shawn T. Blumberg, Jonathon Bohmann

DNA is a classic target of small molecule cancer therapeutics due to the rapid rate of replication in cancer cells and compromised DNA repair machinery, which makes them more susceptible to DNA damage than healthy cells. Unfortunately, DNA-targeting drugs are

notorious for adverse side effects and can even increase the risk of future cancer themselves. Therefore, the design, testing, and development of safer, more selective DNA-targeting drug derivatives remains an important challenge in cancer therapeutic research and development. To date, SwRI's Rhodium™ docking platform has been successfully used for the identification of several potent antiviral and medical countermeasure therapeutics, including therapeutics for filoviruses, SARS-CoV-2, and organophosphate nerve agent antidotes. However, Rhodium™ and other reported docking programs have only been statistically validated for studying protein-ligand interactions. To address the need for a machine learning guided platform for the in silico design of DNA-targeting therapeutics, SwRI recently completed an internal research and development project to adapt Rhodium™ for the screening of DNA-targeting small molecules. This was achieved through a meticulous review of published data to identify highly trustworthy DNA crystal structures as well as unbiased small molecule datasets for training machine learning models to predict DNA binding pose, affinity, and anticancer activity. Finally, the efficacy of this technology was demonstrated by rank ordering the potency of compounds that exhibit anti-cancer activity in several cancer cell models, including breast cancer, leukemia, and liver cancer. In each case, Rhodium™ was able to rank order actual potent compounds above inactive ones with statistical confidence levels ranging from 96% to 99.95%.

P2-07-02: Predicting and validating up to 5-year risk of triple-negative breast cancer using mammogram images.

Graham Colditz, Shu (Joy) Jiang, Debbie L Bennett, Bernard A Rosner

Triple-negative breast cancer (TNBC) drives excess mortality from breast cancer, particularly in Black women. Neither lifestyle variables, mammographic breast density, nor polygenic risk scores—individually or combined—adequately identify at-risk women. Therefore we aimed to identify women undergoing mammographic breast screening at risk for TNBC for appropriate risk management.
We draw on a prospective mammography screening service in an urban health system covering all women regardless of ability to pay. The training cohort included 350 TNBC cases and 10,461 women free from breast cancer (27% of non-cases and 46% of cases were non-Hispanic Black women). The mean age at TNBC diagnosis was 54.6. External validation includes 45 cases and 731 controls. We use full-field digital mammogram images and a generated mammogram risk score. The outcome is discrimination measured by AUC and calibration of TNBC risk.
Established breast cancer risk factors did not differ between TNBC cases and cancer-free women, though more cases had history of pregnancy than non-cases. The mammogram risk score on the first available digital mammogram was substantially higher for women who developed TNBC compared with those who remained cancer-free. The odds ratio for each standard deviation increase in mammogram risk score is 7.1 (95% CI, 6.3, 7.9). In the external validation, the AUC for 5-year risk was 0.85 (95% CI 0.79, 0.91) when including cases diagnosed from the screening mammogram and 0.73 (95% CI 0.65, 0.81) when

excluding cases diagnosed within 6 months of screening. Calibration between predicted and observed risk across quintiles of TNBC risk show good performance. We then plotted the estimated TNBC risk score using mammograms only and observed substantial separation of the baseline risk distribution for women who developed TNBC and those who remained cancer-free.
We assessed predictive performance over time starting from the initial digital mammogram and noticed a slight decrease in AUC as the prediction horizon was extended. In the external validation excluding cases diagnosed <6 months from screening mammogram, the AUC decreased from 0.75 (95% CI 0.67, 0.84) for a 1-year risk to 0.72 for a 2-year risk, and to 0.72 (95% CI, 0.64, 0.80) for a 5-year risk.
Conclusion: The mammogram risk score derived from a diverse mammography screening population offers a tool to identify women at high risk specifically for TNBC. It can be calibrated to population incidence rates allowing consistent classification across screening services. This can open the way for precision management of TNBC risk and recruitment to prevention trials. Evidence-based strategies to reduce TNBC risk are urgently needed.

P2-07-03: Noninvasive imaging of breast cancer and metastasis using TROP2-targeting radiotracer 68Ga-TTP

Yifei Pei, Jingwen Bai, Guojun Zhang

Objective: Molecular imaging technology, such as PET/CT, is of great value for accurate and individualized diagnosis of early tumors and non-invasive target location. Currently, targeted nuclide probes for breast cancer primarily include 18F-FES (ER targeting), 18F-FFNP (PR targeting), and 68Ga-ABY-025 (HER2 targeting). However, these probes are limited to specific types of breast cancer. Therefore, there is an urgent need to develop new targets for all kinds of breast cancer imaging. Human trophoblastic cell surface antigen 2 (TROP2) is a glycoprotein distributed on cell membranes. Researchers have found that TROP2 expression is low or absent in breast tissues but significantly elevated in breast cancer, especially in 93% of triple-negative breast cancer. Based on the successful ASCENT Phase III clinical trial and TROPiCS-02 study, Trodelvy, an antibody-conjugated drug that targets TROP2, - has been approved by the U.S. Food and Drug Administration and the China Drug Administration for treating triple-negative breast cancer or HR+/ HER2- advanced breast cancer. The efficacy of Trodelvy in breast cancer treatment highlights the viability of TROP2 as a reliable marker for breast cancer cells imaging.
Methods: Based on phage libraries, we innovatively screened TTP (TROP2-targeted peptide), a polycyclic peptide with high affinity for TROP2. The peptide was conjugated with the bifunctional chelating agent DOTA-NHS and labeled with 68Ga to obtain the radiotracer 68Ga-TTP. We examined its physicochemical and pharmacokinetic properties, followed by micro-PET/CT imaging to assess its potential for tumor imaging. Then, we evaluated the probe's ability to detect in situ tumors and metastatic tumors using mouse models of breast cancer in situ, lymph node metastasis, and lung metastasis. Additionally, we compared the targeting efficiency of this imaging probe with that of 18F-FDG, the most commonly used

radiotracer in clinical practice.
Results: The radiochemical purity of probe 68Ga-TTP exceeds 95% and demonstrates excellent stability in both vivo and vitro settings. The results from biological distribution and pharmacokinetic experiments indicate rapid clearance and predominant renal metabolism of the probe in mice, with minimal uptake and fast metabolism observed in normal tissues and organs. PET/CT imaging reveals high tumor tissue uptake of 68Ga-TTP in mouse models expressing high levels of TROP2, such as MDA-MB-231-TROP2 and 4T1-TROP2 tumor cells. However, in mouse models carrying MDA-MB-231 and 4T1 tumors with relatively low TROP2 expression, the uptake of 68Ga-TTP in tumor tissues is significantly lower, which can be further blocked by excessive non-radioactive injection. Furthermore, the constructed 68Ga-TTP accurately identifies lymph nodes with tumor metastasis as well as lung metastatic lesions, exhibiting superior clarity and signal-to-noise ratios compared to conventional 2-fluorodeoxyglucose PET/CT imaging techniques. Overall, these findings demonstrate that the developed 68Ga-TTP exhibits excellent targeting capabilities for breast cancer.
Conclusion: This study proposes that TROP2-targeted PET imaging holds potential as a diagnostic tool for breast cancer and metastasis, thereby facilitating its clinical application in PET/CT and PET/MR imaging for breast cancer detection and therapeutic response evaluation to Trodelvy.

P2-07-04: [18F]FluorThanatrace PET imaging correlates with response to PARP inhibitors in breast cancer: a pilot study

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Introduction: PARP inhibitors (PARPi) are approved for BRCA-mutant HER2- breast cancer, and there is clinical interest in expanding indications to include homologous recombination deficient (HRD) breast cancers. Yet, response in these populations remains variable, suggesting clinical utility in developing a better biomarker to select patients for PARPi and predict response. Here, we evaluated the 18F-labeled PARPi, [18F]FluorThanatrace (FTT), as a functional biomarker of PARPi response in breast cancer.

Methods: A single-arm prospective observational trial was conducted at the University of Pennsylvania from May 2017 to March 2022 (NCT03083288, NCT03846167). Subjects ≥18 years of age with known or suspected breast cancer, at least one lesion ≥1 cm on conventional imaging, and willing to undergo an FTT-PET prior to treatment initiation were enrolled. Participants provided written informed consent.

Subjects were scanned on an Ingenuity TF PET/CT (Philips Healthcare) following injection of FTT. To evaluate if FTT uptake associated with PARPi response, primary and metastatic target lesions were selected based on anatomic CT imaging and RECIST 1.1 criteria. Maximum standardized uptake values (SUVmax) were recorded from the tumor with reference to CT scans. Spearman correlation estimated the strength of association

between FTT uptake and progression free survival (PFS). Mann-Whitney test compared FTT uptake in subjects with < or ≥ 5-month PFS. For HRD correlation, somatic sequencing of the index tumor and germline sequencing were utilized and compared with SUV from the index malignancy on PET/CT.

Participants: Twenty-four subjects with primary breast cancer underwent a baseline FTT-PET/CT scan prior to standard of care therapy. FTT uptake and genomic mutations were previously reported and showed a broad range of FTT uptake independent of genomic data. Here, untreated biopsy specimens were sequenced and HRD was calculated for correlation analysis with FTT uptake.
A second cohort of 10 women with stage IV breast cancer underwent a baseline FTT-PET/CT scan prior to the start of a PARPi, and eight women received a second optional scan after a short interval after initiating PARPi treatment. Subjects ranged from 35 to 72 years of age and 3 self-reported as Black and 7 as White. All subjects had a germline or somatic pathogenic mutation in HR repair genes identified by a CLIA certified assay.

Results: Baseline FTT-PET uptake did not correlate to HRD score, supporting that FTT provides distinct information from genetic features. In the PARPi cohort, 22 target lesions were identified across the 10 subjects. Target lesion diameter averaged 2.8 cm (range 1-6.5) and baseline FTT-PET imaging showed variable uptake (SUVmax range 0.5-6.9, mean 4.8). SUVmax did not correlate to tumor CT measurements, supporting that PARP expression or binding of PARPi to its target is not directly related to lesion size.
Baseline average lesion uptake correlated to PFS ($r=0.74$, $P=0.023$, $n=8$), suggesting subjects with greater [18F]FTT uptake had longer survival. Dichotomizing subjects between those with a PFS >5 months and those with <5 months showed subjects with longer survival had higher SUVmax at baseline ($P=0.04$). All eight subjects imaged by FTT-PET/CT post-PARPi had a decline in FTT uptake (13%-61%), indicating variable drug-target engagement. A correlation was seen between PFS and subject-level percent change in SUVmax from baseline to post-PARPi imaging ($r=-0.86$, $P=0.012$, $n=7$), indicating larger declines in FTT uptake were associated with longer times-to-progression.

Conclusion: These early results suggest the potential of FTT-PET to select patients for PARPi treatment and monitor in vivo pharmacodynamics after therapy start. The absence of association with HRD score supports FTT uptake as a novel measure from genomic biomarkers. Larger multicenter trials to evaluate FTT-PET as a biomarker of PARPi response in breast cancer are warranted.

P2-07-05: Performance of the BCSC and MammoRisk (MR) scores with and without PRS313 to predict invasive breast cancer (iBC) risk in the UK Biobank cohort

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Background: As risk-based iBC screening and prevention strategies emerge as promising avenues, the international validation of iBC risk prediction scores becomes increasingly important. BCSC and MR scores have been developed on the US BCSC cohort. We evaluated their performance in predicting iBC risk in the UK Biobank cohort with and without PRS313.

Methods: We included the data from female participants of European ancestry who were enrolled in the UK Biobank cohort (application number 69901) and excluded women with prevalent iBC, history of other malignancy except for non-melanoma skin cancer, and a history of mastectomy or breast augmentation surgeries. The primary endpoint was the diagnosis of iBC. Participants were censored at diagnosis of another malignancy, death, last follow-up, or administratively at 5 years whichever came first. Mammographic density was not reported in this cohort, thus a sensitivity analysis was performed using different strategies for imputing the density of all participants: 1) by B, 2) by C, 3) by the mean prediction of all densities weighted by their respective frequencies reported by Tice et al (JCO 2015) [ongoing]. Both models were calibrated for age and the national incidence of iBC in UK. Their discrimination and calibration were assessed using respectively the time-dependent ROC AUC (tdAUROC), and the calibration curves, observed to expected ratio (O/E) and the integrated calibration index (ICI) for the 5-year iBC risk prediction.
Results: Among the 273,476 women enrolled in UK Biobank, 196,317 were eligible for the present analysis. Most women were ≥ 50 years (77%), postmenopausal (60.8%) and had no reported history of breast biopsy (99.8%). 10.8% had a first-degree relative with iBC. At 5 years, 2,951 participants had a reported diagnosis of iBC.
For mammographic density imputed by density B, BCSC showed an O/E of 1.196 (95% CI 1.191-1.202), ICI of 0.00256 (95% CI 0.0020-0.0031), and tdAUROC of 0.556 (95% CI 0.545-0.566). The addition of PRS313 yielded an O/E of 1.177 (95% CI 1.172-1.182), ICI of 0.00235 (95% CI 0.0019-0.0029), and tdAUROC of 0.638 (95% CI 0.625-0.648). MR showed an O/E of 1.222 (95% CI 1.216-1.227), ICI of 0.00282 (95% CI 0.0023-0.0034), and tdAUROC of 0.558 (95% CI 0.547-0.569). Adding PRS313 to the MR model resulted in an O/E of 1.203 (95% CI 1.197-1.208), ICI of 0.00263 (95% CI 0.0021-0.0032), and tdAUROC of 0.642 (95% CI 0.630-0.652).
For mammographic density imputed by density C, BCSC showed an O/E of 0.848 (95% CI 0.845-0.852), ICI 0.00327 (95% CI 0.0027-0.0038), and tdAUROC of 0.557 (95% CI 0.546-0.566). Adding PRS313 to BCSC yielded an O/E of 0.835 (95% CI 0.831-0.839), ICI of 0.00301 (95% CI 0.0024-0.0036), and tdAUROC of 0.640 (95% CI 0.627-0.650). MR showed an O/E of 0.794 (95% CI 0.790-0.797), ICI of 0.00456 (95% CI 0.0040-0.0051), and tdAUROC of 0.550 (95% CI 0.536-0.560). The addition of PRS313 to MR resulted in an O/E of 0.782 (95% CI 0.778-0.785), ICI of 0.00420 (95% CI 0.0036-0.0048), and tdAUROC of 0.637 (95% CI 0.625-0.646).
Conclusions: PRS313 had a significant additional value for the prediction of the iBC risk for both models which performed similarly. The addition of the PRS to the BCSC and MR scores provided a significant improvement in discrimination and calibration. The mammographic density is essential to provide a reliable prediction for stratified interception with

calibration showing that the BC density imputation impacted the predictions. The clinical utility of PRS313 in combination with MR is being evaluated in the MyPeBS (NCT03672331) trial.

P2-07-06: ClearCoast® MRI System - Intraoperative Magnetic Resonance Imaging for Margin Assessment of Ductal Carcinoma In Situ (DCIS) and Invasive Breast Cancer in Breast Conserving Surgery. MRO - A Controlled Post-Market Study

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Introduction: Breast cancer accounts for 12.5% of all new annual cancers worldwide. Ductal Carcinoma in Situ (DCIS) has increased its incidence more than fivefold in western countries and alone accounts for up to 25% of all newly detected breast cancers. The primary treatment modality for local breast cancer is breast-conserving surgery (BCS), which aims at balancing adequate pathologic margins while minimizing removal of excess breast tissue. However, unaddressed positive margins occur in 20-40% of cases, often requiring re-excision, which is a strong predictor of local and distant recurrence. The ClearCoast™ MRI System (ClearCut. Ltd., Israel) is a CE-marked adjunctive intraoperative margin assessment tool for distinguishing normal and suspected cancerous tissue based on Diffusion-Weighted Imaging (DWI).
Method: A prospective, controlled, non-randomized study conducted in the AGAPLESION MARKUS KRANKENHAUS (AMK), Frankfurt, Germany and in the Helios University Clinic Wuppertal compared patients undergoing BCS with the ClearCoast™ for real-time margin assessment to an historical control group under standard-of-care (SoC) methods. The focus was the system's impact on re-excision rate, feasibility, diagnostic ability, and conservation of breast tissue volume.
Results: 116 patients were included in the trial, 50 patients in Frankfurt, 66 patients in Wuppertal. The results presented in the abstract are focussing on an interim analysis of the 50 patients of the AMK, and 150 margins were evaluated per study group. The ClearCoast™ was associated with a 40% reduction in re-excision rates among the treatment group, and potentially 80% compared to control group. The ClearCoast™ presented highly accurate diagnostic performance of sensitivity 81% and specificity 70% compared to SoC 36% and 86%, respectively. Particularly for DCIS, ClearCoast™ detected 92% compared to Control's 18%, resulting in a significantly lower rate of unaddressed intra-operative positive margins compared to SoC (15% vs. 64% accordingly). More conservative removal of breast tissue was achieved by the ClearCoast™ (69.9cm3 vs. 153.2 cm3) without any increase in operation time.
Conclusions: The ClearCoast™ MRI System showed potential to improve BCS outcomes, by offering surgeons a real-time, accurate approach to intraoperative margin assessment. It decreases the likelihood of unaddressed residual tumor, thus reducing the burden of re-

excision for patients and the health care system without trading time for precision. Further analysis together with the data from the Helios Universitätsklinikum Wuppertal can be awaited for end of 2024.

P2-07-07: Multiparametric Whole-Body MRI for Reclassification of Oligometastatic Disease in Metastatic Breast Cancer Patients: A Prospective Study

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Background: Almost half of patients with metastatic breast cancer (BC) present with oligometastatic disease (OMD), currently defined as the presence of a maximum of 5 metastases safely treatable with metastases-directed therapy (MDT). MDT aims to eradicate visible metastatic lesions, bringing patients to a state of non-evident disease. However, data regarding survival benefit are conflicting and the correct management of OMD remains a matter of debate. Possible explanations include the lack of biological criteria and advanced imaging methodologies for oligometastatic status definition. The aim of our study is to investigate whether multiparametric whole-body magnetic resonance imaging (WB-MRI) may provide a more precise definition of OMD compared to standard imaging modalities (SIT) as computed tomography (CT) and positron emission tomography (PET-CT).
Methods: In our prospective study, WB-MRI was used as an additional staging tool in patients defined as oligometastatic based on SIT. We aimed to assess how often MRI was able to redefine the number of metastases in patients formerly defined as oligometastatic. Here we report the preliminary results of this ongoing study.
Results: Eighteen patients with 1 to 5 metastases detected by standard imaging techniques underwent WB-MRI: 9 patients were staged with CT and 9 with PET. In 11 cases, only one metastasis was detected by SIT. Ductal or lobular histology was found in 9 and 5 cases, respectively. The majority of patients had HR+/HER2-negative disease (14 patients), 3 patients were triple-negative, and only 1 patient was HER-2 positive. WB-MRI revealed additional metastases (bone and liver metastases in 89 % and 11% of cases respectively) in 9 patients compared to SIT. In 8 cases, the oligometastatic status was redefined: 3 patients were reclassified as disease-free, and 5 patients as polymetastatic (having more than 5 metastases).
Conclusions: WB-MRI has proven to be useful in reclassifying OMD, primarily due to higher detection of additional bone metastases compared to SIT. Missing some metastatic lesions with imaging could hamper the complete eradication of the disease sought with MDT. WB-MRI is therefore strongly recommended for the definition of OMD both in clinical practice and clinical trials.

P2-07-08: MRI screening for brain metastases versus SYMptom-directed brain imaging for Patients with Triple neg or HER2+ Metastatic breast cancer: updated primary outcome analysis and preliminary quality of life data from a randomized phase II pilot study

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Background: The role of MRI screening to detect asymptomatic brain metastases (BrM) among patients with HER2+ or triple negative (TN) metastatic breast cancer (MBC) continues to be debated. While early detection and intervention for BrM has potential to improve patients' outcomes, evidence to confirm utility of screening is still lacking.
Methods: We conducted a multicentre phase II pilot study randomizing patients with TN or HER2+ MBC to 1 year of BrM screening (contrast enhanced brain MRI at baseline, 4, 8, and 12 months) versus symptom directed brain imaging (MRI only if symptoms of BrM develop). The primary goal of this study was to determine the feasibility of a future randomized trial of BrM screening versus symptom-directed imaging in this patient population. Key inclusion criteria were age ≥18; TN MBC diagnosed ≤12 weeks prior to study entry or HER2+ MBC with no restrictions regarding time of diagnosis; no symptoms of BrM or known asymptomatic BrM; ECOG ≥ 1 and no MRI contraindications. Overall and neurologic-specific quality-of-life (QoL) as well as cancer-related anxiety were assessed at baseline, 6 and 15 months.
Criteria rendering a future trial "not feasible" were defined a-priori as: <30% of eligible patients enroll in the study, <50% complete the study protocol, and/or >50% of patients allocated to the control arm during the 1 year study period are screened for asymptomatic BrM with CT or MRI.
Results: Between 2018 and 2024, 50 patients from 3 cancer centres enrolled in the study. Metrics regarding the proportion of eligible patients who enrolled in the study were collected from the Sunnybrook Odette Cancer Centre site; of 70 patients approached, 44 (63%) enrolled in the study. Forty (80%) had HER2+ and 10 (20%) had TN MBC. The median age was 52.7 (13.2) years. Twenty-four patients (48%) were randomized to the MRI screening arm and 25 (52%) were randomized to standard of care (SOC) surveillance. So far, 42 patients (84%) have either completed the study (n= 23, 46%) or withdrawn early (n= 18, 36%); 8 (16%) continue on study. 10 patients (42%) in the MRI screening arm withdrew a median of 2.5 months after enrollment and 8 (31%) in the SOC arm withdrew a median of 6.7 months after enrollment; the most common reasons for withdrawal were patients' discontent with their randomised arm (n=6) and claustrophobia (n=5). To-date, 9 patients (18%) developed BrM, 4 (17%) in the screening arm and 5 (19%) in the SOC arm. Eighteen patients (69%) in the SOC arm had brain imaging, 7 (27%) in the absence of neurological symptoms.
Baseline scores for overall QoL (EORTC QLQ BN20) and neurologic-specific QoL (FACT-BR tools) as well as cancer-related anxiety (NCI PRO-CTCAE) were similar in patients in both study arms. Only 5 (24%) of patients in the MRI arm and 8 (36%) in the SOC arm

experienced anxiety frequently at baseline. At 6 month follow-up, the majority of patients [14 (93%) in the MRI arm and 8 (62%) in the SOC arm] had either improved or unchanged level of anxiety compared to baseline (p=0.069). There was no significant difference between baseline and 6-month scores in any of the assessed QoL domains. Data regarding 15-month QoL measures will be reported during the abstract presentation, when longer follow-up is available.
Conclusion: A large, multi-center randomized trial to investigate the utility of MRI-based surveillance for asymptomatic detection of BrM in the proposed patient population is likely feasible based on a-priori criteria. However, a high rate of brain imaging in the control arm of this pilot study and increasing uptake of BrM screening in daily practice need to be taken into consideration. Anxiety scores were similar among patients in both arms of the trial.

P2-07-09: Breast cancer diagnosis using artificial intelligence algorithms

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Background: In our previous study, we developed a 3-protein signature blood marker using a proteomics technique for early detection of breast cancer. This study is to evaluate the breast cancer diagnosis performance of a newly developed 7-protein signature blood marker with artificial interlligence techniques to improve breast cancer diagnosis.
Meterial and methods: From July 2022 to March 2024, 300 breast cancer patients and 150 health controls were enrolled prospectively. The subjects regularly collected blood at regular intervals of 6 months or 1 year. The peptides that are optimal for MS/MS detection were selected through the development of the PepQuant library, a biomarker detection library. The selected proteins were chemically synthesized and then quantified by a multi-reaction monitoring (MRM) method. After discovering and verifying breast cancer biomarkers, algorithms were developed using five types of machine learning techniques for seven candidate proteins (APOC1, CHL1, FN1, vWF, PRG4, CLU, and MMP9). To evaluate the perfomance of breast cancer diagnosis, performance evaluation was conducted based on the sensitivity, specificity, accuracy, false positive rate, false negative rate, positive predictive value, and negative predictive value.
Result: The sensitivity, specificity, and accuracy of the 7-protein signature analyzed using a new machine learning algorithm were 87.6%, 89.7%, and 88.0%, respectively, which outperformed the previous 3-protein markers. The false positive and false negative rates were 20% and 15.4%, indicating a 33% improvement in false negatives over previous markers. In addition, positive and negative predictive value were also recognized to be improved to 85.2% and 87.0%.
Conclusion: Breast cancer diagnosis using a 7-protein signature developed with aritificial intelligence models shows that breast cancer detection can be significantly improved compared to before.

P2-07-10: The role of [18F]16Î±-Fluoro-17Î²-Fluoroestradiol (FES) Positron Emission Tomography (PET) in Predicting Response to Endocrine Therapy (ET)

Nicholas DiGregorio, Hannah Linden, Jennifer Specht, Dan Hippe, Jasper van Geel, Song Shaoli, Liu Cheng, Christine Brand

Background: Seventy percent of breast cancers (BC) are estrogen receptor-positive (ER+). ET improves clinical outcomes in ER+ BC; however, ET effectiveness relies on functional ER. While immunohistochemistry (IHC) is routinely used to quantify ER presence in sampled tissue, its clinical utility has inherent limitations. ER+ by IHC does not confirm function of ER, as illustrated by up to 50% of patients experiencing disease progression while on ET. FES is a radiolabeled form of estrogen and quantification of functional ER binding via FES PET may render important guidance on potential response to ETs, thus optimizing patient management, avoiding futile therapy or associated toxicities, and reducing financial burden. FES PET quantification, as measured by standardized uptake value (SUV), informs on functional ER and ER heterogeneity. This meta-analysis evaluated FES SUVmax and heterogeneity with clinical outcomes following ET.
Methods: A systematic review identified five studies with comparable FES SUV and progression-free survival (PFS) measures; patient-level data was obtainable from three studies. All patients had advanced ER+ BC and received ET (n = 53, Palbociclib + ET; n = 48, Fulvestrant monotherapy); all patients were scanned on a Siemens PET/CT. Using Syngo software, two board-certified nuclear medicine physicians measured FES SUVmax per lesion. The number and percentage of FES-positive (FES+) lesions were calculated per patient. A lesion with FES SUVmax > 1.8 was defined as ER+ and heterogeneity as 1-99% of lesions FES+; other thresholds for FES SUVmax and % heterogeneity were considered. PFS was defined as time from initiating ET until disease progression / death from any cause and was censored at last follow-up. PFS was estimated via the Kaplan-Meier method. Cox proportional hazards regression was used to evaluate associations between FES+ and PFS, summarized via hazard ratios (HRs). HRs were estimated using different thresholds to define FES+ lesions (SUVmax from 1.8-6) and different thresholds for heterogeneity (e.g., 1-X% vs. (X+1)-100% FES+ lesions, X from 50-99%).
Results: The individual-level data was merged, resulting in 101 distinct patients with 878 quantified lesions (median lesions/patient [inter-quartile range]: 7 [4 - 12]). By FES PET, 40% had only visceral lesions; 31% bone and soft tissue; 16% bone only; and 14% soft tissue only.
Lesions were homogeneous (100% FES+) in 75% of patients and heterogeneous in 25%. Overall, 64% of patients progressed or died over follow-up (median PFS: 13.1 months). mPFS was less in the heterogeneous vs. homogeneous group (5.5 vs. 21.6 months), with a corresponding HR for progression or death of 5.4 (95% confidence interval [CI]: 3.2-9.4, $p < 0.001$). When the SUVmax threshold for FES+ was raised from 1.8, the HR for heterogeneous vs. homogeneous decreased, ranging from 1.1 (95% CI: 0.6-2.0) to 3.3 (95% CI: 2.0-5.5). Similarly, when the heterogeneity threshold was lowered from 99%, the HR also tended to decrease compared to when 1-99% vs. 100% was used, ranging from 1.8

(95% CI: 0.4-7.3) to 5.2 (95% CI: 3.0-8.9). No combination of SUVmax or heterogeneity thresholds resulted in a higher HR than the original combination.
Conclusion: To our knowledge, this analysis is the largest individual-level data cohort to date assessing the predictive value of FES in patients receiving ET. FES was strongly predictive of PFS using a threshold of SUVmax > 1.8 for FES+ and 1-99% for heterogeneity threshold. Raising the threshold for SUVmax or heterogeneity did not improve risk stratification. Any amount of heterogeneity was associated with shorter PFS with ET compared to patients with homogeneous FES+ expression. The results support the utility of FES in predicting clinical benefit to ET but warrant further analysis to support more routine use in clinical practice.

P2-07-11: The value of baseline staging tests in early stage breast cancer: A retrospective study from the Antwerp University Hospital

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Background: Breast cancer, the most common cancer among Belgian women, impacts almost 1/8th of the female population. Prognosis of breast cancer patients largely depends on disease stage and the presence of (occult) metastases, therefore many patients undergo imaging to detect distant metastasis as part of their diagnostic work-up. Furthermore, the detection of distant disease will have major therapeutic consequences. However, it is unclear whether the detection of oligometastatic disease before surgery has an impact on survival and numerous international guidelines suggest not to do screening in the early stage setting because of the very low a priori probability of a patient presenting with metastatic disease. Despite these guidelines many Belgian women still undergo baseline staging test with a bone scan (BS), a liver ultrasound (LUS) and chest radiography (CXR), when presenting with early stage breast cancer. This study aims to assess the benefit of staging tests by examining the incidence of metastatic disease in early-stage breast cancer detected by baseline staging tests.
Methods: We conducted a single-center retrospective cohort study including all newly diagnosed invasive breast cancer patients, who had their initial diagnosis and complete treatment at Antwerp University Hospital (Edegem, Belgium) between 1 January 2012 and 31 Augustus 2023. The hospital records were reviewed to obtain clinical-pathological information and data about the baseline staging. Confirmatory investigations and biopsy (anatomopathological confirmation) were conducted after an initial positive result. For each staging procedure, we analyzed the 'prevalence' and a 'false positive rate'. Relationships between staging results and clinicopathological variables were assessed in R studio.
Results: A total of 1589 patients were evaluated in this study of which 1090 had early-stage breast cancer (cT1/2N0). Only 2 patients out of 857 had a positive CXR (0.2%) and 2 out of 902 patients had a positive LUS (0.2%). A positive BS was seen in 12 out of 968 cases (1.2%). Interestingly, false-positive results leading to additional examinations were seen respectively in: 31/857 (CXR), 41/902 (LUS) and 18/968 (BS) cases. Clinicopathological

parameters associated with a positive staging in the early setting were a larger tumor size (P= 0.009) and an elevated CA15.3 (P< 0.001).
In contrast with a negative nodal status, cT1/2 patients that had cN1 disease (230) had more often positive screening results: 7.2% versus 1.5%, P<0.001. Although the prevalence of metastic disease in this cohort was also still low: CXR: 0/147 (0%), LUS: 2/173 (1.1%) and BS: 12/192 (6.3%).
Conclusion: In this real-life university hospital setting, a very low prevalence of metastases in early-stage breast cancer (T1/2N0) was seen. These findings are in line with previous studies and support the omission of routine baseline staging. Although staging can be considered in larger tumors and patients with an elevated CA15.3. Furthermore, our results reveal a substantial number of false positive staging results, leading to psychological distress among patients and increasing the public health care expenses.

P2-07-12: Associations between demographic and clinicopathologic characteristics and convolutional neural network-derived breast cancer risk scores among women with hormone receptor-positive breast cancer.

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Introduction: Deep learning tools applied to standard surveillance mammograms could serve as noninvasive pharmacodynamic biomarkers of response to adjuvant breast cancer (BC) therapies, including endocrine therapy (ET). We previously demonstrated that a convolutional neural network (CNN)-based model applied to surveillance mammograms is an accurate, independent predictor of BC risk, and that short-term change in a CNN-derived BC risk score with adjuvant ET was also associated with breast cancer relapse. We evaluated demographic and clinicopathologic characteristics associated with baseline CNN risk score and with change in CNN risk score over time on adjuvant ET among women with early-stage HR+ breast cancer.
Methods: We conducted a retrospective cohort study among women diagnosed with stage I-III unilateral hormone receptor-positive (HR+) BC at Columbia University Irving Medical Center (CUIMC) from 2007-2017, who received adjuvant ET (tamoxifen, aromatase inhibitors [AIs) and had mammograms of the contralateral breast at diagnosis (baseline) and at 1-2 years on adjuvant ET (follow-up). We extracted demographics, body mass index (BMI, kg/m2), clinicopathologic characteristics including tumor stage and grade, and BC treatments from the electronic health record (EHR) and the New York-Presbyterian Hospital Tumor Registry. We applied the CNN risk model to baseline and follow-up mammograms to estimate baseline CNN BC risk score (range, 0-1, with 1 indicating highest risk), and to calculate absolute change in CNN risk score from baseline to 1-2 years on ET. We conducted multivariable linear regression models to evaluate for potential associations between baseline CNN risk score and change in CNN risk score and prognostic factors including age, race/ethnicity, BMI, tumor stage and grade, and BC treatments.

Result: Among 749 evaluable women, mean age was 59.4 years (standard deviation [SD], 12.4 years), and 38% were non-Hispanic White, 14% non-Hispanic Black, 38% Hispanic, and 10% Asian. Thirty-six percent of women were obese (BMI >30 kg/m2), and three-quarters were postmenopausal at diagnosis. The majority of patients had stage I cancer (59.4%), and received adjuvant aromatase inhibitors (68.1%). After adjustment for covariates, baseline BMI was not significantly associated with baseline CNN risk score (regression slope estimate [ß]=0.001; p=0.424). However, increasing age (ß=0.001; p<0.001), Black race (ß=0.023; p<0.001), tumor stage III (ß=0.023, p=0.005) were associated with higher baseline CNN score, while high tumor grade was inversely associated with baseline CNN score (ß= -0.0150; p=0.028). In multivariable analysis, absolute change in CNN risk score from baseline to follow-up was inversely associated with change in BMI over that period (ß= -0.002; p=0.007) and with high tumor grade (ß -0.014; p=0.039), while older age (ß=0.001; p<0.001), Black race (ß=0.024; p<0.001), tumor stage III (ß=0.023; p<0.001), and low tumor grade (ß=0.022; p=0.024), had significant positive associations with change in CNN score.
Conclusions: We found that poor prognostic factors such as advanced tumor stage and Black race were associated with higher baseline CNN risk score and increase in CNN score on adjuvant ET. However, factors typically associated with more favorable BC diagnosis, including lower tumor grade, older age, and a decrease in BMI on adjuvant ET, were also associated with higher baseline CNN score and an increase in CNN risk score. These associations should be further evaluated in future studies utilizing larger, diverse patient cohorts of patients with early-stage HR+ BC.

P2-07-13: USE OF INDIGO CARMINE FOR DETECTING SENTINEL LYMPH NODES IN BREAST CANCER: INTERIM RESULTS OF A STUDY ON EFFICACY AND SAFETY

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Abstract: Sentinel lymph node biopsy (SLNB) is the standard staging procedure for early breast cancer (BC). Various markers are currently used to detect sentinel lymph nodes, including radioisotopes, methylene blue-based dyes, and fluorescent agents.
Relevance: the reference and most studied method remains the use of radioactive colloid: technetium-99mTc. In addition to the high cost, a limitation of the method is its technical complexity and lack of availability in most clinics due to radiation safety requirements. Therefore, the search for alternative techniques remains relevant.Indocyanine green (ICG) also has high sensitivity and has been recommended as an alternative to radioisotopes. After intradermal injection, ICG binds to albumin, an integral component of lymph, and is therefore an excellent means of visualizing lymphatic vessels and lymph nodes. The disadvantages of this method are the high cost of the dye, the need for expensive additional

equipment (near-infrared camera), and indirect visualization of the surgical field.
Indigo carmine is an affordable and safe dye that has been successfully used in urology to study the excretory function of the kidneys, including the renal pelvis and ureters. In surgery, it is used for visual detection of integrity violations of hollow organs and the extent of fistulous passages. The use of indigo carmine during endoscopic chromoendoscopy has shown high efficiency in visualizing esophageal, gastric, and colorectal neoplasms. The low volume of dye (<2 ml) and intradermal injection have low rates of anaphylaxis (0.031%). In experimental animal studies, with daily administration of indigo carmine at doses 10 times higher than the recommended human dose, it did not have a harmful effect on the main organ systems and tissues (nervous, cardiovascular, hematopoietic, excretory, respiratory), metabolism, general condition, and important physiological homeostasis parameters, which also demonstrates the complete absence of cumulative properties of this dye. However, despite the potential advantages, there is currently insufficient data confirming the efficacy and safety of indigo carmine for lymph node marking in SLNB.
Purpose: The aim of this study was to evaluate the efficacy of performing "sentinel" lymph node biopsy using indigo carmine in patients with breast cancer and to assess the safety and tolerability of indigo carmine for the "sentinel" lymph node biopsy procedure in patients with breast cancer.The objective of this study was to identify lymph nodes marked using indigo carmine, identify lymph nodes marked using the radiopharmaceutical technetium-99mTc, and compare the number of cases of detection of "sentinel" lymph nodes using indigo carmine with the number of cases of detection of "sentinel" lymph nodes using the technetium-99mTc preparation and gamma probe.
Materials and methods: Over 6 months, 117 patients with a diagnosis of breast cancer who were indicated for the sentinel lymph node biopsy procedure underwent sentinel lymph node biopsy using a combined method with the use of indigo carmine and the radiopharmaceutical technetium-99mTc. Indigo carmine and the technetium-99mTc radiopharmaceutical were injected intradermally into the areola of the affected breast in each patient. The "sentinel" lymph nodes stained with indigo carmine were visible to the naked eye without the use of additional equipment, then detected with a gamma probe, and then removed. The frequency of detection of "sentinel" lymph nodes was analyzed and the presence of complications was investigated.
Results: A total of 273 "sentinel" lymph nodes (2-3 lymph nodes per patient) were identified and removed using technetium-99mTc and a gamma probe. > Воронов Михаил Вячеславович: In 262 of the 273 removed lymph nodes, the presence of indigo carmine was detected using blue LED illumination. None of the patients experienced complications related to the use of indigo carmine or blue LED illumination.
Conclusions: Based on the preliminary data, it can be concluded that the use of indigo carmine for lymph node marking in SLNB in BC patients has potentially high efficacy and safety. Given the availability of the method - low cost of the drug and no need for additional equipment (the dye is visible to the naked eye), indigo carmine can be an alternative to ICG. Further research on a larger number of patients, as well as comparison of indigo carmine with other dyes, is required to confirm the efficacy and safety of indigo carmine.

P2-07-14: Head-to-head comparison of the dual-targeting tracer [68Ga]Ga-RM26-RGD and [18F]FDG for PET imaging of breast cancer

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Purpose/Background: Radiolabeled RM26 and RGD peptide analogs have been investigated for imaging gastrin releasing peptide receptor (GRPR) and integrin αvβ3 receptor expression in multiple types of tumors. However, the diagnostic efficacy of the RM26-RGD heterodimer for primary lesions and axillary lymph node metastases in breast cancer patients has not been systematically evaluated. This multi-center prospective study provides the first head-to-head comparison of [68Ga]Ga-RM26-RGD with conventional [18F]FDG PET/CT in breast cancer patients.
Materials and Methods: This prospective study was approved by the Peking Union Medical College Hospital ethics committee (I-22PJ246) and registered at ClinicalTrials.gov (NCT05549024). All patients provided written informed consent to receive both the [68Ga]Ga-RM26-RGD and [18F]FDG PET/CT within 1 week. Thirty-six newly diagnosed female breast cancer patients with pathologically confirmed malignant breast tumours by preoperative ultrasound-guided needle aspiration were consecutively recruited from June 2023 to June 2024. All patients underwent [68Ga]Ga-RM26-RGD PET/CT scan at 44.1 ± 10.6 min and [18F]FDG PET/CT scan at 72.8 ± 15.7 min after intravenous injection. The peripheral normal tissue was considered as background for the calculation. The tumor-to-background ratio (TBR) was calculated for further analysis. A final diagnosis was made based on the histopathological examination of the surgical excision. In addition, immunofluorescence and HE staining of breast tumors and metastatic axillary lymph node sections were also performed to further evaluate the expression of GRPR and integrin αvβ3 receptors in breast tumors.
Results: All patients tolerated the examination well and no significant adverse events related to the study were reported in any of the patients. Both the primary breast tumors and metastases showed positive [68Ga]Ga-RM26-RGD accumulation. In 36 patients, 39 lesions were surgically removed, including 36 invasive ductal carcinoma and 3 ductal carcinomas in situ. In 39 primary breast tumors, both [68Ga]Ga-RM26-RGD PET/CT (38/39) and [18F]FDG PET/CT (37/39) had a high detection rate (97.4% vs. 94.9%, $p > 0.05$). There were no significant differences in SUVmax/bkgmeanTBR (7.6 ± 5.7 vs. 5.8 ± 4.7, $p = 0.07$) and SUVmean/bkgmeanTBR (4.8 ± 3.6 vs. 3.8 ± 2.9, $p = 0.09$) of the primary tumor between [68Ga]Ga-RM26-RGD and [18F]FDG PET/CT. However, [68Ga]Ga-RM26-RGD is superior to [18F]FDG in terms of sensitivity (56.04% vs. 39.56%, $p < 0.001$), specificity (98.11% vs. 94.61%, $p < 0.001$), and accuracy (89.83% vs. 83.77%, $p < 0.001$) in detecting metastatic lymph nodes by McNemar's test. Additionally, the expression of GRPR and integrin αvβ3 receptors in both primary tumor and metastatic lymph nodes was demonstrated by immunofluorescence, verifying the tumor-specific targeting ability of [68Ga]Ga-RM26-RGD.
Conclusion: This study demonstrates that the new dual integrin αvβ3- and GRPR-targeting PET radiotracer has an equivalent performance to [18F]FDG in detecting primary breast

tumors and is superior in detecting metastatic axillary lymph nodes. [68Ga]Ga-RM26-RGD PET/CT may be of great value in the diagnosis of primary breast cancer and tumor staging.

P2-07-15: Metabolic tumor burden measured by [18F]FDG PET/CT and outcomes of patients with HER2-negative mBC treated with sacituzumab govitecan or trastuzumab deruxtecan

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Aim: This study aimed to test the association between pre-treatment biomarkers using fluorine-18 fluorodesoxyglucose positron-emission tomography/computed tomography ([18F]FDG PET/CT) imaging and clinical outcomes in metastatic breast cancer (mBC) patients treated with antibody-drug conjugates (ADCs) Sacituzumab Govitecan (SG) and Trastuzumab Deruxtecan (T-DXd).
Methods: A retrospective and bicentric analysis was conducted on triple-negative mBC (mTNBC) patients treated with SG and hormone receptor positive or negative, HER2-low mBC patients treated with T-DXd, who underwent [18F]FDG PET/CT scans before therapy. Clinical, biological, pathological and PET parameters (tumor SUVmax, total metabolic tumor volume [TMTV]) were evaluated. A multivariate prediction model was developed using Cox models for progression-free survival (PFS) and overall survival (OS).
Results: The study included 128 patients: 71 mTNBC treated with SG and 57 HER2-low mBC treated with T-DXd. Median follow-up was 12.9 months in both cohorts. In the SG/T-DXd cohort, median PFS and OS were 4.8/5.8 and 8.9/9.0 months. In multivariate analyses, TMTV>median (SG: 38.5cm3; T-DXd: 91.8cm3) was associated with shorter PFS (SG: HR 2.3, 95%CI 1.2-4.4 and T-DXd: HR 2.1, 95%CI 1.3-3.9) and OS (SG: HR 2.9, 95%CI 1.3-6.7 and T-DXd: HR 2.8, 95%CI 1.2-7.1).
Conclusion: In this pretreated population, high TMTV, reflecting the metabolic tumor burden, on [18F]FDG PET/CT imaging before SG or T-DXd was associated with poor PFS and OS in mBC patients. Although SG and T-DXd rely on different targets and vectorization strategies, these two ADCs share the same baseline metabolic efficacy-associated biomarkers.

P2-07-16: Rising ESR1 Mutations in Circulating Tumor DNA (ctDNA) Mediate Endocrine Therapy (ET) Resistance to Everolimus and ET but Retain Sensitivity to Fulvestrant in HR+/HER2- Metastatic Breast Cancer (MBC)

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Background: Treatment with endocrine therapy and CDK4/6 inhibitors is the first-line therapy for the management of HR+/HER2- mBC. ESR1 mutations represent a type of acquired resistance in up to 40% of patients after initial ET. Recent advancements and our previous studies (ASCO-2019 #1036, 2022 #1057, and 2023 #1038) in understanding ET resistance in ESR1-mutant HR+/ HER2- mBC have highlighted the pivotal role of circulating tumor DNA (ctDNA) in monitoring treatment response and progression. Here, we report a first-of-its-kind finding of the significance of ctDNA ESR1 mutations as a factor associated with therapeutic resistance in HR+/HER2- mBC.

Methods: This study included 158 HR+/HER2- mBC patients enrolled under an IRB-approved trial (NU16B06) who received systemic treatments from 2016 to 2021 at Robert H. Lurie Cancer Center at Northwestern University. The median follow-up period was 126.3 months from the first diagnosis. 40 patients with ESR1 mutations were included for statistical analysis (Causal Inference) of the correlation between the presence of ESR1 mutations and ET resistance. Patients received various therapies including CDK 4/6 inhibitor, ET and mTOR inhibitor. None of the patients received ESR1-targeted therapy with Elacestrant. Baseline ctDNA was analyzed by Guardant 360 NGS at the time of metastatic spread and subsequent points of progression. Additional clinical, pathologic, therapy, and response data were retrospectively collected and analyzed.

Results: ESR1 mutations were identified in 10 hotspots in 40 patients. The number of patients with ESR1 mutations at each hotspot, along with the mean percentage of ESR1-mutated DNA, was as follows: 13 D538G (average 11.9%), 12 Y537S (average 5.6%), 8 E380Q (average 6.3%), 1 V392I (0.1%), 1 Y537N (1.7%), 1 L536P (3.4%), 1 L536R (11.0%), 1 L536H (23.9%), 1 K520K (0.6%), and 1 K362 (15.8%). Among the 40 ESR1-mutation positive patients, 13 received Fulvestrant and 26 received Everolimus and ET. Patients treated with Everolimus and ET had a significantly higher mean percentage of ESR1-mutated DNA compared to patients who did not receive Everolimus plus ET (17.9% vs. 6.1%, p=0.01). This indicates that a higher percentage of mutated ESR1 may be associated with resistance to Everolimus and ET. Conversely, patients treated with Fulvestrant had a significantly lower mean percentage of ESR1-mutated DNA compared to patients who did not receive Fulvestrant (6.85% vs. 18.1%, p=0.04). This suggests that an increasing percentage of ESR1-mutated ctDNA correlates with an increased treatment sensitivity to Fulvestrant. Interestingly, we identified a high rate of PIK3CA co-mutations in 24 patients (60%) among the 40 patients with ESR1 mutations, with the most common co-occurring ESR1 mutation hotspots being Y537S (8 pts), E380Q (7 pts), and D538G (5 pts). Further treatment response analysis to determine the correlation between these two gene co-mutations is underway.

Conclusions: Our findings indicate that the level changes of ctDNA ESR1 mutations have implications with regards to treatment sensitivity and resistance to various therapies in patients with HR+/HER2- mBC. These results suggest a potentially effective method for monitoring treatment response and guiding future therapy by providing new insights into targeting ESR1 pathways to overcome resistance.

P2-07-17: Palbociclib in women aged ≥70 years as first-line treatment for endocrine-sensitive Hormone Receptor-positive/Human Epidermal Growth Factor Receptor 2-negative locally advanced or metastatic breast cancer: final results of PalomAGE

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Background: Endocrine therapy (ET) combined with a CDK4/6 inhibitor is standard of care for hormone receptor-positive (HR+) / human epidermal growth factor receptor 2-negative (HER2-) locally advanced or metastatic breast cancer (ABC). In Europe, around 40% of BC are diagnosed in women aged ≥70 years, contrasting with their underrepresentation (<5-10%) in clinical trials. PalomAGE is a French prospective, observational, real-life study, assessed the feasibility of palbociclib (PAL) combined with ET in women aged ≥70 years with HR+/HER2- ABC. PalomAGE included a geriatric assessment as recommended in this population when the G8 screening tool identified a probability of frailty. Here, we report the final results in patients with no prior systemic treatment for ABC and no relapse within 1 year after the end of adjuvant ET.
Methods: Data collected at baseline and every 3 months included sociodemographic, clinical, biological, disease and treatment response, quality of life (QoL; EORTC QLQ-C30 and ELD14), geriatric (G8 and Geriatric-COre DatasEt [G-CODE]) and safety parameters. Primary endpoint was PAL discontinuation rate (any reason) at 18 months. Secondary endpoints included time-to-treatment failure (TTF), progression-free survival (PFS), geriatric and QoL assessment, and safety.
Results: From October 2018 to December 2020, 816 patients were included, among whom 412 patients initiated PAL as first-line. Median age was 78 years (range 70-94) with 44.4% of patients ≥80 years, ECOG was ≥2 in 17.5%, visceral metastasis was present in 41.7%. For patients with geriatric questionnaires completed, baseline scores showed G8 ≤14, ADL ≤5, IADL short form ≤3 and Charlson comorbidity score ≥ 4 in 69.1%, 14.6%, 28.7% and 85.5% of patients, respectively. PAL starting dose was 125 mg, 100 mg, and 75 mg in 80.6%, 14.3%, and 5.1% of patients, respectively, combined with an aromatase inhibitor in 93.2% or fulvestrant in 6.8%. Patients initiating PAL at reduced dose were older (median age 83 years), presented more often an ECOG ≥ 2 (22.5%) and poorer geriatric scores (G8≤14, ADL ≤5, IADL short form ≤3, Charlson comorbidity score ≥4 in 82.1%, 25.4%, 35.8%, 91.9%, respectively).
Of all patients, 382 (92.7%) were evaluable for efficacy endpoints. With a median follow-up of 25.2 months (95% CI 21.7-28.4), the 18-month PAL discontinuation rate was 41.4% (95% CI 36.4-46.3) with disease progression (20.7%), toxicity (7.3%), patient's choice (6.8%), death (4.7%), or other reason (1.8%) as main causes. Median TTF and PFS were 23.0 months (95% CI 19.8–26.0) and 30.4 months (95% CI 25.7-not reached). Higher 18-month PAL discontinuation rate, shorter median TTF and PFS values were observed with

PAL initiation at a reduced dose without reaching statistical significance. The univariate analysis showed that baseline factors associated with lower TTF were higher age, poorer ECOG score, greater metastatic sites, G8≤14, IADL short form ≤3, and no person able to provide care and support. The multivariate analysis is in progress. At the end of the study, 93.7% and 38.7% of patients were still alive and under PAL treatment.
80.6% patients presented at least one adverse event (AE) related to treatment, with 63.3% neutropenia all grade (1.0% febrile neutropenia). AE led to PAL dose reduction and permanent discontinuation in 35.2% and 10.7% of patients.
Conclusion: PalomAGE confirms the feasibility of ET combined with PAL as first line for HR+/HER2- ABC in unselected women aged ≥70 years with significant prevalence of comorbidities and G8 impaired. Median PFS of 30.4 months is consistent with results obtained with PAL + ET in ET-sensitive HR+/HER2- ABC in other RCT and RW studies (PALOMA-2, PARSIFAL-LONG, PALBOSPAIN), with no new safety signal.

P2-07-18: Results from PALVEN: A Phase 1b Study of Palbociclib, Letrozole and Venetoclax in ER and BCL2-Positive Metastatic Breast Cancer

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Background: CDK4/6 inhibitors induce growth arrest/senescence in breast cancer cells, which become refractory to apoptotic cell death. In preclinical models, the BCL2 inhibitor venetoclax augmented tumor response to endocrine and CDK4/6 inhibitor therapy, by triggering apoptosis. This is now being tested in PALVEN, a phase 1b dose escalation study in patients (pts) with ER+ and BCL2+ metastatic breast cancer (MBC). The maximum tolerated and recommended phase 2 dose has been defined as letrozole 2.5 mg (d1-28), palbociclib 75 mg (d1-21) and venetoclax 400 mg (d1-21). Here, we present the clinical benefit rate (CBR) and response rate (RR) at 24 wks and report treatment-related toxicity.
Methods: Participants included post-menopausal women with ER+/HER2– (ASCO/CAP) and BCL2+ MBC who had received ≤2 prior lines of systemic therapy in the metastatic setting. Patients (pts) with evaluable disease were included. Tumor assessment occurred every 8 wks. Secondary endpoints included CBR and RR defined by RECIST v1.1 within the first 24 wks of treatment. Adverse events from venetoclax, palbociclib and letrozole were reported using the CTCAE v5.0 grading system.
Results: As of 19 June 2024, sixteen pts had received venetoclax, palbociclib and letrozole treatment and fifteen were DLT-evaluable (median age 50 yrs [range 36-67]), with a median follow-up time of 29.2 months. Of the DLT-evaluable population, 8 pts had de novo MBC and 7 pts had relapsed MBC, with 9 pts having previously received endocrine therapy in either the adjuvant or metastatic setting. Three pts had received prior systemic treatment for MBC; 1st line aromatase inhibitor therapy (2 pts), chemotherapy followed by tamoxifen (1 pt). At the 24-wk tumor assessment, 60% (9/15) of patients had achieved a partial response (PR), 33% (5/15) had stable disease (SD), and one pt had progressive disease. The

CBR (rate of confirmed complete response or PR or SD ≥24 wks) was 93% (14/15 pts). Of thirteen pts with baseline measurable disease, 9 (69%) achieved a PR by 24 wks. In pts who were previously untreated in the metastatic setting, the CBR was 100% (12/12), 89% (8/9) for pts who had previously received endocrine therapy in the adjuvant or metastatic setting. For pts with strong (IHC 3+) BCL2 expression, CBR was 88% (7/8).
An interim safety analysis of all eligible pts that received at least one dose of triple therapy (n=16), determined the most common non-hematological treatment-related adverse events (TRAEs) of any grade were fatigue (75%), nausea (69%), arthralgia (50%), hot flashes (44%), constipation (44%) and diarrhea (44%). Grade ≥3 hematological TRAEs were decreased neutrophil count (63%), reduced white cell count (44%) and decreased lymphocyte count (19%). There were no AEs of special interest since dose level 1 (G3/4 AST/ALT elevations in 2 pts). No treatment-related deaths were reported. Two pts ceased triple therapy due to intolerable TRAE, including one with arthralgia attributed to letrozole alone.
Conclusion: Triple therapy with palbociclib, letrozole and venetoclax in ER+ and BCL2+ MBC is well tolerated and shows promising clinical activity in pts with endocrine sensitive and CDK4/6 inhibitor-naive disease. Our findings suggest that combining a BCL2 and CDK4/6 inhibitor in endocrine responsive breast cancer merits further investigation.
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P2-07-19: Health-Related Quality of Life with Sacituzumab Govitecan versus Treatment of Physician's Choice in Previously Treated Hormone Receptor-Positive/HER2-Negative Metastatic Breast Cancer: A Meta-Analysis of TROPiCS-02 and EVER-132-002 Trials

Hope S. Rugo, Binghe Xu, Anandaroop Dasgupta, Ankita Kaushik, Wendy Verret, Barinder Singh

Introduction: Two phase III randomized controlled trials (RCTs), TROPiCS-02 and EVER-132-002, compared sacituzumab govitecan (SG) versus treatment of physician's choice (TPC) in participants with HR+/HER2- [Human epidermal growth factor receptor 2, (IHC0, IHC1 positive, or IHC2 positive and ISH negative)] locally recurrent inoperable or metastatic breast cancer (MBC) who had progressed after 2-4 prior chemotherapy regimens. Prior CDK4/6 inhibitor (CDK4/6i) treatment was mandatory for inclusion in TROPiCS-02 but not EVER-132-002. In this study, we explored the health-related quality of life (HRQoL) benefits of SG vs TPC in the overall population and determined if results vary by prior CDK4/6i exposure and duration of prior CDK4/6i treatment via a meta-analysis of the two RCTs.
Methods: Meta-analysis was conducted to compare time to first clinically meaningful deterioration (TTD) of SG vs TPC using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Version 3.0 (EORTC QLQ-C30) domains (≥10 points change) and EuroQol 5 Dimensions 5 Levels Visual Analog Scale (EQ-5D-5L VAS) (≥15 points change) in the overall, prior CDK4/6i treated and fast progressor

populations (prior CDK4/6i duration of treatment: ≤12 months). A sensitivity analysis was performed, considering death to be an event. Hazard ratios and 95% confidence interval were estimated for TTD outcomes using a stratified Cox proportional hazards regression analysis.
Results: A statistically significant increase in TTD was observed with SG compared to TPC for 6 of 15 domains of EORTC QLQ C-30, including Global Health Status/QoL, physical functioning, emotional functioning, fatigue, pain, and dyspnea measures in the overall and prior CDK4/6i treated population. Amongst the remaining domains, 4 (role functioning, cognitive functioning, social functioning, insomnia) were numerically favored in SG arm, 3 (appetite loss, constipation, financial difficulty) were numerically favored in TPC arm, while 2 (nausea-vomiting and diarrhea) were significantly favored in TPC arm. Subgroup results for fast-progressors revealed significant TTD findings in similar 6 domains favoring SG, with significant benefit also observed for financial difficulties. In the sensitivity analyses, the findings remained largely consistent in the overall and prior CDK4/6i treated population. However, statistical significance in TTD favoring SG was lost in pain and gained in financial difficulties. Furthermore, SG demonstrated significantly longer TTD over TPC for EQ-5D-5L VAS, with and without considering death as an event for all three patient subgroups (HR ranging from 0.63 to 0.76, p <0.001).
Conclusions: In this meta-analysis, SG significantly improved quality of life compared to TPC for several EORTC QLQ C-30 domains and EQ-5D-5L VAS in the overall, CDK4/6i pre-treated, and fast-progressors populations. The consistency of these results across different patient populations and several time-to-event analyses enhances the generalizability of the individual trials and reinforces the HRQoL benefits associated with SG versus TPC.

P2-07-20: Sociodemographic Disparities in First-Line (1L) Treatment Patterns and Outcomes in Women With Hormone Receptor-Positive (HR+)/Human Epidermal Growth Factor Receptor 2-Negative (HER2–) Metastatic Breast Cancer (mBC) in the United States (US)

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Background: Recent substantial advances in targeted treatments for HR+ (estrogen/progesterone receptor-positive) and HER2– (immunohistochemistry score 0, 1+, or 2+ and fluorescence in situ hybridization test negative) mBC have led to improved outcomes. However, sociodemographic disparities in survival exist and are in part related to differences in access to health care, delays in diagnosis and treatment, income, comorbidities, and underlying disease biology. Further evaluation of treatment patterns and clinical outcomes in women with HR+/HER2– mBC is needed to identify health disparities and improve outcomes.
Methods: This retrospective observational cohort study utilized the nationwide, Flatiron Health electronic health record-derived de-identified database. Women aged ≥ 18 years with HR+/HER2– mBC , who initiated 1L treatment for metastatic disease from Jan 1, 2015

to Feb 28, 2023 were included. Sociodemographic and clinical factors, treatment patterns, time-to-next treatment or death (TTNTD), and real-world overall survival (rwOS) were evaluated by race/ethnicity, baseline socioeconomic status (SES) index (a composite of area-level measures), geographic region, and practice type. Sociodemographic and clinical factors and TTNTD were analyzed descriptively. rwOS was assessed by Kaplan-Meier methods and multivariable Cox proportional hazard regression was used to evaluate the association of rwOS with important clinical and sociodemographic factors.
Results: This study included 6974 women with HR+/HER2– mBC initiating 1L treatment. Median age was 65 years; 64% of women were non-Hispanic White (NHW), 10% were non-Hispanic Black (NHB), 8% were Hispanic/Latina (H/L) and 18% were of other or unknown race. Over one-third (37%) of women were from the Southern US and 83% were treated only in community cancer centers. Women were distributed equally by SES index (15-21% per quintile). NHW women and those treated in community centers were older (median age 66 years for both); women with higher SES index were more likely to be treated in academic centers (22% in the highest SES quintile vs 11% in the lowest quintile). More NHB women (44%) had stage IV (de novo) disease compared with NHW women (36%).
More than half of the women overall (54%) received 1L endocrine therapy + a cyclin-dependent kinase 4/6 inhibitor, with little difference between NHW and NHB women (55% vs 52%). The proportion of women receiving 1L single-agent chemotherapy (overall 15%) was higher among NHB and H/L women compared with NHW women (21% and 24% vs 14%, respectively).
Median rwOS was 41.7 months (95% CI 40.1-44.1) for NHW and 32.1 months (95% CI 28.3-35.7) for NHB. A statistically significant difference remained after adjusting for clinical and sociodemographic factors (hazard ratio [HR] 1.2, $P < 0.01$). Median rwOS was 49.7 months (95% CI 45.8-56.3) for women treated in academic centers and 37.6 months (95% CI 36.2-39.3) for those treated in community centers (HR 0.82, 95% CI 0.75-0.91). There was no statistically significant difference in adjusted rwOS by SES index and geographic region. No statistically significant difference was observed in TTNTD by race, SES index, geographic region, or treatment setting.
Conclusions: This study showed that race/ethnicity and treatment setting continue to play an important role in rwOS in HR+/HER2– mBC. Future studies could elucidate the relationship between sociodemographic factors and disparities. This can inform interventions to improve the outcomes of women with HR+/HER2– mBC.

P2-07-21: A phase II study of pembrolizumab plus fulvestrant in ER positive, HER2 negative advanced/metastatic breast cancer patients

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Background: The role of immune checkpoint inhibitors (ICI) has not been elucidated in ER+/Her2- metastatic breast cancer (MBC). Previous studies have demonstrated a subset of these patients may derive benefit from immunotherapy in combination with endocrine therapy. We hypothesized that pembrolizumab in combination with fulvestrant would demonstrate disease control with acceptable toxicity profile.
Methods: This was a multicenter, single arm, open label Phase II Simon's two-stage optimal design study in patients with ER+/HER2- MBC. The study was conducted through the BIG TEN Cancer Research Consortium. Patients received no more than 2 prior lines of endocrine or chemotherapy in the metastatic setting. Treatment consisted of pembrolizumab 200 mg every 3 weeks in combination with fulvestrant. The primary endpoint was to evaluate the clinical benefit rate (CBR: SD+PR+CR). The anti-tumor activity of pembrolizumab plus fulvestrant was measured by RECIST 1.1, irRECIST, and progression free survival (PFS). Scheduled staging scans occurred every 3 cycles (9 weeks). Secondary endpoints included safety and tolerability. PD-L1 status and next generation sequencing (NGS) by Tempus was performed on available samples to investigate biomarkers of response and the underlying mutational landscape. (NCT03393845)
Results: Forty-seven patients were enrolled, with median age of 61. Majority of patients (n=32, 70%) received 1 prior line of therapy in the metastatic setting (range 0-2). Forty patients (87%) had prior treatment with CDK4/6 inhibitors, with 1 patient who received it in the adjuvant setting on a clinical trial. Eleven patients (24%) received prior fulvestrant, and 1 received chemotherapy in the metastatic setting. Forty-six patients were treated with at least 1 cycle, 44 were efficacy-evaluable, and 43 remained on study at the time of first restaging imaging. One patient came off study due to clinical progression and was included in the final response analysis. Two patients without radiographic or clinical progression withdrew after adverse events unrelated to the study drugs and were not included in the final response analysis. The median PFS in evaluable patients was 3.2 months (range 0.2-23.3). The CBR at 18 weeks was 36.4% (n=16/44) by RECIST 1.1. Of the 43 patients who underwent first restaging scan, 22 (50%) patient achieved disease control, including 17 patients with stable disease and 5 patients with partial response. Amongst patients who achieved disease control, the median duration of response was 6 months (range 2.3-21.3 months). Twenty-two patients had either radiographically confirmed progression on first restaging scan or clinical progression. The most common treatment related adverse effects (TRAEs) were AST elevation (n= 15, 31.9%), ALT elevation (n= 12, 25.5%), and fatigue (n= 14, 29.8%). Majority of TRAEs (93.5%) were G1-2. TRAEs of interest included hypothyroidism (n= 8, 17.0%), hot flashes (n= 3, 6.4%), arthralgia (n= 4, 8.5%), and pneumonitis (n= 1, 2.1%, G2). There were 7 (15%) patients who experienced G3 toxicities (arthralgia, anemia, hypocalcemia, decreased lymphocyte, rash, weight loss) and of these one patient (2.1%) discontinued the study drug due to elevated AST/ALT. There were no G4+ TRAEs. In patients with available tissue for NGS utilizing the Tempus platform (648 gene panel for next generation sequencing, whole exome, and RNA sequencing), genomic alterations and tumor mutational burden were evaluated. Response will be correlated with PD-L1 status by combined positive score (CPS), prior fulvestrant exposure, BMI, and biomarkers identified on NGS as exploratory analyses.

Conclusions: The combination of pembrolizumab and fulvestrant in ER+/HER2- MBC demonstrated a manageable toxicity profile with durable response in patients who achieved disease control. The combination may be considered for further exploration to better understand biomarkers of response to ICI in this population.

P2-07-22: Cyclin-dependent kinase 7 (CDK7) inhibitor samuraciclib combined with selective estrogen receptor degrader (SERD) elacestrant in advanced HR+ breast cancer after CDK4/6i: dose escalation data from the Phase 1b/2 SUMIT-ELA study

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Background: Effects of CDK7 in cancer include: enhanced oncogene transcription; anti-apoptotic gene upregulation; cell cycle acceleration via CDK phosphorylation; and estrogen receptor activation, driving resistance to hormonal therapy. Thus, CDK7 inhibition represents a potential anticancer strategy. Samuraciclib (CT7001), a once-daily oral CDK7 inhibitor, combined with the SERD fulvestrant had a favorable safety profile and clinical activity in patients with HR+/HER2− advanced breast cancer (BC) previously treated with a CDK4/6i [Coombes, 2023]. Patients with no detectable TP53 mutation in baseline ctDNA appeared to have better outcomes. Oral SERDs are of interest due to their pharmacokinetic (PK) properties and convenient administration. The oral SERD elacestrant significantly improves progression-free survival (PFS) versus standard-of-care endocrine therapy in ER+/HER2− advanced or metastatic BC previously treated with a CDK4/6 inhibitor, and particularly ESR1-mutant tumors [Bardia et al. SABCS 2022]. Non-clinical data indicate that the biology underlying the samuraciclib/SERD combination translates from fulvestrant to elacestrant, in addition to overcoming other resistance mechanisms. Thus, clinical evaluation of this combination is warranted [data on file].
Methods: The phase 1b/2 SUMIT-ELA open-label dose escalation and expansion study (NCT05963997) is evaluating the safety, efficacy, and PK of samuraciclib combined with elacestrant, both dosed QD. Eligible patients (n=48) are ≥18 years, have histologically or cytologically confirmed ER+/HER2− advanced or mBC not amenable to resection or radiotherapy of curative intent, have received an AI + CDK4/6i in the adjuvant or advanced setting, are receiving a LHRH agonist if pre/perimenopausal, and have RECIST v1.1 measurable or evaluable disease. All patients undergo baseline Guardant360 ctDNA analysis to assess ESR1 and TP53 mutation status. Prior SERD, mTOR inhibitor, or chemotherapy for advanced BC are not permitted. Patients undergo RECIST v1.1 evaluation at baseline, every 8 weeks until week 48, and every 12 weeks thereafter. The primary endpoints are identification of tolerable combination doses of samuraciclib and elacestrant (phase 1b) and 6-month PFS rate (phase 2). Secondary endpoints are tolerability, clinical benefit rate at 24 weeks, ORR, DoR, best percentage change in tumor size, PK, and associations between ESR1 and TP53 mutation and efficacy/safety.

Results: The following cohorts (C) have been recruited and dosed with samuraciclib + elacestrant as follows: C1 (240+300 mg, n=6); C2 (360+300 mg, n=6); and C3 (360+400 mg, n=5). Dose escalation is complete and C4 expansion at the recommended top doses of both compounds is recruiting (360 mg samuraciclib + 400 mg elacestrant). Co-dosing of samuraciclib and elacestrant had no significant impact on the exposure of either agent. The most frequent all-grade treatment-related AEs were diarrhea, nausea, vomiting, asthenia, and abdominal pain. One subject in C1 (53-year-old female; TP53wt, ESR1wt; liver metastasis) experienced a confirmed partial response with a 37% reduction in the size of target lesions.
Conclusions: The most frequent treatment-related AEs were similar to the known safety profiles from both previous samuraciclib and elacestrant studies. With no drug-drug interactions between the treatments, further study of combination treatment in expansion C4 is supported. Preliminary signs of antitumor activity were observed.

P2-07-23: Targeting Insulin Feedback to Enhance Alpelisib (TIFA): A phase II randomized trial in PIK3CA-mutant hormone receptor-positive metastatic breast cancer

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Background: Fulvestrant+alpelisib significantly improves progression-free survival in patients with PIK3CA-mutated metastatic breast cancer (MBC). However, PI3K inhibition induces hyperglycemia, a major cause of dose interruption and dose reduction. Novel strategies to minimize hyperglycemia and improve alpelisib drug delivery are needed.
Methods: In this phase II trial, patients receiving fulvestrant+alpelisib were randomized to 1) ketogenic diet (KD), 2) low-carbohydrate diet (LCD), or 3) canagliflozin (SGLT2i) (NCT05090358). Eligibility criteria included ≥1 activating PIK3CA mutation, measurable or non-measurable disease, hemoglobin A1c (HbA1c) <8% and fasting glucose ≤140mg/dL. The primary endpoint was the grade 3/4 hyperglycemia-free rate at 12 weeks; this was a cumulative endpoint and any grade 3/4 hyperglycemia event within the first 12 weeks was counted. The study was designed for the endpoint to be tested in each arm separately, such that ≥1 strategy could be deemed successful. Weight and serum glucose levels were measured every 2 weeks for the first 4 weeks and every 4 weeks thereafter. Patients also performed daily at-home point of care glucose and beta-hydroxybutyrate measurements to assess the level of ketosis. Continuous glucose monitoring (CGM) was done in 2-week intervals throughout the 12-week study period. Body composition was assessed on CT scans pretreatment and at 12 weeks. Diet adherence was defined as consuming ≤40g daily carbohydrates for the KD arm and ≤100g for the LCD arm on ≥70% of available complete food log days. Although expected enrollment was 106 patients, this trial closed early due to low alpelisib use.
Results: Among 15 enrolled patients, 8 were evaluable. 6/8 (75%) patients met the primary

endpoint: 2/3 in the KD arm, 2/2 in the LCD arm, and 2/3 in the SGLT2i arm; the grade 3/4 hyperglycemia rate was 25%. Mean (standard deviation, SD) glucose in mg/dL by patient within each arm was as follows: KD arm 139 (39), 100 (19), 190 (54); LCD arm 113 (16), 103 (12); SGLT2i arm 170 (59), 95 (9), 97(7). Mean CGM time in range was 85% across the cohort (25); mean (SD) by patient within each arm were as follows: KD arm 77 (17), 45 (38), missing; LCD arm 98 (6), 96 (5); SGLT2i arm 91 (17), 94 (11), 89 (15). Beta-hydroxybutyrate mean levels (SD) in mmol/L by patient within treatment arm were as follows: KD arm 0.12 (0.08), 0.15 (0.06), 0.69 (0.29); LCD arm 0.64 (0.32), 0.57 (0.28), SGLT2i arm 0.42 (0.31), 0.15 (0.08), 0.30 (0.12). Hemoglobin A1c level 12-week change ranged from 0 to +3.5%. Alpelisib mean RDI over 12 weeks ranged from 120-300mg. All 5 patients randomized to dietary arms adhered to the diet during the 12-week study period. All 8 patients had decreases in BMI at 12 weeks compared to baseline; 4/8 patients had decreases in visceral fat and 6/8 patients had decreases in subcutaneous fat on body composition measurements at 12 weeks.
Conclusions: CGM for percent time in range may be a useful way to monitor PI3K-induced hyperglycemia. Grade 3/4 hyperglycemia was lower with the study interventions than in SOLAR-1 (25% vs. 40.3% in patients with HbA1c <8%), albeit in a small number of enrolled patients; these data support further study of diet and/or SGLT2 inhibition with agents that target this pathway.

P2-07-24: Results from the Phase II Study of ROS1 Targeting with Crizotinib in Advanced E-cadherin Negative Lobular Breast Cancer (ROLo)

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Background: Lobular breast cancers are characterised by loss of function of the cell adhesion protein E-cadherin, which is commonly inactivated via CDH1 mutation. Pre-clinical studies demonstrated synthetic lethality between ROS1 inhibition and E-cadherin loss in models of lobular breast cancer and diffuse gastric cancer. We conducted a phase 2 study with the ALK/ROS1 inhibitor crizotinib to assess the potential of ROS1 inhibition in lobular advanced breast cancer (ABC).
Methods: This phase 2 multi-centre study recruited to two cohorts in parallel: Eligible patients with measurable disease, had previously treated ER+ lobular ABC confirmed E-cadherin negative by immunohistochemistry (ER+ lobular cohort), or advanced diffuse gastric cancer (DGC), lobular triple negative breast cancer (TNBC) or other CDH1-mutated solid tumours (basket cohort). The ER+ lobular cohort received crizotinib and fulvestrant, and the basket cohort crizotinib alone. The primary endpoint was confirmed response rate (RR). Secondary endpoints included clinical benefit rate (CBR) at 6 months, safety, progression-free (PFS) and overall survivals (OS). Optimal Simon 2 stage design with 80% power to exclude p0 5% and target p1 20% RR, with recruitment of 29 ER+ lobular and 29

DGC or lobular TNBC patients. At least 4 confirmed responses in 29 patients were required to consider crizotinib +/- fulvestrant worthy of future study.
Results: Twenty-seven eligible patients were recruited from five centres to the ER+ lobular cohort and 6 to the basket cohort (one DGC, 5 lobular TNBC). The median age for each cohort was 57 and 63 respectively, all patients were female. ER+ lobular patients had received a median of 2 lines of endocrine therapy (range 1-2) and 2 lines of chemotherapy (range 1-3), the basket cohort received a median of 2 lines of chemotherapy (range 1-2) for advanced disease.
One patient in the first 10 patients with ER+ lobular ABC had a partial response, but no further responses were recorded in 17 further assessable patients (4%, 1/27). None of the 6 patients with TNBC or DGC (0%) responded to crizotinib and recruitment was terminated. CBR was 11% and 0% respectively and median PFS was 1.8 months for both cohorts. Median OS was 17.5 months in the ER+ lobular cohort and 4.4 months in the basket cohort.
For the combination of fulvestrant and crizotinib, nausea (20/27, 74%) and raised liver transaminases (20/27, 74%) were the most frequent toxicities reported. Raised ALT and/or AST was grade 3 in 9/27 patients and no grade 4 or 5 events related to the treatment were reported. For crizotinib monotherapy, adverse events were consistent with the known toxicity profile.
Conclusions: Despite promising pre-clinical data, ROS1 inhibition with crizotinib was not effective combined with fulvestrant in pre-treated ER+ lobular ABC, nor as monotherapy in pre-treated lobular TNBC or DGC.
Trial Registration – Clinical Trials: NCT03620643

P2-07-25: Thymidine kinase activity as a prognostic and predictive biomarker in the Phase II PACE trial of CDK4/6 inhibition beyond progression

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Background: There are no validated predictive biomarkers for continuation of CDK4/6 inhibition (CDK4/6i) beyond progression in patients (pts) with estrogen receptor-positive (ER+), HER2-negative (HER2-) metastatic breast cancer (MBC). Thymidine kinase (TK) is an enzyme involved in DNA synthesis that is released into the bloodstream by proliferating cells. Lower baseline serum TK activity (TKa) levels as well as on-treatment changes (TKa decreases with CDK4/6i and increases with immunotherapy) have been associated with better outcomes. The aim of this study is to assess the prognostic value of TKa in pts previously treated with CDK4/6i, as well as its predictive value for response to CDK4/6i beyond progression with or without immunotherapy in the PACE trial.

Methods: The PACE (NCT03147287) multicenter phase II trial randomized 220 pts with ER+, HER2- MBC who had progressed on at least 6 months (mo) of prior CDK4/6i and aromatase inhibitor (AI) to receive fulvestrant alone (F), F plus palbociclib (F+P), or F+P plus the PD-1 inhibitor avelumab (F+P+A). No statistically significant differences in median progression-free survival (PFS) were observed for F+P or F+P+A compared to F. Blood samples were collected at baseline (BL, within 14 days of treatment start) and at cycle 2 day 1 (C2D1); serum TKa was quantified at a central laboratory with the ELISA-based DiviTum® TKa assay (Biovica, USA). TKa analysis was blinded to clinical data. The associations of BL and C2D1 TKa PFS were assessed using stratified Cox models, the predictive associations by testing for treatment-by-TKa interaction. In this study, prespecified TKa cutoffs were defined as 250 DuA (DiviTum unit of Activity) and the median TKa values.

Results: BL samples were available for 198 pts (90.0%), and 176 (80.0%) had paired C2D1 samples. Median BL TKa was 408 DuA (IQR 198-822) in the overall population, 372 (202-604), 465 (203-843), and 328 (190-805) in F, F+P, and F+P+A arms, respectively. Higher BL TKa (>250 DuA) was not associated with inferior PFS (HR 1.0, 90%CI: 0.74-1.36) with all arms combined. Among those with high BL TKa, median PFS was 3.6 mo (90% CI: 1.9-7.3) for F, 5.4 mo (90% CI: 3.5-8.1) for F+P, and 8.1 mo (90% CI: 3.2-14.3) for F+P+A. Higher BL TKa (>250 DuA) was not predictive of differential PFS when comparing F with F+P (interaction p=0.16) or F with F+P+A (interaction p=0.41). Similar results were observed using the median BL TKa as a cutoff. Median TKa at C2D1 was 248 (IQR 136-546) with all arms combined, 290 (161-620), 239 (109-500), and 228.0 (149.0-492.0) for F alone, F+P, and F+P+A, respectively. High C2D1 TKa (> 250 DuA) was associated with shorter PFS (mPFS 2.4 mo vs 7.2 mo, HR 2.3, 90%CI: 1.6-3.1) with all arms combined. TKa at C2D1 was not predictive of PFS when comparing arm F with F+P (interaction P-value 0.74) or F with F+P+A (interaction P = 0.19). To evaluate TKa dynamic change, pts from all arms combined were classified in 4 groups based on TKa change between BL and C2D1 (cutoff of 250 DuA): 44 (25.0%) pts had “high to low,” 45 (25.6%) pts had “stay low,” 15 (8.5%) pts had “low to high,” and 72 (40.9%) pts had “stay high.” PFS differed based on the on-treatment TKa dynamic change: “high to low” mPFS 7.6 mo (90% CI: 7.1-16.3), “stay low” mPFS 5.6 mo (90% CI: 3.3-9.6), “low to high” mPFS 4.9 mo (90% CI: 1.7-8.2), and “stay high” mPFS 2.2 mo (90% CI: 1.4-2.6). Pts with “high to low” TKa decrease had favorable PFS compared to “stay low” (HR 0.58, 90% CI: 0.36-0.92), “low to high” (HR 0.46, 90% CI: 0.26-0.83), or “stay high” (HR 0.29, 90% CI: 0.18-0.45) groups.

Conclusion: In the PACE trial, BL TKa after progression on CDK4/6i was not associated with prognosis or benefit from different therapies. However, high C2D1 on-treatment TKa was associated with inferior outcomes. Early dynamic changes in TKa levels may allow identification of populations with distinct prognoses, possibly facilitating clinical decisions in this context.

P2-07-26: Onvansertib shows synergistic efficacy in combination with paclitaxel in HR+ breast cancer: Mechanistic insights from preclinical models

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Background: Paclitaxel is a first-line chemotherapy for hormone receptor-positive (HR+) metastatic breast cancer patients who have progressed on CDK4/6 inhibitors and endocrine therapy (ET) and have exhausted available ET options. The response rate for paclitaxel ranges from 20-40%, with most patients eventually developing resistance, resulting in limited treatment options. Paclitaxel-resistant tumors exhibited increased expression of polo-like kinase 1 (PLK1), a serine-threonine-protein kinase that is a key mitotic regulator. Onvansertib is a highly selective PLK1 inhibitor in clinical development that showed potent anti-tumor activity in combination with paclitaxel in preclinical models of ovarian cancer and triple negative breast cancer (TNBC). A phase 1b/2 clinical trial to evaluate the safety and efficacy of onvansertib plus paclitaxel in advanced TNBC is ongoing (NCT05383196). In this study, we investigated whether onvansertib synergizes with paclitaxel and overcome paclitaxel resistance in HR+ preclinical models resistant to first-line therapies.
Methods: We assessed the efficacy of paclitaxel combined with onvansertib in HR+ breast cancer cell lines (MCF-7, T-47D, EFM-19, CAMA-1, ZR-75-1, HCC1428 and MCF7/164R-7) and patient-derived xenograft (PDX) models. The effect of this combination on cell viability, cell cycle, mitotic arrest and DNA damage was evaluated in vitro. Eight PDXs resistant to ET and CDK4/6 inhibitors abemaciclib or palbociclib were established from primary breast tumors (n=2) or metastatic biopsies (n=6). The PDX tumors were engrafted in nude mice, and the effect of onvansertib (oral, 45 mg/kg, 5 days a week) and paclitaxel (IP, 15-25 mg/kg, once a week) as monotherapies, and in combination was investigated. The impact of the combination on apoptosis and expression of selected proteins was investigated both in vitro and in vivo.
Results: The combination synergistically inhibited the viability of HR+ breast cancer cell lines. In vivo, the combination was well tolerated with minimal toxicity and exhibited enhanced anti-tumor activity compared to either agent alone across all the eight PDX models. Paclitaxel and onvansertib monotherapies showed little to no anti-tumor activity in five out of eight PDX models (HBCx-139palboR5, HBCx-124palboR25, HBCx-131, HBCx-202, and HBCx-239). Conversely, the combination induced strong anti-tumor activity in all these models. Notably, tumor regression with >50% complete response rates were observed in HBCx-139palboR5, HBCx124palboR25, and HBCx-239 PDXs. Among the eight PDX models, three were sensitive to paclitaxel (HBCx-86, HBCx-137palboR26 and HBCx-3). In these models, compared to monotherapy, the combination demonstrated greater activity, inducing tumor regression and achieving a higher rate of complete response. The antitumor activity of the combination was very durable, showing a robust delay in tumor relapse after treatment cessation. Mechanistic studies revealed pronounced mitotic arrest and DNA damage with the combination treatment in cell lines. Compared to monotherapies, the combination induced marked apoptosis, both in vitro and in vivo. A significant decrease in

c-Myc protein expression was observed in both cell lines and tumors from mice treated with the combination compared to single agents. Studies are ongoing to further understand the regulation of c-Myc with this combination therapy.
Conclusions: Our findings strongly suggest that combining paclitaxel with onvansertib extends its benefit and overcomes paclitaxel resistance. This presents a promising therapeutic strategy and merits further clinical investigation for advanced HR+ breast cancer patients who are resistant to standard-of-care therapies.

P2-07-27: Long-term administration of endocrine therapy on the gut microbiota of breast cancer patients between disease-free and recurrence

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Background: In recent years, there has been a dramatic proliferation of research concerned with the gut microbiota and breast cancer. It is well known that the best-known mechanism of the gut microbiota on breast cancer comes from "estrobolome", which refers to the gut bacterial genes whose products are capable of metabolizing estrogens. However, not only does the gut microbiota affect the metabolism of estrogen, but estrogen can also impact the composition of the gut microbiota. The isoflavone-rich diet, soy isoflavone, or long-term administration of estrogen could impact the diversity and composition of the gut microbiota. To date, little is known about the effect of oral medication with endocrine therapy (ET) on the gut microbiota, especially in long-term administration of human studies. Thus, in this study, we aimed to investigate the long-term effect of oral medication with tamoxifen and letrozole on the human gut microbiota, especially in patients between disease-free and recurrence during ET.
Methods: A total of 48 female participants were included and divided into four groups including groups I and II of Tam-T and Let-L: patients were only administered with tamoxifen or letrozole; group III of CLet-CL: patients have received chemotherapy (CTx) with anthracycline–taxane sequence plus letrozole; group IV of Recur-R: patients have received CTx plus letrozole and have a recurrence during the period of ET. Fecal samples were serially collected from patients at two different time points. At the first point, fecal samples were collected before patients received any ET, CTx, or surgery (T1, L1, CL1, and R1). At the second point, fecal samples were collected after taking tamoxifen or letrozole for about 1 year (T2, L2) and collected after taking CTx plus letrozole (total about 1 year, CL2 and R2). The fecal samples were analyzed using 16S rRNA sequencing and further analyzed with operational taxonomic units (OTUs), α/β-diversity, linear discriminant analysis (LDA) effect size (LEfSe), heat tree analysis, random forest algorithm, and Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) prediction.
Results: In comparisons with the same regimen of third arms at the time point of the CL1 group, we found the α-diversity was significantly reduced in patients with recurrence of ET at the time point of the R1 group ($p = 0.03$). In comparison with the group of T1, L1, and L2, the α-diversity was also reduced in the R1 group. At the OTUs level, the relative abundance of major taxons did not observe significant differences among T1 and T2, L1 and L2, CL1

and CL2 but with high abundance of Fusobacteria appeared in the R2 group. The PERMANOVA analysis showed that there was no significant difference between the first-time point and the one-year ET administration of the second-time point. However, in patients with recurrence of ET, the microbial composition was different from patients with non-recurrence. In addition, there is no significant difference in the abundance of hierarchical taxonomic among T2 vs T1, L2 vs L1, and CL2 vs CL1. In contrast, several abundances of hierarchical taxonomic have existed in Recur-R versus Let-L and CLet-CL. We found the abundance of Fusobacterium, Ruminococcus, and Parabacteroides were significantly higher in patients with recurrence of ET than in patients with non-recurrence, especially in the second-time point of R2. Interestingly, we also found that the microbiome in the R1 group was specifically enriched in the pathway of Parkinson's disease than in the R2 or non-recurrence group.
Conclusion: To our knowledge, we provide the first preliminary results of long-term administration of ET on the gut microbiota in human study. We found long-term administration with ET (one year of tamoxifen or letrozole) did not significantly impact the total microbial composition. However, we found the abundance of several critical microbial markers was significantly higher in patients with recurrence of ET than in patients with non-recurrence, especially in the second-time point of R2. In the future, accumulating case numbers and introducing multiomics approaches will be needed on this topic.

P2-07-28: Treatment de-escalation by omission of chemotherapy & the effect of adding the CDK4/6 inhibitor ribociclib in HER2-positive and hormone-receptor positive metastatic breast cancer– second interim efficacy analysis of the randomized DETECT V trial

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Background: Metastatic breast cancer (MBC) is still regarded as an incurable disease with improvement of survival and maintenance of quality of life (QoL) being equally important treatment goals. In patients with HER2-positive MBC, taxane-based chemotherapy in combination with dual HER2 targeted therapy with trastuzumab (T) and pertuzumab (P) is the standard of care therapy for first line patients. However, given that adverse events associated with cytostatic treatment can seriously impact the patients' QoL, the less toxic synergistic combination of dual HER2-targeted therapy with endocrine therapy might offer a better treatment option for patients with HER2-positive and hormone-receptor (HR) positive MBC compared to cytotoxic chemotherapy-based treatment regimen.
Methods:Between 9/2015 and 11/2022, the German multicenter phase III DETECT V trial randomized 271 patients with HER2-positive and HR-positive (i.e. ER positive and/or progesterone-receptor positive) MBC in the 1st-3rd line setting 1:1 to receive T and P combined with either endocrine therapy or chemotherapy followed by maintenance

therapy with T, P and endocrine therapy. Chemotherapy and the endocrine agents could be chosen from a variety of available regimens according to physicians' choice. Based on emerging data strongly suggesting an additional benefit of CDK4/6 inhibitors, an amendment came into effect in January 2019 (after 124 patients had been randomized) with the addition of ribociclib to both treatment arms. The primary objective of DETECT V is to compare tolerability between the chemotherapy-free and chemotherapy-containing treatment arm. Secondary objectives comprise the comparison of overall survival (OS), progression-free survival (PFS) and safety between chemotherapy-free and chemotherapy-containing treatment, as well as the evaluation of the effect of adding ribociclib to both treatment arms. Here we report results of the second interim efficacy analysis with data cut off April 3rd 2024 (as based on the full ITT set of 271 patients; 54 patients still in follow up). Results:Median patient age was 60 years, 209 (77.1%) of patients were in the first line setting and 139 (51.3%) patients had a metastasis-free interval exceeding 12 months. Overall survival (OS) and progression-free survival (PFS) did not differ between patients receiving chemotherapy-free and chemotherapy-containing treatment (median OS not reached vs. 46.1 months, hazard ratio 1.07, 95% CI 0.65 – 1.77, p = 0.79; median PFS 19.1 vs. 22.4 months, hazard ratio 1.19, 95% CI 0.84 – 1.69, p = 0.34). Both OS and PFS were significantly improved by the addition of ribociclib (median OS not reached vs. 46.1 months, hazard ratio 0.42, 95% CI 0.24 – 0.74, p = 0.002; median PFS 27.2 vs. 15.6 months, hazard ratio 0.52, 95% CI 0.37 – 0.75, p < 0.001). However, exploratory analyses showed that the effect of adding ribociclib seemed to be more pronounced in the chemotherapy-containing arm (OS: hazard ratio 0.25, 95% CI 0.10 – 0.62, p = 0.003; PFS: hazard ratio 0.36, 95% CI 0.21 – 0.62, p < 0.001) as compared to the chemotherapy-free arm (OS: hazard ratio 0.65, 95% CI 0.31 – 1.36, p = 0.255; PFS: hazard ratio 0.71, 95% CI 0.44 – 1.15, p = 0.159). The 2-way interactions between randomization arm and addition of ribociclib approached significance both for OS and PFS (p = 0.087 and p = 0.088, respectively). Conclusion:Our preliminary results suggest that chemotherapy-free treatment for patients with HER2-positive and HR-positive MBC might be an effective alternative, while the addition of ribociclib may further improve survival.

P2-07-29: DNADX in advanced hormone receptor-positive and HER2-negative (HR+/HER2-) breast cancer following endocrine therapy with or without palbociclib: a correlative analysis from the GEICAM/2014-12 FLIPPER phase II randomized trial

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García-Palomo, Yolanda Fernández, Sonia González, María Rodríguez de la Borbolla, Vega Iranzo, Catherine M Kelly, Maccon M Keane, Patrick G Morris, Conleth G Murphy, Charles M Perou, Jesús Herranz, Joel S Parker, Marta Portela, Patricia Villagrasa, Rosalia Caballero, Ana Vivancos, Federico Rojo

Background: DNADX, a novel machine learning-based approach, utilizes DNA copy-number aberration (CNA) data from tumor tissue or plasma to identify clinically relevant phenotypic tumor features and classify breast cancer into 5 groups (Prat et al. Nat Comm 2023). Here, we evaluated DNADX's ability to predict prognosis and treatment benefit in advanced HR+/HER2- breast cancer following endocrine therapy and a CDK4/6 inhibitor.
Methods: FLIPPER (NCT02690480) was a multicenter phase 2 clinical trial which randomized 189 patients with HR+/HER2- advanced breast cancer to receive (1:1 ratio) first line fulvestrant with either palbociclib or placebo. DNADX was evaluated centrally in pre-treatment baseline plasma and tumor samples. Shallow whole genome sequencing (shWGS) was performed on ctDNA and tumor tissue DNA to determine the RB-LOH signature, and the 5 DNA-based groups (called Proliferative, Basal-related, Luminal-high, , CNA-flat, and TF-low [with a tumor fraction < 3%]). The primary objective was to evaluate the association of the RB-LOH signature and the DNADX subtypes determined in plasma and in tissue with progression-free survival (PFS). The secondary objective was overall survival (OS). Stratified Cox regression models were used to calculate hazard ratios (HRs) after adjusting for treatment arm and other potential prognostic factors.
Results: DNADX information was obtained from 175 pre-treatment plasma samples and 111 tumor samples. In total, 183 patients, representing 96.8% of the FLIPPER population, had either baseline plasma or tumor samples available and were included in this study. Overall, DNADX identified 57.9% pts with TF-low (n=106), 0.5% with CNA-flat (n=1), 33.3% with Luminal-high (n=61), 7.7% with Proliferative (n=14) and 0.5% with Basal-related (n=1). The median PFS in pts classified as i) TF-low, ii) CNA-flat or Luminal-high and iii) Basal-related or Proliferative was 33.8m, 24.5m and 16.5m, respectively (HRs of 2.05 and 3.42, all $p<0.001$). Results remained consistent in patients treated with palbociclib or placebo and after adjusting for clinicopathological variables. The RB-LOH signature was significantly associated with PFS (HR=1.17, 95%CI 1.02-1.34, p=0.027). In terms of OS, the median OS in pts classified as i) TF-low, ii) CNA-flat or Luminal-high and iii) Basal-related or Proliferative was 79.0m, 55.1m and 45.5m, respectively (HRs of 2.4 and 3.9, all $p<0.001$). Results remained consistent in the multivariable analysis. The RB-LOH signature in plasma was also significantly associated with OS (HR=1.31, 95%CI 1.10-1.55, p=0.002). DNADX subtypes were significantly associated with PFS and OS in plasma but not in tissue samples.
Conclusions: Liquid biopsy-based DNADX assay identifies substantial biological heterogeneity in advanced HR+/HER2- breast cancer and was a strong prognostic biomarker beyond standard clinical-pathological variables and treatment in the FLIPPER trial.

P2-07-30: Exploring Treatment Options Following Progression to CDK4/6 Inhibitor in Hormone Receptor-Positive/HER2-Negative Advanced Breast Cancer: A Systematic Review and Network Meta-analysis

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Introduction: The treatment algorithm following progression on CDK4/6 inhibitors (CDK4/6i) remains uncertain. This study aimed to conduct a comprehensive systematic review and the first network meta-analysis to evaluate the efficacy and safety of new drugs (or combined new drugs) for the treatment of Hormone Receptor-Positive/HER2-Negative (HR+/HER2-) advanced breast cancer (ABC) in patients who have progressed after first-line treatment with CDK4/6i.
Methods: PubMed, Embase, and Cochrane Central were searched from 2000 to 2024 and conference proceedings from major congresses of the last two years were retrieved. Two authors independently performed the screening, three authors extracted data, and discrepancies were resolved by consensus. The primary endpoint was progression-free survival (PFS), with overall survival (OS), objective response rate (ORR), and adverse events (AEs) as secondary endpoints. The new drugs (or combined new drugs) were compared to either standard chemotherapy (CT) or endocrine therapy alone (ET) in second-line and beyond treatment settings. Network meta-analyses were performed using random-effects models, calculating risk ratios for dichotomous outcomes and hazard ratios (HR) for time-to-event outcomes with 95% confidence intervals (CI). Heterogeneity was assessed using the I^2 statistic and chi-square test.
Results: A total of 4034 papers were identified, after removing duplicates. Out of the 67 studies screened in full-text, 26 clinical trials were selected, encompassing a total of 7083 patients and five different drug classes. Specifically, 9 studies included biological agnostic therapy (CDK4/6i, mTOR inhibitors, immunotherapy), 7 novel ET (including selective estrogen receptor degraders – oral SERD), 5 targeted therapies (PI3K, AKT and PARP inhibitors), 3 antibody-drug conjugates (ADCs: trastuzumab deruxtecan [T-DXd] and sacituzumab govitecan), and 2 CT (eribulin, VEX metronomic). Control arms consisted of either ET alone (mainly fulvestrant) or standard CT (paclitaxel, capecitabine, eribulin, gemcitabine, or vinorelbine). All network estimates coincided with pairwise estimates as no direct comparison between the new drugs existed in the literature. Among all therapies, T-DXd showed the largest PFS benefit compared to standard CT [HR 0.51 (CI: 0.45 – 0.57)], followed by ribociclib plus ET compared to ET alone [HR 0.57 (CI: 0.45 – 0.72)]. Interaction tests conducted among the five drug classes showed a significant different positive effect on PFS by targeted therapies [HR 0.61 (CI: 0.57 – 0.66)] compared to ET alone (p-value 0.009) but failed to identify significant differences in PFS among the different drug classes compared to CT (p-value 0.16), although ADCs showed a larger effect in PFS [HR 0.63 (CI: 0.50 – 0.80)]. Although ADCs showed the largest OS benefit compared to standard CT [HR 0.72 (CI: 0.58 – 0.89)], the interaction test among the drug classes was not significant (p-value 0.10), and similarly no interaction was observed for ORR. Conversely, the interaction

test for toxicity indicates a significant difference between the five drug classes compared to ET, with biological agnostic therapy showing greater toxicity [HR 4.81 (CI: 3.18 – 7.25)] compared to other treatment options.
Conclusions: This network meta-analysis showed that targeted therapies, such as PI3K, AKT, and PARP inhibitors, demonstrate significant improvement in PFS compared to ET with a positive interaction test among the different drug classes. Biological agnostic therapies offer some survival improvement but at the cost of increased toxicity as compared to other options. These findings could help tailor the most suitable treatment for each patient, determining the optimal sequence following progression to CDK4/6i for patients with HR+/HER2- ABC.

P2-08-01: Engaging breast cancer survivors in the United States in research: clinical characteristics and demographics from Susan G. Komen's ShareForCures® first cohort

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Background: Lack of diversity in research and reliance on small, siloed datasets limits the availability of real-world evidence, hampering the practice of precision medicine and optimization of treatments and care for all breast cancer patients.
To address these limitations, the Susan G. Komen® breast cancer foundation launched ShareForCures® (SFC). This online, IRB-approved, patient-centered breast cancer research registry engages breast cancer survivors and patients from diverse backgrounds as research partners. By facilitating the exchange of information between survivors, patients and researchers, SFC aims to drive innovative breast cancer research and improve outcomes. Guided by focus groups and pilot testing, SFC began enrolling adults living in the U.S. and diagnosed with breast cancer in July 2023.
Methods: We implemented a mixture of tactics to recruit eligible individuals to SFC. Emails, earned and paid media, digital advertising, social media posts, blogs, and in-person events were used to spread awareness and educate individuals about the registry. Potential participants could connect with the SFC team via contact forms, email, and phone for assistance.
To fully onboard, participants had to register by creating an account in the SFC online platform, accept the informed consent and medical records release forms, and complete the "About You" survey. We implemented tailored communication strategies to aid potential participants in completing the onboarding process.
Once fully onboarded, participants were assigned tasks to provide additional data: surveys (quality of life, family health history, social determinants of health), medical records, and a saliva sample for germline whole genome sequencing were collected.
Integrated data from the first cohort of SFC participants were analyzed to evaluate recruitment and engagement efforts and to provide descriptive statistics.

Results: As of June 26, 2024, 773 participants registered, with 399 fully onboarding (median age = 56 years [range: 27-82], 99% women, 81% non-Hispanic White) and 31 declining (92% participation, average of 33 enrolled/month). Most participants (92%) enrolled with no assistance, and only 3 withdrew after completing enrollment (99% retention). Participants were geographically dispersed across 86% of U.S. states. Fifty percent of participants were from communities historically under-represented in biomedical research. Most participants self-reported their first breast cancer diagnosis as invasive ductal carcinoma (61%), with 39% self-reporting ER+/HER2– disease and 77% reporting early stage (Stage 0, 1, or 2) disease. Participants also self-reported living with metastatic breast cancer (17%) and experiencing a recurrence (19%). At least one survey or data collection activity (saliva or medical records) was completed by 62% of participants (45% saliva samples, 35% medical records collected).
Conclusions: In the first year of SFC, we recruited breast cancer survivors and patients from across the U.S. using a variety of broad, direct-to-patient methods, and participants remained engaged with the registry through completion of surveys and data collection activities at or above expected rates. Continued focused recruitment of participants from communities historically under-represented in biomedical research is still needed. Participants' breast cancer diagnoses were consistent with national prevalence estimates, with an over-representation of metastatic breast cancer in SFC.

P2-08-02: Effectiveness of cyclin inhibitors in patients with Hispanic metastatic breast cancer: Real-world evidence from the Instituto Oncológico Nacional in Panama

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Introduction: The incorporation of cyclin-dependent kinase inhibitors (CDKi) has been accompanied by significant improvements in the prognosis of advanced breast cancer with positive hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) negative status. However, Hispanic patients are frequently underrepresented in clinical trials. Consequently, this study aims to assess the efficacy of CDKi in routine clinical practice within our specific population. Methods: We conducted a retrospective analysis of patients treated with a CDKi in combination with endocrine therapy at our institution from August 2018 to December 2022. We examined demographic, clinical, therapeutic, and prognostic variables. We used the chi-square test to compare variables and estimated survival using the Kaplan-Meier method, the log-rank test, and Cox proportions for multivariable analysis. Results: 299 patients were evaluated. The median age was 62 years (range 24-90). 225 (75%) were postmenopausal, and 73 (25%) were premenopausal. ECOG performance status was as follows: 24% ECOG 0, 68% ECOG 1, 6% ECOG 2, and 2% ECOG 3. 56% of patients received hormonal therapy with or without chemotherapy as adjuvant treatment. The most common sites of metastasis were the bone, lung, and liver. 62% of patients were treated in the first line, and 38% were treated in the second line. The overall response rate

was 30% in the first line and 20% in the second line. Grade 3 neutropenia was observed in 36% of patients, 5% experienced QT interval prolongation, and 32% required dose adjustment. At a median follow-up of 29.5 months, 227 patients experienced tumor progression, and 166 patients (92 in the first line and 74 in the second line) died. The median first-line progression-free survival was 22.8 months (95% CI 17.5-31.5); in the second line, it was 12.5 months (95% CI 9.3-15.6). The median overall survival in first-line patients was 49.7.4 months (95% CI 35.9-63.5), while in second-line patients, it was 31.7 months (95% CI 26.9-36.5). Independent factors associated with progression-free survival were age over 60 years (HR 0.64, 95% CI 0.48-0.85, p=0.002, use of iCDK in the second line (HR 1.72 95% CI 1.31-2.23, p= 0.001), as well as an ECOG score equal to or greater than 2 (HR 2.23, 95% CI 1.39-3.58, p=0.001) Conclusion The real-world progression-free survival and overall survival outcomes of our Latino patients using CDK4/6 inhibitors closely align with those observed in clinical trials.

P2-08-03: Real-World Treatment Challenges in HR+/HER2- mBC: Results From the RETRACT Survey of 150 US Oncologists

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Background: There are few published studies that evaluate the real-world barriers to the optimal management of patients with breast cancer, especially patients with metastatic disease (mBC). Clinical oncologists have a unique insight into barriers that exist both at the patient level and at the level of the health care system. The RETRACT (REal-world TReatment patterns And Considerations of Toxicity in HR+/HER2- mBC) survey was developed to explore real-world treatment patterns in patients with HR+/HER2- mBC by surveying a relatively large sample of oncologists working in academic and community centers. Topics included challenges to optimal patient treatment, providing a unique insight into this question from the provider perspective.
Methods: A survey was distributed to clinical oncologists using an internal database. The survey was accessible via an online digital platform using an invitation link and individualized credentials. Responses were collected from December 2023 through April 2024. The results were analyzed using descriptive statistics. This sub-analysis concerns the responses to the following survey question: “What are the key challenges that hinder optimal management of patients with mBC?" Respondents could choose any number of 6 pre-filled options, and an optional free text response was also available.
Results: The survey was sent to approximately 1000 oncologists working at academic or community medical centers, 187 registered on the digital platform, and 150 completed the survey, including 145 respondents who completed the item regarding key challenges to optimal patient treatment. The highest number of respondents, 118 (81%), chose treatment toxicity as a key challenge to optimal patient management. In addition, 25 (17%) respondents cited treatment efficacy and/or a lack of enough treatment options in the free-text responses. Most respondents, 84 (58%), chose financial barriers, and 12 (8%) cited

insurance barriers in the free text responses: “high copays for targeted therapies such as CDK4/6 inhibitors that require significant work on the part of pharmacy staff in order to secure financial assistance for patients, which can sometimes result in delayed start of medication.” There were also 5 (3%) respondents who cited socioeconomic barriers in the free text including “transportation” and “patients need home care for themselves, other family members, or children.” Minorities of respondents identified low health literacy among patients (32; 22%) and poor treatment adherence (30; 21%). Other free text responses included difficulty in enrolling patients onto clinical trials due to lack of availability or accessibility (5; 3%), symptoms of progression and metastasis (4; 3%), comorbid conditions (4; 3%), lack of biomarkers for treatment decisions (4; 3%), patients diagnosed at an advanced stage (2; 1%), difficulty in coordinating complex care (2; 1%), and 1 (1%) each for medical misinformation and lack of enough oncologists.
Conclusions: Oncologists who participated in the RETRACT survey identified several challenges to optimal care of patients with mBC. A large majority chose treatment toxicity, highlighting an urgent need for more tolerable treatments. In addition, education for providers can help to improve toxicity management. Financial and insurance-related challenges were frequently cited and can reduce access to existing medications due to high patient costs, increased burdens on providers’ time, and delays in treatment. These challenges are difficult to address through medical practice and are of particular concern among patient populations with fewer resources and other socioeconomic burdens such as needing childcare, lacking transportation, or lacking social support. These results show there are opportunities to improve care of patients with mBC throughout the health care pipeline.

P2-08-04: A Cohort Study of the effectiveness and tolerability of Trastuzumab Deruxtecan For The Treatment of Metastatic or Locally Advanced HER2-positive Breast Cancer.

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Background: For over 20 years, patients (pts) with human epidermal growth factor receptor-2-positive (HER2+) advanced breast cancer (BC) have been routinely treated with HER2-targeted therapies. In December 2019, the FDA approved the use of a noval antibody drug conjugate (trastuzumab deruxtecan [T-dxd]) for the treatment of pts with HER2+ progressive advanced BCs based on the impressive results of a phase 2 trial (DESTINY-B01). A subsequent phase 3 trial (DESTINY-B03) comfirmed its clinical efficacy with improvements in both progression-free and overall survivals compared to trastuzumab emtansize (T-DM1) (HR 0.33 and HR0.64; respectively). Notwithstanding, T-dxd was associated wtih increased absolute risk (AR) of clinically relevant adverse events (AEs) such as intersticial lung disease (ILD), ie all-grade AR of 15%. There is pressing need for improved knowledge on the effictiveness and tolerability of this novel agent in a real-world population.

Methods: After IRB approval, we conducted a retrospective single-institution cohort study of pts with HER2+(per ASCO/CAP guidelines) metastatic or unresectable locally advanced BC treated wtih T-dxd. We assessed the safety, tolerability, and effectiveness of T-dxd in this real-world population. Deidentified patient-, tumor- and outcome-related data including the AR of AEs of interest, and treating-physician assessments of tumor response, progressive-free and overall survivals were collected and summarized. Kaplan-Meier methods were applied to survival analyses and stratified analyses of interest were conducted. A two-sided P value < .05 was considered statistically significant.
Results: A total of 84 women and 1 man with HER2+ advanced BC treated with T-dxd between 01/2020 and 06/2024 were included. Most women were post-menopausal (75.3%), had hormone receptor + BC (58.8%) and visceral metastasis (94.1%). At the time of initiation of T-dxd, 17.6% had ECOG PS of 3-4 and 28% were smokers; median age in years was 57 (28-76). In addition, 69.4% of the pts had 1-2 prior lines of therapy for metastatic BC, 12.9% had 4 or more prior lines of therapy. Up to 44.7% of the pts had prior treatment T-DM1 and 69.4% of the pts started T-dxd at full dose regimen (5.4mg/kg every 3 wks). The estimated median progression-free survival wa 12.7 months (95% CI, 9.7-19.5) and no significant diferrences were appriciated in stratified analyses according to tumor HR expression, presence of visceral disease or prior diagnosis of CNS disease metastatic disease or prior treatment wtih T-DM1. The medial overall survival for this cohort was estimated at 28.5 months (95% CI, 17.3-NE). As many as 40% of the pts required dose reductions and fatigue was the most common reaso for dose reduction (9.4%), only 16.5% of the pts permanently discontinued T-dxd due to AEs. Common all-grade AEs were ILD (7.1%), alopecia (14.1%), diarrhea (42.4%), vomiting (49.4%) and nausea (80%), fatigue (95.3%). Grade 3 or higher AEs were observed as follows: ILD (1.2%), peripheral neuropathy (1.2%), LVEF decrease (1.2%), increased AST (2.4%), increased alkaline phosphatase (2.4%), nausea (3.5%), leukopenia (8.3%), neutropenia (10.6%), anemia (13%), and fatigue (16.5%). No grade 5 AEs were observed.
Conclusion: T-dxd showed effectiveness to treat pts with HER2+ metastatic or advanced BC in this real-world cohort of patients with pre-treated high-risk pts. Pts treated wtih T-dxd are at risk of clincally relavant but manageable AEs and require close monitoring.

P2-08-05: Pembrolizumab (P) added to Neoadjuvant Chemotherapy (NACT) for Triple Negative Breast Cancer (TNBC) in a community practice setting: Real World Evidence (RWE) for the effectiveness of the KN -522 regimen

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Background: NACT with platinum is the standard of care approach for operable TNBC. The KN-522 trial (N Engl J Med 2022; 386:556-7), demonstrated superior outcomes with the addition of P to NACT (KN-522 regimen), the pathological complete response (pCR) was

65% and significantly superior to 51% NACT alone (NEJM 2022; 386:556-67). The event-free survival at 36 months was 85% in the P-NACT group, compared to 77% for placebo-NACT ($p<0.0001$). The KN522 regimen was approved by the FDA in July 2021, with rapid uptake in community practices. However, RWE demonstrates an efficacy-effectiveness gap between reported outcomes in phase 3 clinical trials to practice, including TNBC. In the control arm of the KN-522 study, 51% of patients had a pCR rate with NACT (without P). RWE for NACT is much lower, with pCR rate of 22% (n=255) from MD Anderson Cancer Ctr, TX (JCO. 2008; 26: 1275-81), and a pCR rate of 24.5% (n=421) from the BC Cancer registry (JCO 2024; 42: 16_suppl: e23274).
Methods: Ironwood Cancer & Research Centers , Phoenix, AZ (ironwoodcrc.com) is a large oncology practice (includes medical, radiation oncologists, and breast surgeons) in Phoenix, AZ. We reviewed medical records for all operable TNBC cases from August 2021 to May 2024. Eligible patients had pathologically documented TNBC, operable disease, received NACT+P and were scheduled for breast surgery in the time period. This project involved retrospective chart reviews and was deemed IRB review exempt.
Results: Patient characteristics: N=93, all were female. Age 29-86 yrs (median 60). 40.9% were > 65 yrs and 69.9% were post-menopausal. Performance status: ECOG 0 (54.8%), ECOG 1(12.9 %) and not determined before NACT (30.1%). Nodal status was positive in 41.9% and clinical staging was 1 (5.8%), 2 (54.8%) and 3 (39.8%). NACT+P was initiated in all 93 patients, following completion of NACT, 85 had surgery (91.4%). Lumpectomy was performed in 35.5% and mastectomy in 55.9%. The pCR rate (n=85) was 51.8%. Adjuvant therapy following surgery with P was given to 77.3% of patients. Most patients who received NACT+P met relevant selection criteria similar to the KN522 study.
Conclusions: Our analysis is notable for having older patients, lower percentage of ECOG 0 and higher percentage of stage 3 disease compared to the KN522 study. Major differences in our population compared to the KN522 study are : Age > 65 yrs (40 vs 11%), ECOG 0 (55 vs 87%) and stage 3 disease (39 vs 25%) respectively. The pCR (RWE) for NACT without P in TNBC is 22-25%. To the best of our knowledge, this analysis is the first RWE for the KN522 regimen. The pCR is doubled to 51.7% in our study with P+NACT, results support the community use of the KN522 regimen for operable TNBC patients.

P2-08-06: Comparative Analysis of Pathological Outcomes and Toxicity in Hispanic vs Non-Hispanic Patients Receiving Keynote 522 Regimen: A Retrospective Single Center Experience

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Introduction: There is limited data on the comparative outcomes between Hispanic and non-Hispanic patients in real-world settings, as this patient population was underrepresented in the original trial. This retrospective real-world study examines pathological responses and treatment-related toxicities among Hispanic and non-Hispanic patients undergoing the Keynote 522 regimen for locally advanced triple-negative breast cancer.

Methods: We conducted a retrospective analysis of patients with early stage TNBC treated on KN522 regimen and undergoing surgery at our institution from July 2021 to June 2024. Data collection included demographics, clinical stage, HER2 status, node status, and treatment pattern. Outcomes measured were pathological complete response (pCR) rate, and incidence of adverse effects (AEs). Statistical analyses to compare outcomes between Hispanic and non-Hispanic patients was performed using R v4.2.
Results: The study included 59 female patients, with 66.1% being Hispanic and a median age of 52. Pathological complete response (pCR) was achieved in 56% of patients overall, with no differences based on node status or tumor size. pCR rates were similar between Hispanic (46.1%) and non-Hispanic (50.7%) patients. Non-Hispanics had a significantly higher rate of drug dose modification (40% vs 12%), but treatment delays, drug omissions, and time from last treatment to surgery were comparable. Hispanic patients experienced higher fatigue rates (89% vs 60%). Hospitalization rates and severe (≥grade 3) toxicities, including neutropenia, anemia, and neuropathy, were similar between both groups.
Conclusion: This retrospective study supplements trial data from the real world with comparable pCR rates, with no difference based on tumor size or node status. It revealed no statistical difference in pCR rates between Hispanic and non-Hispanic patients undergoing the Keynote 522 regimen for locally advanced triple-negative breast cancer. Although non-Hispanic patients required more frequent dose modifications, overall treatment delays, drug omissions, and time from last treatment to surgery were similar across groups. Notably, Hispanic patients reported higher rates of fatigue. Hospitalization and severe toxicities, such as neutropenia, anemia, and neuropathy, were comparable between the two groups. These findings highlight the need for tailored management strategies to address specific toxicity profiles and support equitable outcomes across diverse patient populations.

P2-08-07: Benefit of neoadjuvant Pertuzumab/Trastuzumab therapy on pCR in Peruvian patients with HER2-Positive Breast Cancer

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Background: Trastuzumab has significantly improved survival outcomes in HER2-positive early-stage and metastatic breast cancer. Moreover, clinical trials have shown that combining trastuzumab and pertuzumab with chemotherapy as neoadjuvant therapy further increases pCR rates in HER2-positive breast cancer (BC). In Peru, real-world evidence is currently limited because this healthcare technology was recently implemented in 2023.
Methods: A statistical analysis was conducted to evaluate the efficacy of combined Pertuzumab/Trastuzumab treatment compared to Trastuzumab alone in patients undergoing neoadjuvant therapy at the Instituto Nacional de Enfermedades Neoplásicas (INEN, Lima, Peru). Initially, we described the clinical and pathological characteristics of the patients, comparing variables between the treatment groups. Logistic regression was used

to assess the likelihood of achieving pathological complete response (pCR) across all patients. Subsequently, stratified univariate logistic regression analyses were performed within different patient subgroups to identify those who significantly benefited from the combined treatment. The statistical analysis was conducted using R programming language with the dplyr, gtsummary, and readxl libraries. A p-value less than 0.05 was considered statistically significant.
Results: A total of 109 BC HER2-positive patients were included in this analysis; 33.9% (n=37)received TCHP, while 66.1% (n=72) received TCH. Notably, pCR was significantly higher in the group that received TCHP (62.2%) than TCH (36.1%) (p=0.01). Additionally, significant differences were observed in the N stage (0.042), clinical stage (p = 0.044) and RCB (p = 0.013) between these two treatments. The overall benefit of TCHP in reaching pCR was shown in our cohort (OR=2.91, 95% CI: 1.3-6.73, p = 0.01). Significant benefits were also observed in pre-menopausal patients (OR = 4.96, 95% CI: 1.45-18.91, p = 0.01), patients with histologic grade I/II tumors (OR = 3.85, 95% CI: 1.12-14.81, p = 0.04), ER-positive (OR = 5.06, 95% CI: 1.65-16.81, p = 0.01) and PR-positive patients (OR = 6.00, 95% CI: 1.55-26.77, p = 0.01), HER2 2+ patients (OR = 2.91, 95% CI: 1.25-7.07, p = 0.01), those with node status N2/N3 (OR = 19.25, 95% CI: 2.4-425.96, p = 0.02), and clinical stage III patients (OR = 3.94, 95% CI: 1.43-11.46, p = 0.01).
Conclusions: Given the recent implementation of this healthcare technology in Peru, our findings provide early and crucial evidence supporting the adoption and widespread use of combination therapy in this patient group in the local context. Our study confirms that the combination of trastuzumab and pertuzumab with chemotherapy as neoadjuvant treatment results in a significantly higher pathological complete response rate (pCR), similar to what is observed in pivotal studies.

P2-08-08: Adjuvant Cyclin Dependent Kinase 4/6 Inhibition in Inflammatory Breast Cancer

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Background: Cyclin Dependent Kinase 4/6 inhibitors (CDKI) combined with endocrine therapy (ET) are used in the adjuvant setting for patients with high-risk hormone receptor positive (HR+), HER2 low/negative (HER2-) breast cancer. Our research showed that patients (pts) with the highly aggressive subset of breast cancer, inflammatory breast cancer (IBC) demonstrate a poor response to CDKIs in the metastatic setting, with a relatively short median time on treatment (ToT) of 7 months. However, the efficacy of CDKI in the adjuvant setting or after completion of induction therapy for de novo stage IV IBC who achieve no evidence of disease (NED) status has not been reported. Therefore, we analyzed outcomes in these settings for the first time.
Methods: We analyzed pts with stage III or de novo stage IV HR+HER2- IBC who received a CDKI combined with ET in the adjuvant/post-induction setting enrolled to the MD Anderson

Cancer Center (MDACC) IBC Registry. Two stage III pts received palbociclib based on the PALLAS clinical trial; the remainder received abemaciclib as part of standard care. In addition, 9 stage IV de novo pts took adjuvant CDKI after neoadjuvant systemic therapy and local therapy having achieved NED status (5 palbociclib, 2 ribociclib, 2 abemaciclib). ToT was calculated from the date of first CDKI treatment until date of last treatment. Patients last known to be continuing treatment are censored on date of last known treatment. Progression-free survival (PFS) was calculated from the date of first CDKi treatment until progression or death, whichever came first. Patients alive and free of progression were censored on their date of last follow-up. Median, 95% confidence interval (CI), and 36-month ToT and PFS are presented with Kaplan-Meier estimates from SAS 9.4.
Results: Thirty patients had stage III, and 9 had de novo stage IV disease. In the stage III cohort, median age was 49 years. Four pts were Hispanic or Latino, 1 patient was American Indian, and the remainder were White. One pt achieved a pathological complete response (pCR). At a median follow-up of 15.8 months (mos) from initiation of CDKI, median (95% CI) ToT was 25.6 (17.4, 39.5) mos. Reasons off treatment include toxicity (n=3), planned completion (n=2), one progression, one each for death unrelated to breast cancer, financial issues. Median PFS was 38.5 (20.3 - 38.5) months with 83% surviving progression-free at 3 years. One patient died without progression while one patient experienced recurrence of disease in the bone and lymph nodes. In the de novo stage IV cohort, median age was 44 years. Two patients were Hispanic or Latino and the remainder were White. No one achieved a pCR. At a median follow-up of 43.4 mos from CDKI start, median ToT was 25.0 (4.2, not reached [NR]) mos. Four pts came off treatment due to progression. Median PFS was 23.3 (4.2, NR) mos with a 3-year estimate of 44.4%.
Conclusions: Despite data demonstrating relatively short response to CDKI in patients among IBC pts in the metastatic setting, stage III and stage IV de novo pts (achieving NED after multidisciplinary management in the neoadjuvant setting) receiving CDKI and ET in the adjuvant setting appear to derive benefit with meaningful follow up time and low recurrence rates. Ongoing follow up is required to definitively determine benefit of CDKI in this population/different settings. Biomarkers to identify long responders are needed.

P2-08-09: Predictors of inadequate ovarian function suppression (OFS) in premenopausal women with hormone receptor (HR)-positive breast cancer receiving a gonadotropin-releasing hormone (GnRH) agonist in combination with endocrine therapy

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Background: In premenopausal women with early-stage hormone receptor (HR)-positive breast cancer and a higher risk of recurrence, the addition of ovarian function suppression (OFS) to endocrine therapy has been shown to improve disease outcomes, as demonstrated by combined analysis of the SOFT/TEXT trials. Estradiol (E2) is the primary source of estrogen in premenopausal women and is synthesized from the ovaries through indirect

control of gonadotropin-releasing hormone (GnRH). Endocrine therapy with a GnRH agonist has become a mainstay of treatment in premenopausal women for ovarian suppression. However, studies have shown that up to 25% of patients do not achieve adequate ovarian suppression within the first year of treatment. Data remains limited regarding whether monitoring of OFS should routinely be performed and is not part of current NCCN guidelines. The aim of our study was to evaluate premenopausal women in a 'real world' setting at our institution who did not achieve adequate OFS and to assess potential contributing factors.

Methods: This was a retrospective, descriptive study of premenopausal women ≥18 years of age at our institution from 2020-2022 with stage I-III HR-positive breast cancer receiving OFS in combination with endocrine therapy with either an aromatase inhibitor or tamoxifen. OFS was achieved by use of a GnRH agonist with leuprolide or goserelin. Patients who had two or more consecutive tests showing non-suppressed estradiol levels (E2 level >20 pg/mL) were considered to have inadequate ovarian suppression. Data regarding patient age at the start of treatment, BMI, receipt of neoadjuvant and/or adjuvant chemotherapy, history of prior pregnancies, history of diabetes mellitus, and choice of initial GnRH agonist were examined. The rate of change to another GnRH agonist upon ovarian suppression failure was also determined.

Results: Twenty-six patients (age range: 27-50) were identified at our institution who received either leuprolide or goserelin. Of these patients, 25 received OFS every month (96%) and 1 received OFS every three months (4%). The rate of inadequate OFS among all patients was 30.8%. The average age at the start of treatment was 40.1 years for those with adequate OFS compared to 37.9 years for those with inadequate OFS. Average BMI was 26.7 in the adequate OFS group and 30.3 in the inadequate OFS group. In the adequate OFS vs. inadequate OFS group, 17% vs. 38% had received neoadjuvant chemotherapy, and 39% vs. 63% had received adjuvant chemotherapy, respectively. 17% of patients in the adequate OFS group had a history of diabetes mellitus, compared to 25% in the inadequate OFS group. In the adequate OFS versus inadequate OFS group, 33% versus 50% had a history of at least one term pregnancy, respectively. For those who received leuprolide initially, 67% had adequate OFS. In comparison, for those who received goserelin initially, 33% had adequate OFS. Among the patients with inadequate OFS, 50% had their treatment subsequently changed to the alternative GnRH agonist.

Discussion: This study showed that inadequate OFS was detected in 31% of the patients, half of whom underwent a subsequent change in therapy to the alternative GnRH agonist. Patients who received leuprolide initially were more likely to have adequate OFS compared to those who received goserelin. A higher BMI, history of diabetes, and history of term pregnancy were found in patients with inadequate OFS. Further research is needed to establish monitoring guidelines for estradiol and the optimal degree of ovarian suppression for premenopausal patients receiving OFS.

P2-08-10: Real World Use of Ovarian Function Suppression in Women Younger Than 35 with Early Stage ER+Her2- Breast Cancer

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Background: Premenopausal women with estrogen receptor positive (ER+) human epidermal growth factor receptor 2-negative (Her2-) breast cancer may be considered for adjuvant endocrine therapy with tamoxifen alone or ovarian function suppression (OFS) with tamoxifen (tam) or an aromatase inhibitor (AI). Results from the SOFT and TEXT trials have demonstrated an improvement in disease free survival and reduction in distant recurrence with the addition of OFS, particularly in women who are found to be at high risk and received chemotherapy or in young women age < 35. For this reason, addition of OFS should be considered for women age < 35 with ER+ breast cancer. Use of OFS, however, may be associated with an increase in toxicity and requires shared decision making between patients and clinicians. There are also no clear guidelines in place for the appropriate agent to accomplish chemical OFS with a gonadotropin releasing hormone agonist (GnRHa), dosing, or monitoring. This has led to significant variability in clinical practice. The goal of this study is to evaluate real world use of OFS in women age < 35 and describe the chosen methods for OFS in a large multidisciplinary community breast oncology center.

Methods: We analyzed the medical records of premenopausal women with Stage 1-3 HR+Her2- breast cancer from January 2012 to December 2023. We then selected women who were diagnosed at age < 35 and collected additional demographic, clinicopathologic characteristics, and treatment information. The method of OFS, either with bilateral salpingoopherectomy (BSO) or use of GnRHa, was collected including agent, dosing, and duration of therapy. Patients with non-invasive breast cancer were excluded from this study.

Results: A total of 118 women were diagnosed with early-stage breast cancer at age < 35 at our institution between 2012-2023. Of these, 48 met eligibility of having an ER+Her2- breast cancer. Ages ranged from 23-34. Node-positive disease was present in 43% of women. 35 patients were Caucasian, 6 Asian, 5 African American and 2 identified as other. 33 of 48 patients elected mastectomy. Chemotherapy was administered in 79.2% (38) of patients and the majority were treated with a 3-drug regimen (68%). Adjuvant endocrine therapy was administered in 43 of 48 patients. Of those who received endocrine therapy, 25 were treated with tam alone, 10 women received OFS + tam, and 8 women received OFS + AI. When a GnRHa was utilized for OFS, goserelin was utilized in the majority (85%) and leuprolide in 15%. GnRHa frequency was most commonly q28 days with only 6 women treated with 3 month dosing. Estradiol monitoring was inconsistent but 14 women had an estradiol level checked during their treatment with a GnRHa. A BSO was completed in 9 women during their treatment course.

Conclusion: It is well established that young women are at high risk of breast cancer

recurrence. Data from the SOFT and TEXT trials support consideration of maximal hormone suppression with OFS plus tam or an AI in women under 35. Our study shows significant variability of uptake of OFS use even in this high-risk population in the community, likely due to concern for increased toxicity. Additional research regarding optimal GnRHa dose, frequency, and monitoring is needed.

P2-08-11: Real-world safety of sacituzumab govitecan in patients with advanced triple receptor-negative breast cancer in a tertiary centre in the United Kingdom

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Background: Sacituzumab Govitecan (SG) is a new treatment option in the pre-treated advanced triple negative breast cancer (aTNBC) setting. Since the regulatory approval of SG in the UK in August 2022, there remains little real-world evidence on its safety for patients with aTNBC. Its registration study documented high rates of myelosuppression in this population. In this analysis, we investigated the incidence of neutropenia in patients with aTNBC treated with SG in a large tertiary centre in the UK and its impact on patient management.
Methods: We retrospectively collected data on patients with aTNBC treated with at least one dose of SG between September 2022 and September 2023 at the Royal Marsden Hospital, London. We collected clinical data from the electronic patient records and analysed them with descriptive statistics.
Results: Thirty-nine patients were included in the analysis. Of these, 28/39 (71.7%) experienced neutropenia of any grade. This was grade 1 in 15/28 (53.5%), grade 2 in 6/28 (21.4%), grade 3 in 7/28 (25.0%) and grade 4 in 1/28 (3.6%). Four patients (14.8%) experienced febrile neutropenia requiring antibiotics and hospitalisation. In 15/28 (53.6%), neutropenia occurred prior to Day 1 of treatment and in 13/28 (46.4%) neutropenia occurred prior to Day 8. Out of the 11 patients who did not experience neutropenia, 7 (63.6%) had upfront GCSF. Out of the 28 patients experience neutropenia, 13 (46.4%) required a dose delay, 5 (17.8%) had a Day 8 dose omitted and 8 (28.6%) had a dose reduction. All patients experiencing neutropenia were prescribed secondary GCSF prophylaxis: 11 (39.3%) following the first episode of neutropenia and 3 (10.7%) after the second episode.
Conclusion: Our analysis documented a substantial rate of neutropenia in patients with aTNBC receiving SG. This is consistent with clinical trial data. Our findings support the use of primary GCSF prophylaxis to avoid preventable haematological toxicity and dose adjustments in this setting.

P2-08-12: Prediction of Chemotherapy Benefit by MammaPrintÂ® in HR+HER2- Early Stage Breast Cancer Revealed by the FLEX Registry of Real World Data

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Background: Gene expression assays play a key role in personalizing adjuvant chemotherapy (CT) treatment decisions for patients (pts) with hormone receptor (HR)-positive, HER2-negative (HR+HER2-) early stage breast cancer (EBC). The 70-gene signature, MammaPrint, determines distant recurrence risk in EBC and has demonstrated its ability to guide CT de-escalation in pts with genomically Low Risk tumors based on the MINDACT trial. In the FLEX Registry of Real World Data (RWD), we evaluated MammaPrint as a continuous variable to predict adjuvant CT benefit in HR+HER2- EBC.
Methods: This study included 1002 HR+HER2- EBC pts treated from 2017 - 2020 with endocrine therapy (ET) only or ET plus chemotherapy (ET+CT), with 5-year (yr) median follow up in the prospective, observational FLEX RWD study (NCT03053193). ET treated pts were propensity score matched to ET+CT treated pts based on menopausal status, tumor stage, and lymph node (LN) stage (n = 501 each). The MammaPrint Index (MPI) categorizes 4 risk groups: UltraLow (+1.000 to +0.356), Low (+0.355 to +0.001), High 1 (0.000 to -0.569), and High 2 (-0.570 to -1.000). The primary endpoint was Distant Recurrence Free Interval (DRFI), defined as time from diagnosis to distant recurrence or breast cancer specific death per STEEP 2.0 criteria. Kaplan Meier analysis estimated 5-yr DRFI risk as a continuous function of the MPI for each treatment group separately, with predicted 95% CIs. Cox proportional hazards model was used to test for interaction between CT treatment and clinical variables or MPI risk.
Results: Most pts were postmenopausal (73%) and LN-negative (76%), with tumor stages T1 (40.7%), T2 (46.9%), or T3 (10.8%). Tumor grades were Grade 1 (27%), Grade 2 (54.4%) or Grade 3 (19.0%). Clinical features were comparable between treatment groups, except for Grade 3, which was significantly higher in the ET+CT pts. The MammaPrint Index exhibited strong predictive accuracy for 5-yr risk of a DRFI event in both ET only ($R2 = 0.99$, $p < 0.001$) and ET+CT pts ($R2 = 0.90$, $p < 0.001$).
In ET only pts, 5-yr risk of a DRFI event significantly increased with higher MPI risk group, ranging from 0.6% to 2.2% (average [avg] 1.0%) in UltraLow, 2.2% to 4.5% (avg 3.2%) in Low, 5.6% to 14.6% (avg 10.0%) in High 1, reaching highest risk estimates of 14.8% to 24.8% (avg 19.1%) in High 2. In the ET+CT treated pts, 5-yr risk of a DRFI event ranged from 0.1% to 1.0% (avg 0.4%) in UltraLow, 1.0% to 2.1% (avg 1.5%) in Low, 2.6% to 6.4% (avg 4.4%) in High 1, and 6.5% to 10.6% (avg 8.2%) in High 2. Significant DRFI risk differences were observed between treatment groups as MPI risk increased. CT benefit ranged from 3.1% to 8.2% (avg 5.6%) in High 1, and was highest in High 2, ranging from

8.3% to 14.2% (avg 10.9%). Minimal CT benefit was observed for Low (1.7%) and UltraLow (<1.0%) Risk groups.
In a subgroup of Clinical Low Risk, MammaPrint High Risk pts (n = 209), the ET+CT group had lower risk of a DRFI event (1.8%) than the ET only group (4.5%). The multivariate Cox model demonstrated CT benefit was dependent on increasing MPI risk (HR = 0.15; 95% CI 0.02-0.97, p = 0.047). CT benefit was also significantly associated with premenopausal status, but not age, T stage or LN stage. The CT benefit was not associated with Grade 3 (HR = 0.99, 95% CI 0.10-9.76, p = 0.695).
Conclusion: In this Real World Evidence prospective, propensity score matched study, increasing CT benefit was observed with increasing MPI risk (High Risk). Patients with increasing MPI risk had significantly lower 5-yr risk of a DRFI event when treated with ET+CT than ET alone. Patients with MammaPrint indices within Low and UltraLow Risk ranges did not derive significant CT benefit, consistent with findings from MINDACT. These RWD confirm MammaPrint's comprehensive utility, as prognostic of recurrence risk and predictive of adjuvant CT benefit in HR+HER2- EBC.

P2-08-13: Effectiveness of Combined Neoadjuvant Pembrolizumab and Chemotherapy in Early Triple-Negative Breast Cancer: Real-World Evidence from Multiple Centers in Asia

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Background: Triple-negative breast cancer (TNBC) is associated with a poor prognosis. The KEYNOTE-522 study established the combination of pembrolizumab and neoadjuvant chemotherapy (NAC) as the recommended treatment for early TNBC. However, real-world evidence, especially in the Taiwanese population, is lacking. This multicenter retrospective study aimed to identify clinical characteristics and treatment variables associated with response to pembrolizumab plus NAC in early TNBC.
Methods: We retrospectively reviewed the multicenter medical records of patients with early TNBC who were treated with pembrolizumab plus NAC between May 2018 and October 2023. The primary endpoint was pathologic complete response (pCR). Kaplan-Meier curves were used to evaluate event-free survival (EFS) and overall survival (OS). Statistical analyses included two-sample t-test, chi-square test, and univariate analysis with a significance threshold of 0.05.
Results: Among the 72 patients in our study, the pCR rate was 51.4% (37/72). Median age at diagnosis was 50.5 years old (IQR 43.5–57.6), and mean body weight showed 58.1±9.4 kg. The rates of pCR for Stage II and Stage III patients were 56% and 41%, respectively. Kaplan-Meier curves for EFS according to pCR status after neoadjuvant treatment demonstrated a significant difference (log-rank test p = 0.003), although no significant difference was observed in overall survival (OS) with a median follow-up of 1.6 years (IQR 1.0–2.3). The probability of recurrence events was significantly associated with pCR (p = 0.001). In the pCR group, the recurrence rate was 0% (0/37), whereas in the non-pCR group, it was 25.7% (9/35). Additionally, optimizing neoadjuvant pembrolizumab dosage in

a 100 mg setting of patients showed a remarkable difference in pCR rate, achieving 68% (p = 0.01) with a per-weight dose exceeding 1.8 mg/kg. Only achieve four NAC cycles of anthracyclines along with cyclophosphamide were shown to be substantially linked with achieving pCR, with a rate of 88% (p = 0.023) in univariate analysis.
Conclusions: This real-world study provides valuable insights into the efficacy and tolerability of pembrolizumab in early TNBC. It highlights the importance of optimizing neoadjuvant pembrolizumab dosage to a per-weight dose exceeding 1.8 mg/kg in the 100 mg setting, which significantly improves the pCR rate and the completion of AC regimen cycles necessary for achieving pCR in Asian patients with early TNBC.

P2-08-14: Prognostic Value of Real-World Immunohistochemical Changes in Breast Cancer Following Neoadjuvant Chemotherapy

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Objectives: To evaluate the rate of change in immunohistochemistry (IHC) after neoadjuvant chemotherapy (NAC) in patients with breast cancer and how this can influence the disease-free survival (DFS) and overall survival (OS).
Methods: this was a retrospective, cohort study, that included female patients over 18 years of age, with a diagnosis of non-metastatic breast cancer undergoing NAC. Patients who did not achieve complete pathological responses were evaluated regarding the change in IHC before and after NAC. Quantitative and qualitative factors related to the changes in IHC were evaluated. Additionally, the prognosis of these patients were assessed by examining OS and DFS. The study was submitted and approved to the research ethics committee (CAAE 80127724.1.0000.5463).
Results: We included 369 patients and most of them (215/58.3%) did not change the IHC profile. The average age did not differ between the groups (P= 0.986) as well menopausal status (p = 0.112), histological type (p = 0.998), clinical staging (TNM) and Ki-67 index. Most of the IHC changes was seen in HR- (56.4%) when compared to HR+ (43.5%) p < 0.0001. HER2+ group showed the most change when compared to HER2- (p < 0.0001). It is interesting to notice that in HR+/HER2+ patients only 29.2% remained in the same profile, with 70.8% loosing HER2 expression and changing to HR+/HER2-. When looking at HR-/HER2+ we saw that 50% maintained with the same profile with 30.5% loosing HER expression and 19.5 gaining HR. The HR+/HER2- group had minor changes with 86.4% maintaining the same profile. The HR-/HER2- group showed interesting results with only 34.9% maintaining this profile and 14.1% acquiring HER2 and 50.9% gaining HR. It is interesting to note that among HR+/HER2+ patients, only 29.2% remained in the same profile, with 70.8% losing HER2 expression and changing to HR+/HER2-. In the HR-/HER2+ group, 50% maintained the same profile, while 30.5% lost HER expression and 19.5% gained HR. The HR+/HER2- group experienced minor changes, with 86.4% maintaining the same profile. The HR-/HER2- group showed notable results, with only 34.9% maintaining

this profile, 14.1% acquiring HER2, and 50.9% gaining HR. With a median follow up of 47.7 months (1.7-154) there was local-regional recurrences in 38 patients (10.3%), distant metastasis was observed in 109 patients (29.5%) and 94 (25.5%) patients died. Overall survival (OS) was 48.5 months, and disease-free survival (DFS) was 47.2 months. There was a worse prognosis in patients who experienced changes in their initial IHC profile (P=0.002) but there was no difference in local recurrence and distant metastasis. It is interesting to point out that patients that weren't HR-/HR- and became after NAC had worse prognosis (P< 0,001).
Conclusions: Our data suggests that changes in immunohistochemical profiles post-NAC is common and is associated with higher mortality rates, especially those who lost HR and HER2 underscoring the importance of IHC profiles as potential prognostic indicators in patients undergoing NAC for breast cancer.

P2-08-15: From Clinical Trials to Real-World Practice: Treatment Regimens, Pathological Complete Response Rates, and the Addition of Platinum Salts in Patients with Triple- Negative Breast Cancer

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Purpose: Comprehensive real-world data on current treatment methods for early triple-negative breast cancer (TNBC) are limited. To validate optimal treatments demonstrated in randomized controlled trials analyses of data obtained from routine clinical care are essential. This study aims to analyze the treatment outcomes of patients with early-stage TNBC using data from a large, population-based cancer registry.
Methods: This retrospective, non-interventional, population-based singlemulti-center study utilized data from TNBC patients diagnosed between January 1, 2010, and December 31, 2018, at the cancer registry Tumor Centre Regensburg. The data included demographics, pathology, treatment, recurrence, and survival, with follow-up extending to December 2023. 319 patients with TNBC who received neoadjuvant chemotherapy were identified. The outcomes measured were pathologic complete response (pCR), overall survival (OS), and recurrence-free survival (RFS).
Results: A total of 319 patients were included. Of these, 132 (41.4%) received neoadjuvant chemotherapy (NACT) with Epirubicin/Cyclophosphamide (EC) and Paclitaxel (EC-T), 74 (23.2%) received NACT with EC-T and Platinum (EC-P/T), and 22 (6.9%) received NACT with EC-P/nabPaclitaxel (EC-P/nabP). Other NACT protocols were administered to 91 (28.5%) patients. A pCR was achieved in 49.8% of NACT patients, with a rate of 37.1% in the subgroup without platinum. The addition of platinum significantly increased the pCR rate to 54.1% compared to EC-T (OR 3.476, 95% CI 1.655-7.300, p=0.001). EC-P/nabP led to a significant increase in the pCR rate to 77.3% (OR 8.767, 95% CI 2.421-31.744, p<.001).
Conclusion: This real-world evidence study demonstrates the increased use of platinum-based neoadjuvant therapies in alignment with current clinical guidelines, resulting in higher pCR rates. This trend is consistent with findings from randomized controlled trials.

Platinum salts should be a standard of care in the chemotherapy regiments for patients with TNBC.

P2-08-16: Comparative effects of Ribociclib, Palbociclib, and Abemaciclib on circulating and tumor infiltrating myeloid cells in metastatic breast cancer patients

Nikhila Kethireddy

Background: Cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) are routinely used in the treatment of advanced hormone receptor positive (HR+) breast cancer (BC) patients. CDK4/6i are primarily thought to inhibit cancer cell proliferation. Recent pre-clinical evidence has demonstrated the capacity for CDK4/6i to alter immune cell activity and composition. In BC, tumor infiltrating lymphocytes (TILs) correlate with both extended survival and response to chemotherapy. In contrast, tumor associated macrophages have been associated with chemotherapy resistance possibly due to inhibition of TIL cytolytic activity. Given the important relationship between TILs, tumor associated myeloid cells, and patient outcomes, a clearer understanding of the impact of CDK4/6i on tumor immune surveillance and systemic immune composition is needed.
Methods: Patients with metastatic HR+ BC who are starting first-line ribociclib, palbociclib, or abemaciclib (ribo/palbo/abema) plus aromatase inhibitor are actively being recruited to observe changes in tumor and systemic immune composition over the course of CDK4/6i treatment. This prospective cohort will include n=20 each ribo/palbo/abema CDK4/6i-treated patients with serial blood testing collected prior to initiation and at the time of progression, with an optional on-treatment biopsy. In parallel, a retrospective cohort will be identified from archived pre- and post-treatment tumor biopsies of patients with known clinical response to treatment (N=15-20 each ribo/palbo/abema) and will be used to validate potential immune biomarkers of response identified in the prospective cohort. Collected samples are interrogated by a combination of clinical complete blood cell counts, high-parameter, flow cytometry, multiplex immunofluorescence, and single cell RNA sequencing to assess biological changes over the course of treatment and in association with patient outcomes. Enrollment began in 2023 and is currently ongoing (NCT05244434).
Results: In samples assessed thus far, we observe notable changes in circulating immune cell abundance and composition between pre-treatment and on-treatment timepoints. White blood cell counts (WBCs) and absolute neutrophil counts (ANCs) were significantly reduced in 4/4 patients. Similarly, absolute lymphocyte counts (ALCs) and absolute monocytes counts (AMCs) were reduced in 3/4 patients. Preliminary data suggests that ANCs (53% reduction) and AMCs (55% reduction) are more affected than ALCs (21% reduction) by CDK4/6i treatment. These dynamics resulted in an average decrease in a neutrophil-to-lymphocyte ratio (NLR) of 2.2 to 1.3 from pre-treatment to C2D1. Work is ongoing to comprehensively identify circulating and tumor infiltrating immune cell

phenotype changes induced by CDK4/6i, and how these changes differ between patients treated with ribociclib, palbociclib, and abemaciclib.

P2-08-18: De-Escalating Axillary Surgery in Nigeria: a phase II Single Arm Trial Protocol

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Introduction: Breast cancer is the most common cancer and cause of cancer related mortality in Nigeria. The majority of women present with locally advanced disease. The current standard of care for these women is neoadjuvant chemotherapy (NAC) followed by modified radical mastectomy, including complete axillary lymph node dissection (ALND). Within the last decade, the de-escalation of axillary surgery has been extended to women who present with clinically node positive (cN1) disease who are converted to clinically node negative (cN0) after NAC. In this setting, a sentinel lymph node biopsy (SLNB) is performed using a dual-tracer localization technique with methylene blue and Tc-99 sulfur colloid. This is prohibitive to axillary de-escalation in many low- and middle-income countries (LMICs) where the nuclear medicine infrastructure, to support access to Tc-99 sulfur colloid lymphoscintigraphy, is limited.
To overcome the challenge posed by the dearth of Tc-99 radiocolloid in Nigeria, this study seeks to evaluate the feasibility and efficacy of pre-operative axillary ultrasound (aUS) and a single agent (methylene blue) SLNB to stage the axilla in women converted from cN1 to cN0 disease after NAC.
Methods: This is a phase II, single arm trial being conducted at Obafemi Awolowo University Teaching Hospital (OAUTH) Ile-Ife, Nigeria. Initial inclusion criteria include: women ≥ 18-70 years of age, biopsy confirmed invasive carcinoma of the breast and clinically palpable N1 disease (cN1). Eligible patients have an aUS and if abnormal nodes are present the most suspicious undergoes core biopsy and clip placement. Patients with pathology confirmed TanyN1M0 disease will undergo NAC with curative intent as per institutional standards at the discretion of the treating surgeon.
Patients with a clinical complete response (cN0) at the completion of NAC will have a pre-op aUS. At the time of the operation, 5ml of 1% methylene blue is injected in an intra-parenchymal fashion with a 5-minute message. A sentinel lymph node will be classified as blue and/or suspicious by the operating surgeon. Surgeons performing the procedure have obtained additional training in the SLNB technique. Each sentinel lymph node will be removed and labelled separately and sequentially. A completion ALND (level 1-2) will be performed as per institutional practice and the contents labelled separately. Performance of the pre-operative clinical exam, aUS, SLNB and clipped node will be determined for each eligible patient. The study as research ethics board approval from OAUTH and was registered on clinicaltrials.gov prior to study initiation (NCT06039956).
Results: As of June 2024, 37 patients have met initial inclusion criteria and have been

consented and enrolled into the study. Nine of these have completed NAC and 6 have gone on to receive surgery as per the study protocol with a methylene blue only SLNB followed by completion axillary dissection. We expected to enrol 50 patients within the first year of the study. Target enrolment is 210 patients, which assumes a pCR of 30% and a FNR of 20% with 80% power to report on performance characteristics of the study intervention with 95% confidence intervals.
Discussion: Tc-99 sulfur colloid is not readily available in many LMICs. We have designed and commenced the implementation of a protocol to study the de-escalation of axillary surgery for women who present with locally advanced, node positive breast cancer in a low-resource environment using readily available technology.

P2-08-19: Delineating The Role of Normal Cell Types In Breast Cancer (Breast Cell Atlas)

Nelly Hernandez, Ivan Marin, Margarita Riojas-Barrett, Jessica Montalvan, Bernardo Martinez-Leal, Chandandeep Nagi, Sebastian Winocour, Nicholas Navin, Alastair M. Thompson

Background: The goal for the ATLAS study is to understand normal breast tissue. By identifying all cell types and cell states in collected disease-free breast tissue and establish an unbiased reference of normal breast cells that can be used to understand tumor growth in cancer patients. Previous studies have focused intensely on mammary epithelial cells however, there is a gap in knowledge concerning the non-epithelial cell types. Both cell types affect each other for normal breast development, lactation after pregnancy, and the more important focus on our protocol contribute to tumorigenesis. Excess tissue from healthy subjects as well as subjects who are at higher risk for developing breast cancer will be taken from women undergoing standard of care procedures such as reduction mammoplasties, prophylactic bilateral mastectomies and contralateral mastectomies from the unaffected breast. Factors such as ethnicity, age and menopause can be predictors in the breast cell type and cell state composition. Significant drawbacks that create bias could arise due to the lack of diversity in a study population. Any bias must be addressed to advance our understanding of diseased breast tissue. In this study we plan to collect clinical data from patients at the time of enrollment. Data such as participant's parity, social and economic status, lactation history, known density of the breast, estrogen, progesterone and HER2 receptor status will help to provide specific answers to the correlation between underlying cell types and the patient's association with the cancer and other genomic alteration.
Methods: This is a trial in progress. Tissue has been collected prospectively from normal breast tissue removed during prophylactic mastectomy, reduction mammoplasty or delayed asymmetry correction. This study will collect contralateral and adjacent breast tissue from 150 patients along with clinical data. Once all planned tissue collections are completed, the patients will be taken off the study.
Results: The site population has an accrual of 125 female patients. 65.6% African American,

Caucasian (Non-Hispanic) 23.2%, Caucasian (Hispanic or Latino) 5.6%, Asian 1.6%, American Indian .8%, Arabic .8% and 2.4% declined to state their race. The ages range from 18-65, with most having an unknown BRCA status. Of the 125 participants only 11.5% have BRCA 1 or 2 mutation, PALB2 mutation or CDH1 positive. Gravida ranges from 0 to 10 and Parity ranges from 0 to 7.
Conclusion: Instead of focusing on historical morphological features, the new data will perform unbiased single-cell RNA and epigenomic profiling to characterize these cell types which will serve as a reference 'Human Breast Cell Atlas' to study cell types in tumors, and how they contribute to tumor progression. While also allowing us to understand how we can improve the outcomes for women of all backgrounds.

P2-08-20: ELCIN: Elacestrant in women and men with CDK4/6 inhibitor (CDK4/6i)-naïve estrogen receptor-positive (ER+), HER2-negative (HER2-) metastatic breast cancer (mBC): An open-label multicenter phase 2 study

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Background: Endocrine therapy plus a CDK4/6 inhibitor (ET+CDK4/6i) is the mainstay treatment in first-line ER+/HER2- mBC; however, a subset of patients is unable to tolerate CDK4/6i, and resistance to ET arises. Intrinsic resistance mechanisms include alterations in the PI3K/AKT/mTOR or cell cycle pathways; acquired resistance mechanisms include estrogen receptor gene alpha (ESR1)-mut developing during ET in mBC. In EMERALD, single-agent elacestrant significantly prolonged progression-free survival (PFS) vs standard-of-care (SOC) ET and was associated with a manageable safety profile in patients with ER+/HER2- advanced or mBC previously treated with ET+CDK4/6i, leading to its approval as the first clinically available oral SERD. Elacestrant significantly reduced the risk of progression or death compared with SOC ET by 30% in the overall population (HR = 0.70; 95% CI, 0.55 to 0.88; P = 0.002) and by 45% in patients with ESR1-mutated tumors (HR = 0.55; 95% CI, 0.39 to 0.77; P = 0.0005) [Bidard, 2022]. To provide an option for patients unable to receive CDK4/6i and address resistance, the ELCIN trial aims to evaluate the efficacy and safety of elacestrant in patients with ER+/HER2- mBC who received prior ET but did not receive a prior CDK4/6i in the metastatic setting.
Methods: ELCIN (NCT05596409) is a single-arm phase 2 trial. Eligible patients are women or men with ER+/HER2− mBC who received 1-2 prior ET and no prior CDK4/6i or chemotherapy in the metastatic setting. Patients must have measurable disease per RECIST v1.1 or ≥1 lytic or mainly lytic bone lesion (in patients with bone disease only), ECOG PS ≤1, and no active or newly diagnosed central nervous system metastases. Patients will receive elacestrant 345 mg (400 mg elacestrant hydrochloride) once daily. The primary endpoint is investigator-assessed progression-free survival (PFS). Secondary endpoints are objective response rate (ORR), duration of response (DoR), clinical benefit rate (CBR), overall survival (OS), patient-reported outcomes (PROs), health-related quality of life (HRQoL), and

safety. Exploratory endpoints include assessing elacestrant efficacy according to ESR1-mutation status at baseline, assessing changes in biomarkers, including allele mutation frequencies in cell-free nucleic acids (cfNAs) in blood and the relationship between efficacy endpoints and allele mutation frequencies (at baseline and post-baseline). ELCIN has a planned sample size of 60 patients; recruitment is ongoing worldwide.

P2-08-21: ELEGANT: Elacestrant versus standard endocrine therapy in women & men with node-positive, estrogen receptor-positive, HER2-negative, early breast cancer with high risk of recurrence in a global, multicenter, randomized, open-label phase 3 study

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Background: Adjuvant endocrine therapy (ET) is the standard of care (SOC) for the treatment of estrogen receptor-positive (ER+) human epidermal growth factor receptor-2 negative (HER2-) early-stage breast cancer. Despite optimized adjuvant treatments for patients with high risk of recurrence, patients continue to experience local and distant relapses, and new therapies are warranted. In the phase 3 EMERALD trial, single-agent elacestrant was evaluated vs SOC ET in ER+/HER2− metastatic breast cancer. Elacestrant significantly prolonged progression-free survival (PFS) vs SOC ET in the overall population (HR = 0.70; 95% CI, 0.55-0.88; P = 0.0018) and in patients with ESR1-mut tumors (HR = 0.55; 95% CI, 0.39-0.77; P = 0.0005) (Bidard, JCO 2022). In those pts with ≥12 months of prior ET+ CDK4/6 inhibitor (CDK4/6i) and ESR1-mut tumors, median PFS with elacestrant was 8.6 vs 1.9 months with SOC ET (Bardia, SABCS 2022). In patients with tumors without detectable ESR1-mut, a numerical difference was observed (HR = 0.86; 95% CI: 0.63-1.19). As elacestrant significantly prolonged PFS in the metastatic setting relative to endocrine monotherapy, with more pronounced activity in patients with endocrine-sensitive tumors, it is hypothesized that elacestrant should prolong invasive breast cancer-free survival (IBCFS) in the earlier adjuvant setting among patients who received prior adjuvant ET with or without a CDK4/6i. In addition, elacestrant can antagonize the estrogen receptor in tumor cells with a non-degradative antagonist function. Unlike other oral SERDs in development, elacestrant exhibits both degradative and partial agonist properties (Wardell, ERC 2015). Elacestrant could offer a new class of medication in the adjuvant setting and merits further therapeutic evaluation for patients with ER+/HER2- breast cancer with a high risk of recurrence.
Methods: ELEGANT (NCT06492616) is a global, multicenter, randomized, open-label phase 3 study designed to evaluate elacestrant compared with SOC ET (aromatase inhibitor or tamoxifen) in patients with early breast cancer and a high risk of recurrence. A total of 4,220 patients will be randomized 1:1 to continue SOC ET or switch to elacestrant therapy for a duration of 5 years. Eligible patients are women or men with ER+/HER2− node-

positive breast cancer who have completed 24 months (but not more than 60 months) of adjuvant ET and have ECOG PS ≤1. Patients who received a prior CDK4/6i or a poly ADP-ribose polymerase (PARP) inhibitor must have already completed or discontinued these treatments. Exclusion criteria include inflammatory breast cancer, any history of prior invasive breast cancer, and >6 months continuous interruption of prior SOC adjuvant ET or discontinuation of adjuvant ET >6 months prior to randomization. The primary endpoint is IBCFS. Key secondary endpoints include distant relapse-free survival (DRFS) and overall survival (OS). Additional secondary endpoints include invasive disease-free survival (IDFS), safety, and patient-reported outcomes. Exploratory endpoints include PK and biomarker analyses. Time-to-event endpoints will be reported using Kaplan-Meier estimates. Baseline demographics and other characteristics will be descriptively summarized.

P2-08-22: Effectiveness and implementation of a decision support tool to improve surgical decision-making in young women with breast cancer: The CONSYDER Study

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Background: Young women with early-stage breast cancer are increasingly choosing contralateral prophylactic mastectomy (CPM) despite longstanding data demonstrating that breast conservation is equally effective as mastectomy, with comparable overall and disease-specific survival. Life-stage specific factors (e.g., breastfeeding, body image) can make surgical decisions particularly complex for young women and young women report high levels of decisional conflict regarding the surgical decision. The use of decision support tools has been shown to improve decision quality and reduce decisional regret. To optimally support young women making decisions about breast cancer surgery, we developed CONSYDER, a web-based decision aid (DA) tailored to the unique concerns of young patients. We aimed to test the effectiveness and implementation of the DA in a multi-center, pragmatic trial.
Methods: The CONSYDER Study incorporates a Type II hybrid effectiveness-implementation, stepped-wedge design. Using a mixed-methods approach, including surveys, interviews, focus groups, and audio-recordings with patients and providers, we will test the efficacy and evaluate the implementation of the CONSYDER DA across 4 sites (Weill Cornell Medicine, Yale Cancer Center, Dana-Farber Cancer Institute, Duke Cancer Institute) over a 30-36 month period. Eligibility criteria include: female and age 18-44 years with a new diagnosis of stage 0-III unilateral breast cancer. All sites have a 6-month “run-in” period (T1) where patients will not be sent the DA. Sites will then be randomized to begin delivery of the DA as part of standard clinical care during subsequent 6-month blocks (T2, T3, T4, or T5). The primary study aims are to: 1) test the effectiveness of the DA on reducing patient-reported decisional conflict (assessed by the Decisional Conflict Scale [DCS]) prior to breast cancer surgery; 2) evaluate the implementation and mechanisms of DA use.

Secondary aims include determining the impact of the DA on decision-making preferences, breast cancer knowledge, treatment goals and preferences, anxiety, decisional regret, and self-efficacy in communication. An exploratory aim will evaluate how the DA impacts surgical choice. A sample size of 800 patients (200 per site) was selected to have approximately 80% power to detect a minimum effect size of 0.35 (clinically meaningful DCS effect sizes range 0.3 to 0.4). For the primary endopoint, we will use generalized linear models to assess if the DCS score differs between the intervention and usual care groups. An assessment for differential effects of the DA on women of different races, ethnicities, and ages (e.g., 40-44 vs. 35-39 vs. <35) will be performed by testing for a treatment by variable (e.g., race, ethnicity, age group) interaction, assuming sufficient accrual numbers for different patient subsets. As part of the implementation evaluation, we will perform semi-structured interviews with a subset of participants. Surgeon attitudes and approaches around discussing surgical options will be evaluated with a survey of surgeons prior to the implementation of the DA and at the end of the study. To complement the surgeon surveys, we will randomly sample a subset of surgeons at each site post-implementation and audio-record 4-8 consultations per surgeon to characterize how the DA may impact patient-provider communication and shared surgical decisions. We will also conduct interviews and focus groups with providers/staff involved with the delivery of the DA about perceived barriers to DA implementation at their site/practice and opportunities to improve sustainability. The CONSYDER Study opened to enrollment in March 2024. As of June 2024, 57 participants are enrolled. Clinical Trials Information: NCT06275126

P2-08-23: Evaluation of Pathological Complete Response & Immunomodulatory Effects Following Intratumoral Injection of INT230-6 Prior to Neoadjuvant Immuno-chemotherapy in Early-Stage Triple Negative Breast Cancer: A Phase II Study INVINCIBLE-4-SAKK 66/22

Ursina Zürrer-Härdi, Andreas Müller, Arianna Calcinotto, Zsuzsanna Varga, Magda Marcon, Olivier Tredan, Katrin Gysel, Katrin Eckhardt, Roch-Philippe Charles, Lewis H. Bender, Markus Joerger

Background: Triple negative breast cancer (TNBC) poses significant challenges due to its aggressiveness, high relapse rates, and increased mortality. Neoadjuvant immuno-chemotherapy (NAIC) is now a common treatment for early-stage TNBC before surgery. NAIC aims to eliminate viable cancer in the tumor, lymph nodes and possible occult distant metastases, and shrink tumors to improve surgical outcomes and prevent disease recurrence. The Keynote-522 (KN522) study revealed a 84.5% 3-year event-free survival (EFS) in early-stage TNBC patients using NAIC. The KN522 regiment also improved pathologic complete response (pCR) rates from 51.2% with neoadjuvant Chemotherapy to 64.8% with neoadjuvant immuno-chemotherapy. Larger tumors pose an increased risk for resulting in a non-pCR and breast cancer recurrence post-surgery. A new potential method to improve clinical outcome and induce immune activation pre-surgery is through novel local therapies in combination with immuno-chemotherapy that could cause increased cell

death, create personalized tumor antigens and potentially increase pCR. Arnaout et al. conducted a randomized, phase 2 neoadjuvant window-of-opportunity trial using intratumoral (IT) INT230-6, a drug product candidate comprising vinblastine (VIN), cisplatin (CIS) and a tumor dispersion and cell penetration enhancer molecule (SHAO), evaluating clinical and biological effects in patients with early-stage operable breast cancer (the INVINCIBLE trial, NCT04781725). Results in T2 to T4 tumors showed significant necrosis in74% of subjects at the time of surgery, with some patients having >95% tumor necrosis following a single IT administration of INT230-6. Gene expression analysis showed a significant difference between baseline biopsies and surgical specimens. Pathway analysis identified genes associated with TCR signaling, B-cell and T-cell activation, with increasing effects in post-treatment samples (SABCS 2023 #PS16-03).

Objectives & Endpoints: The primary objective of this study is to determine the clinical activity of IT INT230-6 in patients with early TNBC in combination with NAIC to NAIC alone. The primary endpoint is pCR in the primary tumor (ypT0/Tis) and affected lymph nodes (ypN0)

Design: This is a randomized, open-label multicenter phase 2 clinical study to determine the clinical activity, safety, and tolerability of INT230-6 in patients with early-stage, operable TNBC who undergo NAIC treatment. Key inclusion criteria include newly diagnosed, previously untreated, locally advanced non-metastatic TNBC stage cT2-4c N0-3 M0 per American Joint Committee on Cancer (AJCC) version 8. Multifocal and multicentric primary tumors are allowed, with the most advanced T stage being used for eligibility. In case of multifocal or multicentric disease, TNBC needs to be confirmed for each focus. Patients have to have measurable disease in the breast with at least one lesion with a diameter ≥2cm that is evaluable per RECIST v1.1, visible in ultrasound and injectable. Patients are either male or female with age ≥ 18 years, ECOG performance status <2, adequate bone marrow function, hepatic and renal function.
The sample size calculation for both cohorts is determined based on a single-stage phase II single-arm clinical trial design according to A'Hern. The hypothesis for the trial is as follows: Null hypothesis (H0): pCR rate ≤ 0.6, Alternative hypothesis (H1): pCR rate ≥ 0.8. The specified parameters for the sample size calculation are: Type I error: 10% (one-sided), Power: 80%. The accrual duration is expected to be 12 months, and the trial therapy per patient will be eight months. The duration of follow-up is 36 months.

P2-08-24: Exploring the correlation between Superparamagnetic Iron Oxide counts at the axilla and Ductal Carcinoma In Situ size after Primary Breast Surgery: Preliminary Study Results

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Background/Objectives: Approximately 25% of Breast Cancer in the United States is categorized as Ductal Carcinoma In Situ (DCIS). Traditionally patients undergoing mastectomy for DCIS undergo Sentinel Lymph Node Dissection (SLND) which puts the patient at risk of lymphedema. Superparamagnetic Iron Oxide (SPIO) nanoparticles have proven similar detection rate compared to Radioisotope (Technetium 99) and blue dye in detecting SLN, and avoiding unnecessary SLND. An ongoing study involving SPIO for the detection of SLN revealed that 79% of the enrolled participants to the Sentinot2 Study have avoided unnecessary SLND. This interim analysis attempts to demonstrate a positive correlation between the Magtrace (SPIO) counts obtained at the axilla after primary breast surgery, and DCIS size in the subjects that received delayed SLND. The rates of Lymphedema in the SLND group will be a secondary outcome.
Methods: Subjects with a preoperative diagnosis of DCIS, enrolled in the Sentinot2 study at Baylor College of Medicine from November 2021 to July 2024 who underwent a delayed SLND as part of their treatment. Pearson's correlation coefficient will be used to verify the correlation strength between the Magtrace counts and DCIS size.
Results: For the interim analysis, 4 subjects received delayed SLND. The average age was 57.5; average BMI was 32.15kg/m2; The average size of the largest measurement of their DCIS on mammogram prior to primary breast surgery was 24mm. The Magtrace counts obtained at the axilla after the primary breast surgery ranged from 0 to 1920, with a mean of 696. The average size of the largest measurement of their DCIS after their primary breast surgery was 23mm. The correlation between the Magtrace counts at the axilla and the DCIS size after the primary breast surgery revealed a Pearson's r of 0.958 with a p-value of 0.042. The Lymphedema rate was 0% in this group of subjects that underwent delayed SLND.
Conclusions: The present study showed a strong positive correlation between Magtrace counts at the axilla and the size of the DCIS after the primary breast surgery. We plan further data collection to determine if this result is consistent.

P2-08-25: FITWISE: Feasibility study of tirzepatide for weight loss intervention in early stage hormone receptor positive/HER2 negative breast cancer

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Background: In obesity, significant hormonal and inflammatory changes create a mitogenic microenvironment and promote tumorigenesis. Obesity at time of breast cancer (BC) diagnosis and following treatment is associated with poorer BC survival, particularly for hormone receptor positive (HR+)/human epidermal growth factor receptor 2 negative (HER2-) BC. Furthermore, weight gain is a common side-effect of the adjuvant endocrine therapy necessary to decrease the risk of recurrence. Obesity disproportionately affects

Black women 57.2% vs. 38.2% Non-Hispanic White women, with central obesity and higher adiposity being associated with higher all-cause and breast cancer-specific mortality among Black BC survivors. Previous reports have indicated that a 5% weight loss is sufficient to reverse the metabolic disarray induced by obesity. Tirzepatide, an analogue of gastric inhibitory polypeptide (GIP), and a dual GIP and GLP-1 Receptor Agonist, a first -in-class new drug, demonstrated substantial and sustained reductions in body weight (up to 21%) and reversal of cardiometabolic disarray in a 72-week weight loss clinical trial. We hypothesize that tirzepatide will result in ≥5% in body weight reduction in obese patients during the adjuvant treatment of HR+/Her2- BC.
Trial Design: This is a single arm, phase II, non-randomized trial for the weekly administration of tirzepatide for two years of weight loss intervention during the adjuvant treatment of early-stage HR+/HER2- BC. Patients fitting the obesity body mass index (BMI) criteria, BMI ≥30 kg/m2 or ≥27 kg/m2 with at least one weight related comorbidity will be eligible. This study will enroll 20 Black and 20 Non-Black BC patients at the Rutgers Cancer Institute/RWJ Barnabas Health System. This will enrich the cohort of trial participants with the Black population that has an established disparity in HR+/Her2- breast cancer and obesity related outcomes.
Specific Aims: The primary endpoint is to determine how many patients achieve a 5% or more body weight reduction at the end of study treatment with tirzepatide. Secondary endpoints include safety and tolerability and to determine the feasibility of using tirzepatide for weight loss intervention during the adjuvant treatment for HR+/Her2- BC, based on discontinuation rates. Changes in different obesity measurements such as BMI, body fat distribution via Waist/Hip Ratio (WHR) and waist circumference will be assessed. 3-year invasive disease-free survival, 3-year distant relapse-free survival will be determined and changes in obesity related metabolic markers and ctDNA will be assessed. Exploratory objectives investigating adipokines and their receptors, metabolomic pathways and immune cell metabolism will be conducted.
Statistical Methods: Our outcome of interest will be the percentage of patients with a weight loss of more than 5 percent. We plan to carry out a 5 percent level one-sided test of proportions in a single-arm study. With 40 patients, we have 80 percent power to detect an increase from 0.42 (the derived estimated proportion with weight loss exceeding 5 percent with standard therapy) to 0.617 in patients treated with tirzepatide.
Results: This is a clinical trial in progress with IRB approval (approval number: Pro2024000646) and can be found on clinicaltrials.gov. Results will be presented at a future date.
Conclusion: Successful outcomes from this trial would demonstrate that tirzepatide administration can lead to substantial weight loss and significant improvement in cardiometabolic health in obese BC patients, and may help reduce the risk of BC recurrence during the adjuvant treatment for HR+/HER2- breast cancer. The exploratory objectives may provide biological insights and potential targets for treatment.
Grant Support - Ludwig Institute for Cancer Research/The Hilton Foundation. Contact information: Coral Omene, MD/PhD. email: co273@cinj.rutgers.edu.

P2-08-26: FLEX: A Real-World Evidence, Full Transcriptome Study in 30,000 Patients with Early-Stage Breast Cancer

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Background: The advent of subtype-specific treatments, particularly hormone therapies and HER2-targeted therapies, has significantly improved survival rates and quality of life for breast cancer (BC) patients. Over the last decade genomic signatures have enabled improved classification of BC into distinct molecular subtypes, providing prognostic and/or predictive information about the metastatic potential of the tumor beyond those of clinicopathologic features. Despite marked progress, BC remains the most frequent cause of cancer death among women globally, accounting for almost 15.5% of all new female BC cases in the US. These poor clinical outcomes warrant further understanding of tumor heterogeneity and identifying genomic signatures, particularly variation within the subset of ER positive early-stage breast cancer (EBC). Pairing the full genome expression data with comprehensive clinical information enables further tumor stratification and a deeper understanding of tumor biology driving EBC. The ongoing, multi-center FLEX study (NCT03053193) seeks to enroll 30,000 patients to create a large-scale, diverse, population-based registry of full genome expression data matched with clinical data to investigate new gene expression signatures of prognostic and/or predictive value in a real-world setting. Efforts are focused on increasing clinical trial enrollment of racial/ethnic minorities and other historically underrepresented groups in clinical trials in the US to promote efficacy in outcomes and health equity. Additional objectives include supporting investigator-initiated sub-studies to address yet unresolved clinically relevant questions in EBC over 5-10 years of follow-up. Methods: FLEX is a large, multi-center, prospective, observational trial that enrolls patients (male or female) who are ≥ 18 years old with histologically proven stage I-III breast cancer. All patients with up to 3 positive lymph nodes who receive standard of care MammaPrint (70-gene signature risk of recurrence), with or without BluePrint (80-gene signature molecular subtype) on a primary breast tumor and consent to clinically annotated full transcriptome data collection are eligible for enrollment. FLEX fosters collaboration across 95 sites in the US, 2 sites in Canada, 1 site in Greece, and Israel. This initiative encourages investigator-initiated sub-studies, promoting diverse research perspectives and potentially enhancing the scope and robustness of the overall study. Within 7 years of trial initiation, FLEX total enrollment amounts to 16,980 EBC patients. To address racial/ ethnic disparities in clinical trials, a concerted effort has led to the inclusion of 1,377 Black, 530 Latin American (LA)/Hispanic, 353 AAPI, out of 14,330 EBC patients with self-reported race and ethnicity, making FLEX the most diverse study on EBC patients to date. Such diversity in FLEX sets a valuable precedent for future research aiming to improve healthcare outcomes for all groups. Currently FLEX supports 12 in-progress investigator initiated sub-studies in 2024, with over 45 abstracts accepted at congresses internationally (2018-2024), including 11 presentations and 2 poster spot-light sessions that address the underlying differences in tumor biology and clinical outcomes in Black, LA,

and AAPI populations. Overall, as FLEX continues to grow, the study strives to leverage full transcriptome data to enhance precision medicine in EBC. By identifying molecular subtypes and predictive biomarkers, the trial intends to equip clinicians with enhanced tools for tailoring treatment strategies more effectively in EBC. The FLEX trial represents a pioneering effort in integrating genomic data and clinical information on a large scale to improve outcomes and reduce disparities in EBC. Its emphasis on diversity, comprehensive data collection, and collaborative research pursuits places it at the forefront of precision medicine in EBC.

P2-08-27: ICARUS-BREAST02: Safety, Tolerability, & Anti-tumor Activity of patritumab deruxtecan (HER3-DXd) monotherapy & combinations in patients (pts) with inoperable advanced breast cancer (ABC) following progression on trastuzumab deruxtecan (T-DXd)

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Background: The human epidermal growth factor receptor 3 (HER3) is a key member of the ErbB family overexpressed in 50-70% of breast cancers. HER3 plays a critical role in promoting cancer progression, metastasis development across all breast cancer subtypes, being as such associated with poor patient outcomes. Furthermore, HER3 has a key role in driving therapeutic resistance to anti-EGFR1/2 inhibitors, endocrine therapy and PI3K-pathway targeted therapies. HER3-DXd is an investigational antibody drug conjugate containing a human anti-HER3 immunoglobulin G1 monoclonal antibody conjugated via a cleavable peptide linker to an exatecan derivative payload (topoisomerase-I inhibitor), with a drug to antibody ratio of 8:1. HER3-DXd as single agent has shown promising activity in patients with heavily pretreated ABC, across different subtypes and regardless of HER3 expression levels [Krop I et al, Journal of Clin Oncol 2023]. Given that T-DXd has become a standard therapy in pts with HER2-positive (HER2-pos) and HER2-low (IHC 1+ or IHC 2+ ISH neg) ABC and since HER3 plays a key role in resistance to anti-HER2 targeted therapies, our study aims at evaluating the efficacy and safety of HER3-DXd in pts with HER2-pos or HER2-low ABC who had disease progression on prior treatment with T-DXd. HER3-DXd will be given either as single agent or in combination with therapies that have shown to improve HER3-DXd activity in preclinical data.

Patients and Methods: ICARUS-BREAST02 is an ongoing platform study, currently composed of 2 modules: module 0 explores HER3-DXd as single agent in pts with HER2-low ABC (HER2-low cohort) in the safety run-in and dose-expansion part; module 1 evaluates the combination of HER3-DXd and olaparib in pts with HER2-pos ABC (HER2-pos cohort) in the dose-finding part and in both cohorts in the dose-expansion part. HER3-DXd

monotherapy (IV 5.6 mg/kg Q3W) or combined with olaparib (starting dose 100 mg b.i.d PO days 8-14 every 21 days) will be administered until disease progression and/or unacceptable toxicity. The study started on March 21May15thst, 2024 with part 1a (HER3-DXd monotherapy in HER2-low cohort). If none out of 3 pts or <2 out of 6 pts experience dose-limiting toxicity during the first cycle, the study will move to part 1b in which dose-escalation and de-escalation of olaparib combined with HER3-DXd will be assessed using the Bayesian optimal interval (BOIN) design.. In the dose-expansion part (part 2), we will monitor the efficacy endpoint using the Bayesian optimal phase 2 (BOP2) design, with predefined optimized stopping boundaries for interim analysis.
Eligible pts are women or men with ABC whose ECOG PS is ≤1, with ≥1 non-irradiated measurable lesion, who progressed while on T-DXd or within 2 months from its discontinuation for any reason. Prior trastuzumab and CDK4/6 inhibitors are required in HER2-pos and HER2-low cohort, respectively. Pts are excluded if they have, but not limited to, a history of interstitial lung disease/pneumonitis, clinically significant corneal disease, evidence of leptomeningeal disease or unstable/active brain metastases. The primary endpoint of part 1 is safety as measured by DLTs, frequency and severity of any adverse event (AE) graded by NCI-CTCAE v5.0; proportion of treatment discontinuations, interruptions, and dose reductions due to any AEs; frequency and severity of laboratory abnormalities defined by NCI-CTCAE v5.0; while the primary endpoint of part 2 is investigator-assessed objective response rate (ORR) as per RECIST v1.1. Mandatory collection of tumor and blood samples (including circulating tumor cells (CTC) and ctDNA) is performed pre-treatment, on treatment and at resistance for exploratory translational analyses. New combination treatment modules could be added during the study, via protocol amendment.

P2-08-28: Implementing geriatric assessment for dose Optimization of CDK 4/6-inhibitors in older Breast Cancer patients (IMPORTANT trial) – a pragmatic randomized-controlled trial

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Background: Current evidence from both randomized trials and real-world evidence studies suggests that older patients with advanced hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-) breast cancer derive clinical benefit from the addition of cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors to endocrine therapy. However, a higher risk for adverse events due to CDK4/6 inhibitors among older patients is evident, leading to a trend of initiating CDK4/6 inhibitors at a lower dose in clinical practice, though without evidence. The aim of the present randomized trial is to investigate whether lower initial dose of CDK4/6 inhibitors combined with endocrine therapy is comparable to

full dose in older patients with advanced HR+/HER2-breast cancer that are assessed as vulnerable/frail based on comprehensive geriatric assessment (CGA).
Trial Design: We designed a multi-national, open-label, pragmatic randomized controlled trial with non-inferiority approach. Patients ≥70 years old with advanced HR+/HER2-breast cancer with no curative intention, no prior treatment in an advanced setting, and suitable to receive a combination of CDK4/6 inhibitors plus endocrine therapy according to treating physician, are eligible for study inclusion. After informed consent, included patients fill out a self-administered CGA tool and categorized accordingly to fit, vulnerable, or frail, depending on the number of impaired CGA domains. Fit patients receive full dose of CDK4/6 inhibitors and endocrine therapy whereas vulnerable/frail patients are randomized to either -1 level lower CDK4/6 inhibitor dose or full dose and endocrine therapy. The randomization is stratified by type of CDK 4/6 inhibitor, type of endocrine therapy, and level of vulnerability. The treating physician decides on the choice of CDK4/6 inhibitor (abemaciclib, ribociclib, palbociclib) and endocrine therapy (aromatase inhibitors, fulvestrant). The primary endpoint is time to treatment failure whereas secondary endpoints include overall treatment utility, progression-free survival, overall survival, time to chemotherapy initiation, toxicity, quality-of-life, time to quality-of-life deterioration and cost-effectiveness. The evaluation of disease progression, toxicity assessment, and the follow-up strategy resembles clinical practice to enhance the pragmatic design approach of IMPORTANT trial. The trial also enables a hybrid decentralized approach where the initial patient visit is in-person whereas the visits for efficacy and toxicity evaluation can be performed digitally according to local practices. In total, 495 patients are planned to be included to allow 346 vulnerable/frail patients to be randomized.
Enrolment: The trial is open for recruitment since February 2024 with a recruitment period of 30 months.
Clinical Trial Registration: NCT06044623 (ClinicalTrials.gov)

P2-08-29: Lenvatinib and Pembrolizumab in Endocrine Resistant Breast Cancer with Letrozole in the Advanced setting – a Phase II Study (LaPemERLA)

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Background and Rationale: There is an unmet need to develop more effective immune-based therapies in the hormone receptor (HR)+HER2- breast cancer (BC) subtype, especially in the metastatic setting. We hypothesise that lenvatinib, a multi-kinase inhibitor with activity against vascular endothelial growth factor receptors 1-3, fibroblast growth factor receptors 1-4 and ret protooncogene, will enhance the immunogenicity in HR+ BC through normalisation of the tumor vasculature, suppression of myeloid-derived

suppressor cells and regulatory T cells, increased T-cell infiltration of the tumor, and possibly increased apoptosis. Since the estrogen receptor (ER) pathway has been implicated in the immune escape of HR+ BCs, letrozole will be required to suppress the HR pathway concurrently.

Study DesignPhase II open-label, non-randomized study will recruit up to 40 subjects with advanced ER and/or progesterone receptor positive HER2- BC which has progressed on standard endocrine therapy. The treatment will be defined in 6-weekly cycles. Lenvatinib and letrozole will be given orally daily from Day -14, while pembrolizumab will be administered intravenously during week 1 of each cycle from Cycle 1 Day 1 (C1D1), with daily lenvatinib and letrozole. Ovarian suppression will be required for premenopausal, perimenopausal and male patients. The study treatment will be continued for up to a maximum of 18 6-weekly cycles (approximately 2 years) per patient. Treatment may be discontinued upon disease progression, intolerable toxicities, or patient's withdrawal request. Subjects will undergo at least 1 mandatory biopsy – pretreatment before Day -14 lenvatinib and letrozole run-in phase. Posttreatment biopsy at the following timepoints will be optional: 1) at or up to 3 days before C1D1, 2) within 3 weeks of receiving pembrolizumab in C1, 3) post-progression.

Eligibility: Key inclusion criteria: age ≥21 years; histologically confirmed HR+/HER2- metastatic BC; disease progression after at least 1 line of palliative endocrine therapy (prior adjuvant/palliative letrozole is allowed); any number of lines or prior targeted therapy (except lenvatinib) is allowed; ≤1 line of palliative chemotherapy; measurable disease based on RECIST 1.1; availability of tumor sample and be a candidate for tumor biopsy. Key Exclusion Criteria: unstable intracranial disease; contraindications to lenvatinib or pembrolizumab.

Objectives: Primary objective: to determine the efficacy of pembrolizumab, lenvatinib and letrozole in patients with advanced HR+HER2- BC in terms of the objective response rate (ORR).

Secondary Objectives: To assess the anti-tumor activity of this combination treatment in advanced HR+HER2- BC (progression-free survival, duration of response, clinical benefit rate and overall survival) as well as safety.

Exploratory Objectives: To identify biomarkers in peripheral blood/plasma, tumor and faecal specimens that are predictive of efficacy and toxicity to this triplet combination therapy; To investigate differences in tumour characteristics in biopsies taken post treatment with lenvatinib + letrozole, and post treatment with pembrolizumab in addition to lenvatinib + letrozole versus baseline.

Statistical Method: A Simon's 2-stage design will be used to allow early stopping if there is no strong evidence suggesting that the treatment regimen is active; testing for an ORR of 30% versus 10% with one-sided alpha of 5% and power of 90%. A total of 22 evaluable patients will be analyzed in the first stage and the trial will stop early if there are fewer than 3 responders. If 3 or more patients experience objective response, trial accrual will continue to the second stage. An additional 18 patients will be accrued for a total of 40 patients.

This study is registered with clinicaltrials.gov (NCT05286437)

P2-08-30: Longitudinal measurement of serum Progranulin (PGRN/GP88) in metastatic breast cancer patients enrolled in a prospective study.

Ginette Serrero, Binbin Yue, Paula Rosenblatt, Jennifer Latteri, Amy Avergas, Kevin Hu, Ghazal Kango, Srindi Radhakrishnan, Daikripa Radhakrishnan, Katherine Tkaczuk

The ability to monitor response to therapy and disease progression in metastatic breast cancer (MBC) patients is a major step in patient management. Imaging remains the method of choice for the assessment of disease status and the monitoring of disease progression. However, this approach remains expensive, exposes patients to radiation and thus is performed every 2-3 months. During this time interval, the disease may progress on ineffective treatment with an increased risk of treatment related toxicities due to the inability to detect progression at earlier times. Circulating levels of tumor associated biomarkers such as CA15-3 and CEA are used to track the disease status of MBC as a complement to imaging. However, even though they can provide information about disease progression, the monitoring of disease status through the measurement of drivers of disease can provide a complementary approach to existing strategies used in the standard of care in order to fine-tune monitor the disease status and improve the management of MBC patients undergoing treatment.
Progranulin, also called Glycoprotein 88kDa (PGRN/GP88) is an autocrine growth factor overexpressed in breast cancer. PGRN plays a significant role in breast tumorigenesis. PGRN/GP88 overexpression in invasive ductal carcinoma (IDC) is associated with malignant phenotype, estrogen independence, increased proliferation, survival, angiogenesis, and drug resistance. High PGRN/GP88 tumor expression measured by immunohistochemistry in estrogen receptor positive IDC is an independent prognostic marker associated with increased risk of recurrence and mortality. Clinical studies have demonstrated that serum PGRN levels determined by sandwich enzyme immunoassay are elevated in breast cancer patients, compared to healthy individuals. In MBC patients, low serum PGRN levels correlate with increased overall survival. Based on these observations, the measurement of serum PGRN/GP88 levels in MBC patients can provide additional information to monitor MBC disease status. A prospective study was established to identify whether there is a statistically significant change in serum PGRN/GP88 levels associated with time to progression of breast cancer as measured by RECIST 1.1 criteria in MBC patients. Under IRB approved protocols at the University of Maryland Greenebaum Comprehensive Cancer Center and at two Baltimore Medstar Health Facilities, a total of 103 female breast cancer patients with measurable or evaluable metastatic disease will be consented and enrolled. For inclusion, patients have been re-staged within 4 weeks and will continue or begin new therapy. Currently, we have enrolled sixty-five subjects at the three facilities with blood samples collected every 2-3 months or whenever there is an event such as clinical progression. Standard laboratory assessment and radiographic imaging/staging every 2-3 months on study will complement blood collection. The samples are stored at -70C until evaluation of PGRN/GP88 using an enzyme linked immunoassay developed by A&G Pharmaceutical. The poster will provide a status on the prospective trial and provide preliminary analysis of PGRN level in correlation with disease status determined as

responder, stable or progressing based on the RECIST criteria.
This study is supported by grant R44CA210817 from the National Cancer Institute to Ginette Serrero

P2-09-01: Preliminary Findings from the Breast Cancer Combined Visualization And Characterization Tools (bCOMBAT): Low-Dose PEM and Liquid Biopsy Study

Vivianne Freitas, Oleksandr Bubon, Samira Taeb, Frederick Au, Supriya Kulkarni, Sandeep Ghai, Shayna Parker, Michael Waterston, Alla Reznik

Purpose: This study evaluates the effectiveness of low-dose Positron Emission Mammography (PEM) and liquid biopsy in detecting and characterizing breast abnormalities in high-risk patients.
Materials and Methods: Approved by the research ethics board, this prospective study involves high-risk women from the Ontario Breast Screening Program (OBSP) at the University Health Network (UHN). Participants scheduled for MRI-guided biopsy of suspicious lesions detected by standard MRI are subjected to low-dose PEM using 74 MBq of fluorine 18-labeled fluorodeoxyglucose (18F-FDG) and liquid biopsy for plasma analysis.
Preliminary Findings: Among the first high-risk 24 patients analyzed (median age 41; range 33–66) from a planned cohort of 100, four had biopsy-confirmed cancer, including two cases of ductal carcinoma in-situ (DCIS) and two of invasive cancer. Of these, low-dose PEM imaging identified three malignancies and missed one DCIS, with no false positives reported. Liquid biopsy, measuring tumor content and performing fragmentomic analysis on cell-free DNA, did not detect any cancers. Further analysis using cell-free methylated DNA immunoprecipitation and high throughput sequencing (cfMeDIP) is pending.
Conclusion: Early results indicate that low-dose PEM is more sensitive than liquid biopsy for detecting breast cancer in high-risk populations and maintains high specificity relative to MRI.

P2-09-02: Two Years Post-COVID-19: Evaluating the Impact on Brazil's Mammographic Screening Program

André Mattar, Marcelo Antonini, Denise Joffily Pereira da Costa Pinheiro, Marina Diógenes Teixeira, Andressa Gonçalves Amorim, Odair Ferraro, Francisco Pimentel Cavalcante, Felipe Zerwes, Marcelo Madeira, Eduardo de Camargo Millen, Antônio Luiz Frasson, Fabrício Palermo Brenelli, Luiz Henrique Gebrim, Ruffo Freitas-Junior

Objectives: The objective of this study was to assess the impact of the COVID-19 pandemic, after two years, on mammographic screening in Brazil evaluating BIRADS® results, breast cancer diagnosis rates, and breast cancer stage.
Study Design: This was an ecological observational study was based on retrospective data from Brazil's mammographic screening program from 2015 to 2023.

Methods: Data were obtained from the national screening database DATASUS – SISCAN (Cancer System Information) and retrieved in March 2024. Inclusion criteria comprised completeness of mammogram data (incomplete records were excluded), female participants aged 50 to 69 years, and mammograms exclusively performed for screening purposes. The study analyzed the number of mammograms conducted during the specified period, focusing on BIRADS® test results.
Results: Out of 23,851,371 mammograms performed between 2015 and 2023, 15,000,628 were included for analysis. A significant reduction of 39.6% in mammograms was observed in 2020 compared to 2019, followed by a 12.6% decrease in 2021. Notably, a substantial rise in BIRADS categories 4 and 5 examinations was seen post-pandemic. Breast cancer staging analysis revealed a shift towards more advanced stages (III and IV) diagnosed post-pandemic, suggesting potential delays in detection and diagnosis.
Conclusions: In conclusion, the study highlighted significant discrepancies in mammographic screenings and breast cancer diagnosis rates over nine years, with the pandemic reflects the significant influence on the timing and stage at diagnosis, suggesting potential delays in detection and diagnosis that resulted in later identification of more advanced disease stages.

P2-09-03: USING OF SULPHUR HEXAFLUORIDE (SF6) FOR DETECTION OF SENTINEL LYMPH NODE IN OUTPATIENT PRACTICE: PRELIMINARY RESULTS FROM AN EFFICACY AND SAFETY STUDY

Maria Sharavina

Introduction: Sulfur hexafluoride (SF6) is a contrast medium widely used in sonography to visualize lymph nodes. The use of SF6 in outpatient practice can reduce the time and improve the quality of diagnostics, which contributes to more effective treatment of patients.One of the main advantages of this contrast agent is its safety and good tolerability, which makes it preferable in clinical practice.
The purpose of this study was to evaluate the efficacy of fine-needle aspiration biopsy (TAB) of sentinel lymph nodes with sulfur hexafluoride on an outpatient basis in patients diagnosed with breast cancer but with no data on metastasized lymph nodes and to evaluate the safety and tolerability of SF6 for this procedure.
The objective of this study was to identify SF6-labeled sentinel lymph nodes at the outpatient stage and verify them with fine-needle aspiration biopsy.
Materials and method: 30 patients diagnosed with breast cancer but with no evidence of metastasis-affected lymph nodes, who were scheduled for surgical treatment as the first stage, underwent the procedure of staining axillary lymph nodes with SF6 contrast agent in the tissue around the tumor. Sonography was performed using a 7.5 MHz linear transducer to visualize the contrast stained lymph nodes. Next, TAB of the lymph nodes was performed and visualized on an ultrasound scanner. The material was sent for cytologic examination (CI). The frequency of detection of "signal" lymph nodes was further analyzed and the presence of complications was investigated.

Results: Two out of 30 patients with sulfur hexafluoride (SF6) identified metastasis-affected lymph nodes by cytologic examination, which allowed a change of treatment plan to neoadjuvant first-stage chemotherapy. None of the patients had complications related to the use of sulfur hexafluoride.
Conclusions: The use of SF6 allows for improved visualization of sentinel lymph nodes at the outpatient stage and improved diagnostic accuracy, thus allowing for minimally invasive diagnosis without hospitalization of the patient and planning more effective treatment for patients.

P2-09-04: A Randomized Study Assessing Interventions to Improve Comfort During Screening Mammography

Nikki Verma, Mazo Canola, Kenneth Kist, Joel Michalek, Lillian Franco, Pamela Otto, Virginia G. Kaklamani

Introduction: Multiple publications have demonstrated that screening mammography reduces breast cancer mortality through early detection. However, the discomfort and anxiety reported by patients constitutes a significant barrier to screening. Despite this, there is little research on strategies to reduce discomfort and anxiety associated with mammography screening and improving patient compliance with national screening recommendations. Furthermore, literature suggests lower rates of mammography in particular racial and ethnic groups. Studies considering barriers to screening mammography in racial minority groups are limited and in need of further investigation.
Objective: To determine in a double blinded randomized clinical trial with a 2x2 factorial design if the use of local analgesia and calming music can improve patient satisfaction during routine mammographic screening.
Methods: A total of 251 women who presented for mammographic screening were randomized to receive breast lidocaine gel 4% at a dose of 1200 mg on one breast and placebo gel on the other and experience calming music or no music. Patients with history of mastectomy, breast cancer or recent analgesic use were excluded. Pain was measured with a 5-point face pain scale.
Results: Of the 251 patients who were randomized between June 2017 and October 2019, 195 self-identified as Hispanic (77%). Patients were randomized to a partially balanced blinded 2×2 factorial of Music (M) [Yes, No] by Lidocaine (L) [Lidocaine, Placebo] with sample sizes: Music and Lidocaine N=79, Music and Placebo N=47, No Music and Lidocaine (N=51) and No Music and Placebo (N=74).A total of 126 patients were randomized to music [M] vs 125 to no music. Significance was not observed for all 9 outcomes (Age, Hispanic ethnicity, and 7 measures of pain). In particular, the ML interaction remained non-significant throughout, suggesting that the effect of Music did not vary with Lidocaine, and the effect of Lidocaine did not vary with Music. Pain expectation across all groups was similar. Despite this, the majority of patients in both groups expressed no reluctance for future mammograms.
Conclusion: The addition of calming music and breast lidocaine gel did not show a

statistically significant difference in the level of pain perceived by patients getting screening mammography. There seems to be a trend for less severe pain (pain score 4 and 5) when these strategies are combined. Further prospective trials with larger patient populations are needed to explore these interventions. Despite the perceived pain most patients were still willing to return for a mammogram the following year. This study was conducted in a primarily Hispanic population and lends itself to further trials and guideline development in this demographic.

P2-09-05: Evaluating the Impact of Mammographic Screening on 5-Year Breast Cancer Survival: A Propensity Score-Adjusted Retrospective Cohort Study in a Tertiary Hospital in São Paulo, Brazil

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Background: In Brazil, breast cancer (BC) is the most prevalent and deadly form of malignant neoplasia among women. Despite therapeutic advances, late detection remains a significant barrier to achieving a reduction in mortality rates comparable to those observed in developed countries. Organized mammographic screening is advocated as the most effective preventive measure for early detection of cancer in asymptomatic women. The Brazilian government recommends biennial mammography for women aged 50 to 69; however, population coverage is low, and screening is often conducted opportunistically. This situation underscores social inequalities in healthcare access among different groups of women affected by this disease, frequently resulting in poorer prognoses. This study, conducted at the Instituto do Câncer do Estado de São Paulo (ICESP), aims to estimate the 5-year overall survival based on the detection method, while controlling for potential confounding factors among asymptomatic women diagnosed with BC through mammography and those diagnosed after the onset of symptoms.
Methods: This retrospective cohort study included patients selected from ICESP's hospital-based cancer registry, admitted between Jan 1, 2016, and Dec 31, 2017, and followed until Feb 28, 2024. Patients were divided into two groups: asymptomatic women diagnosed with breast cancer through mammography (group 1 – G1) and those diagnosed after the onset of symptoms (group 2 – G2). We described the demographic, diagnostic, and therapeutic characteristics of patients in the two study groups according to the method of breast cancer detection. Five-year survival rates were reported as percentages and compared using the log-rank test and multivariate Cox regression analysis. A propensity score was employed to adjust for the effect of mammographic screening on breast cancer while adjusting for covariates previously associated with the detection method. A 95% confidence level was used in all analyses.
Results: A total of 1,812 medical records were analyzed, and 1,507 patients were included in the statistical analyses. Among these, 313 (20.8%) asymptomatic patients were

diagnosed due to abnormal mammography findings (G1), and 1,194 (79.2%) presented symptoms before screening exams (G2). Significant differences between the study groups were identified in age, race/color, religion, marital status, nulliparity, and molecular subtype. Additionally, the groups differed in histologic grade, pathologic tumor, lymph node stage, and therapies received, such as conservative surgery, adjuvant chemotherapy, and radiotherapy. Median overall survival (OS) was not reached in either group. Overall survival at the fifth year was 69.5%, with a median follow-up of 72 months. The cumulative mortality at the fifth year, according to the detection method, was 10.93% (95% CI, 7.9%-14.9%) in G1 and 34.7% (95% CI, 32%-37.4%) in G2 ($p<0.001$). The detection method was associated with a substantial 5-year mortality reduction in univariate analysis (RR=0.42, 95% CI: 0.28-0.63). However, when adjusted for sociodemographic confounders associated with the detection method (age, marital status, religion, race, and previous pregnancy) using a propensity score, the reduction in mortality attributable to mammographic screening was 13.7% (95% CI: 8.9-18.5%, $p<0.001$), and 8.1% (95% CI: 1.4%-14.8%) when further adjusted for molecular subtype, tumor size, node status, and treatment modalities. Conclusion: The method of detection significantly impacts breast cancer survival despite demographic, clinical staging, molecular subtype, and therapeutic differences between screen-detected and symptomatic cases. The observed mortality reduction was lower than that reported in experimental studies and in countries with organized screening programs. Improving access to screening and screening coverage is crucial to achieving greater reductions in breast cancer mortality.

P2-09-06: Bi-annual mammography does not detect more local recurrences than annual mammography in Asian female patients with breast cancer

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Background: According to NCCN recommendations, patients who have undergone breast-conserving surgery and radiation therapy are advised to have annual mammography 6 to 12 months after completing radiation therapy. However, mammography sensitivity is only 40-60% in Asian females[JL1] , who have a higher rate of dense breast tissue. The authors evaluated whether bi-annual mammography can detect more locoregional cancer recurrence than annual mammography in the early period after breast conserving surgery (BCS) in Asian women with breast cancer.
Methods: The data of 736 female patients who had received BCS with radiotherapy between 2011 and 2015 at Kyungpook National University Chilgok Hospital was reviewed. All patients were followed up after completing adjuvant treatments biannually for the first 3 years and annually for a further 7 years. Locoregional recurrence or distant metastasis was evaluated with blood tests, tumor markers, mammography, breast ultrasonography or MR, chest and abdominal CT scans, bone scans and PET/CT. In addition, locoregional recurrences was analyzed by examining which imaging modalities detected them.

Results: The mean age of the patients was 51.3 (SD, ±10.1) years and the average BMI was 23.6 (SD, ±0.5) kg/m2. During 10 years of follow-up, there were 47 cases (6.4%) of lococoregional recurrence, 31 cases (4.2%) of distant metastasis, and 44 cases (6.0%) of death. The organs where local recurrence occurred as single or multiple, were the ipsilateral breast (n=10, 21.3%), contralateral breast (n=20, 42.6%), and regional lymph nodes (n=18, 38.3%). There was a total of 29 cases of recurrence in the breast, with 8 cases confirmed within the first 3 years. In image surveillance performed at 6-month intervals, 5 cases (62.5%) of cumulative result were detected via mammography, and 7 cases (87.5%) via ultrasound. The incidence of recurrence showed in biannual mammography was as follows: no cumulative results at 6 and 12 months in the first year, 2 cumulative results at 18 and 24 months in the second year, and 5 cumulative results at 30 and 36 months in the third year. The results of the biannual analysis of breast cancer recurrence through mammography over the first 3 years were the same as those of the annual analysis.
Conclusion: In detecting early in-breast recurrence in Asian women with breast cancer, it was confirmed that biannual mammography did not detect more recurrences than annual mammography.

P2-09-07: Diagnostic performance of TILs-US Score and LPBC in biopsy specimens for predicting pathological complete response in patients with breast cancer

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Background: TILs-US score is a non-invasive method for predicting lymphocyte-predominant breast cancer (LPBC) in surgical specimens. The diagnostic performance of TILs-US score for predicting pathological complete response (pCR) was compared with LPBC in biopsy specimens.
Methods: TILs ≥ 50% in biopsy specimens was defined as biopsy-LPBC, and a TILs-US score ≥ 4 was categorized as TILs-US score high. Basic nomogram for pCR was developed using stepwise logistic regression based on the smallest Akaike Information Criterion, and LPBC and TILs-US score nomograms were developed by integrating biopsy-LPBC or TILs-US scores into basic nomogram. The diagnostic performance of the nomograms was compared using area under the curve (AUC), categorical net reclassification improvement (NRI), and integrated discrimination improvement (IDI).
Results: This retrospective study evaluated 118 breast cancer patients, including 33 cases (28.0%) with biopsy-LPBC, 52 cases (44.1%) with TILs-US score high, and 34 cases (28.8%) achieving pCR. The sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and AUC for predicting pCR were 0.53, 0.82, 2.96, 0.57, and 0.68, respectively, for biopsy-LPBC, and 0.76, 0.69, 2.47, 0.34, and 0.73, respectively, for TILs-US score. The LPBC nomogram showed significant improvements in categorical NRI ($p = 0.023$) and IDI ($p = 0.007$) compared to the basic nomogram, but not in AUC ($p = 0.25$). Meanwhile, the TILs-US

nomogram exhibited significant improvements in AUC ($p = 0.039$), categorical NRI ($p = 0.010$), and IDI ($p < 0.001$).
Conclusions: The TILs-US score demonstrated diagnostic performance comparable to biopsy-LPBC to predict pCR in breast cancer treated with neoadjuvant chemotherapy.

P2-09-08: Correlating Breast Density with FDG-avidity on PET Imaging

YiLi Zhao, Mary Salvatore

Purpose: It is well documented that breast density is an independent risk factor for the development of breast cancer[1]. Unlike traditional mammograms, PET-CT is uniquely positioned to analyze both the anatomy and metabolism of the breast parenchyma. FDG-avidity seen on PET-CT has been shown to correspond to the proliferation and number of tumor cells, though uptake in different types of breast cancer is variable, [2,3]. High FDG activity is not specific to malignancy, however, and can reflect increased metabolic activity in physiologic tissue like brown adipose tissue [4]. Increased metabolic activity in breast fibroglandular tissue could help explain the increased risk of malignancy in dense breasts. This study evaluates the correlation between FDG-avidity and tissue density of the breast in patients without breast malignancy.
Patients and Methods: Radiology reports for PET-CTs from Jan. 1 - Sept. 1, 2023 were extracted from a single hospital site. Ten reports were selected in each age category (groups 30-39, 40-49, 50-59) in reverse chronological order. Inclusion criteria included female gender and complete visibility of bilateral breast tissue on PET-CT. Exclusion criteria included patients with primary breast cancer or with known metastases to the breast tissue (n=7), and prior breast procedures including implants (n=1).
Each breast was scored on a scale of 1-4 on CT based on BI-RADS guidelines for breast density [5]. On attenuation-corrected PET imaging, each breast was scored on a scale of 1-4 based on the percentage of tissue with FDG-avidity.
Results and Conclusion: There was a positive correlation between breast density and FDG-avid breast tissue with correlation coefficient (cc) of 0.53 ($p<0.01$, n=30). The positive correlation was maintained within each age category, cc= 0.45 in ages 30-39 ($p=0.04$, n=10), cc= 0.75 in ages 40-49($p<0.01$, n=10), and cc= 0.67 in ages 50-59 ($p<0.01$, n=10). For most patients, the FDG-avidity of the breast correlated directly with the amount of fibroglandular tissue, though exceptions included a patient with extremely dense breast tissue (4) and minimal FDG-avidity (1) and one with scattered fibroglandular tissue (2) but high FDG-avidity (4). Given this positive correlation, increased metabolic activity in breast fibroglandular tissue may explain the association between breast density and development of breast cancer.
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P2-09-09: Image-based breast cancer risk assessment based on dense mammographic tissue subtypes

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Introduction: Cancer prediction models that incorporate mammographic breast density measurements with other clinical factors report area under the ROC curve (AUC) ranges from 0.56 to 0.62 [1]. A major challenge in exploring the links between cancer risk and breast density lies in tumor microenvironment changes that promote a breast tumorigenic environment [2, 3]. The organization of stromal and glandular breast tissue subtypes plays a crucial role in influencing tumor invasion [4]. However, there has been limited research on subtypes of mammographic dense tissue and their potential links to risk. To address this, we developed a method that can segregate mammographic dense tissue into two textural subtypes: active, which is structurally reorganizing, versus passive [5, 6]. We found that tissue restructuring associates with early tumor onset and may be detectable before radiological diagnosis [7]. On an exploratory set of longitudinal screening mammograms, we demonstrated that the composition and temporal dynamics of dense tissue subtypes may influence cancer risk [7]. Now, using 13,323 mammography screenings from the OPTIMAM mammography image database [8, 9], we present a retrospective case-control analysis demonstrating the potential of using density subtypes as predictive metrics for breast cancer at three time points prior to diagnosis.
Methods: Standard bilateral mammographic screening views acquired on Hologic's Selenia units were categorized into three timeline groups: "Time 0": screening visits no more than 1.5 years prior to diagnosis (for n=798 cases) or their last assessment episode (for n=3747 controls); "Time 3": those within 1.5 and 4.5 years (n=341 cases; n=4908 controls); and "Time 6": those within 4.5 and 7.5 years (n=97 cases; n=3429 controls). Mammograms were analyzed following the method in [5, 6] to obtain the amount of each mammographic tissue subtype: fatty tissue, passive dense tissue, or active dense tissue. A generalized linear model was developed to predict cancer vs. control status with the predictive variables consisting of the amounts of fatty, passive dense, and active dense tissue from the four mammographic views. The model is strictly image-based and does not consider any other information. Our main objective was to assess the predictive accuracy of this model for the Time 3 group through calculation of the AUC. We also assessed the model for the Time 6 and Time 0 groups. A Kolmogorov-Smirnoff (KS) test was used to test the age populations.
Results: The Time 3 patient ages between the cases and controls were from the same distribution (KS test p-value = 0.14). The predictive model yielded AUC of 0.603 for the

Time 3 group. The AUC was 0.595 and 0.614 for the Time 6 and the Time 0 groups, respectively.
Conclusion: Our model is at least comparable or outperforms models from the literature (AUC 0.56 to 0.62), but without additional factors beyond the mammographic views, which shows potential for an eventual integration into clinical models [1]. The images and data used in this publication are derived from the OPTIMAM imaging database [8]. We would like to acknowledge the OPTIMAM project team and staff at the Royal Surrey NHS Foundation Trust who developed the OPTIMAM database, and Cancer Research UK who funded the creation and maintenance of the database.
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P2-09-10: Accuracy of stereotactic vacuum-assisted breast biopsy for investigating suspicious calcifications in 2,274 patients a Public Hospital in Brazil

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Backgroud: The cost and morbidity associated with surgical biopsy for breast cancer diagnosis have led many physicians to explore less invasive alternative procedures. Recently, image-guided percutaneous core-needle biopsy has become a commonly used method for diagnosing both palpable and non-palpable breast lesions. While core-needle biopsy has high sensitivity rates for nodules it has several disadvantaged in microcalcifications because of the potential for histological underestimation. Vacuum-assisted stereotactic biopsy (VAB) was developed to address some of these limitations, particularly in cases with uncertain malignant potential in pathological finding.
Objectives: To evaluate the accuracy of vacuum-assisted stereotactic biopsy (VAB) in the investigation of non-palpable suspicious calcifications.
Methods: In a retrospective study from July 2012 to November 2023 we included 2,274 women with suspicious calcifications detected on mammography (BI-RADS 4 and 5) that had VAB performed at Hospital da Mulher, São Paulo, Brazil. Fragments were obtained and sent to anatomopathological study; a metal clip was placed on the biopsy site. Four groups were analyzed, based on the biopsy results: benign, uncertain malignant potential (UMP), Ductal Carcinoma In Situ (DCIS) and invasive carcinoma (IC).

Results: Patients median age was 56y. Pathology results on VAB were classified respectively as benign n=1,525 (67.06%), UMP lesions n=87 (3.83%), DCIS n=488 (21.46%) and IC n=174 (7.65%). Benign and UMP lesions results were clustered to form a new group (lower risk lesions) and so DCIS and malignant lesions (higher risk lesions). The sensitivity of the method was 91.7 %, specificity was 97.1%, false negative rate was 3%, positive predictive value (PPV) was 92.4%, negative predictive value (NPV) was 96.9%. Interestingly 129 patients (5,67%) with benign results were submitted to surgery and 35.66% of them the results showed IC (n=23), DCIS (n-23) or UMP (n=25).
Conclusion: VAB has a good accuracy to distinguish lower and higher risk lesions groups. It has high predictive value in both benign and malignant lesions, guiding therapeutic planning. Correlation between the anatomopathological result and the imaging is crucial since patients with negative results who were submitted to surgical investigation presented a important percentage of malignant findings or findings of uncertain potential.

P2-09-11: Improved Prediction of Axillary Lymph Node Metastasis in Early-Stage Breast Cancer Using Deep Learning on Routine Mammography

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Background: Sentinel lymph node biopsy is the standard axillary nodal staging procedure and is routinely performed. With a trend towards de-escalation of axillary surgery, recent studies indicate that prediction models incorporating imaging modalities can reassess the necessity of surgical axillary nodal staging. However, the clinical utility of MRI is constrained by accessibility, and ultrasound is highly operator-dependent. Mammography, the primary imaging modality for all breast cancer patients, has drawn little attention in nodal staging. This study aims to employ advancements in deep learning (DL) to comprehensively evaluate the potential of routine mammography for predicting nodal metastasis in preoperative clinical settings.
Methods: The study included 1,281 breast cancer patients (age: 62.7 ± 11.5 years, tumor size: 15.3 ± 8.0 mm) diagnosed between 2009 and 2017 at two hospitals in Region Skåne, Sweden. Among these patients, 378 were node-positive and 903 were node-negative. Patients diagnosed in 2017 (n=126) were assigned to the test set, while those from 2009-2016 (n=1,155) were used for model development and cross-validation by period and region (2009-2012 at site 1, 2015-2016 at site 2). Input characteristics included routine preoperative clinical data and features from core needle biopsies: age, BMI, menstrual status, mode of detection, histological grade, histopathological type, and molecular subtype. DL models were constructed in two steps. First, a vision transformer was developed to learn task-specific mammographic features through supervised learning, predicting tumor size, multifocality, lymphovascular invasion, and lymph node metastasis, using two resolutions: the region of interest (ROI) emphasizing the tumor and the full mammographic image. Next, prediction models were trained using both clinical and mammographic features.

Results: Double cross-validation on the development set showed that models using only preoperative clinical variables achieved an area under the receiver operating characteristic (ROC) curve (AUC) of 0.629 ± 0.020. Incorporating ROI-based mammographic features increased the AUC to 0.670 ± 0.018, while utilizing full images resulted in a comparable AUC of 0.669 ± 0.019. Predictive accuracy was improved by 3.4% for patients from 2009 to 2012 at site 1 and by 11.9% for patients from 2015 to 2016 at site 2. Notably, on the independent test set from 2017 at site 2, the combined model of preoperative clinical variables and full mammograms achieved an AUC of 0.784 ± 0.044, outperforming the clinical model that used postoperative tumor size and multifocality (AUC of 0.731 ± 0.051). Thus, DL applied to routine mammography enhanced the prediction of nodal metastasis by 10.6% compared to preoperative clinical models.
Conclusion: Our findings underscore that routine mammograms, particularly full images, can enhance nodal status prediction in clinical models. They have the potential to compensate for key postoperative predictors such as tumor size and multifocality, aiding in patient stratification prior to surgery. Interestingly, the added value of mammography for nodal staging varied largely across different periods and regions due to advancements in screening equipment and procedures.

P2-09-12: A Public Health Initiative To Enhance Screening Mammography (MMG) In Northeast Argentina Utilizing A Novel Thermal Screening Device & Breast Cancer Risk Assessment

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Background: Breast cancer (BC) is the leading cause of cancer mortality in Argentina, with approximately 30% of cases diagnosed at an advanced stage. MMG is the cornerstone of BC screening; however, many obstacles remain. Many women either opt out or have poor access to screening MMG. Given these challenges, changes are needed. We hypothesized that empowering primary care providers to stratify patients (pts) based on BC risk & imaging urgency would enhance MMG rates.
Methods: A public health initiative was implemented in Corrientes, Argentina—the province with the 3rd highest BC incidence in the country. The initiative incorporated the use of Celbrea® (CEL) a single-use class I breast medical device designed to detect asymmetric temperature changes, FDA and ANMAT-cleared as an additional clinical support tool for breast disease detection (86% sensitivity, 87% specificity). The program adopted a prioritization system using BC risk assessment and CEL results. Pts at high risk for breast cancer and with a significant CEL result had the highest priority and underwent MMG within ≤2 weeks. Those at high risk with a non-significant CEL result, or average risk and significant CEL result, had intermediate screening priority, with a screening MMG performed within <2 months. Pts with average breast cancer risk and non-significant CEL had the lowest screening priority, with MMG performed within ≤12 months.
Results: 10,343 pts underwent CEL use, with 712 positive tests. The median age for the entire cohort was 43 [interquartile range (IQR) 34-52]. Other baseline demographic factors

(IQR): median BMI: 28.2 (24.6-32.4); current smoker: 13%; daily alcohol intake: 16%; median age of menarche: 13 (12-14); prior childbirth: 83%; median age first childbirth: 21 (18-25); % menopause: 32%; median age of menopause: 48 (43-50); family history of breast or ovarian cancer: 38%; dense breasts: 17%. The prior MMG rate was 41.8% (67.6% in pts with MMG officially recommended [n=3,532]), while 9.2% and 20.9% had MMG in the last 12 and 24 months (14.9% and 35.3% in pts with MMG officially recommended), respectively. In June-Dec 2022, 2,437 MMGs were performed. In Jun-Dec 2023, following implementation, the number increased to 4,127 (69% increase; p=0.008).
Conclusions: Baseline BC risk assessment and Celbrea® utilization for prioritizing MMG screening, led to a significant increase in screening MMG rates. This initiative encouraged many women at higher BC risk, who had never undergone a screening MMG, to have their first screening. Further prospective follow-up is warranted.

P2-09-14:Diagnostic Accuracy assessing residual disease in breast cancer patients receiving neoadjuvant systemic therapies (NST) is comparable between contrast enhanced mammography(CEM)& breast magnetic resonance imaging(MRI)across various cancer subtypes

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Purpose: To compare the diagnostic accuracy of post-treatment contrast-enhanced mammography (CEM) to MRI in the detection of residual tumor following neoadjuvant systemic therapies (NST). Methods: This retrospective study included women with newly diagnosed breast cancer who underwent post NST imaging with either MR and/or CEM imaging from September 2014 to December 2023. Imaging findings on post-NST was compared with surgical pathology. For this study, pathological complete response (pCR) was defined as the absence of invasive cancer and/or DCIS within the breast at surgery. Radiological complete response (rCR) was defined as no residual tumor seen on post-contrast post-treatment imaging. Accuracy was further stratified by various breast cancer tumor subtypes. Sensitivity, specificity, PPV and NPV values were calculated. Results: We identified 397 unique patients with pathologically confirmed invasive breast cancer who underwent either post-treatment contrast-enhanced imaging, 338 (85%) with CEM, 313 (79%) with MR imaging, and 254 (64%) patients who had both CEM and MR. Mean age was 54 (range 25–83) years. 57% of patients were post-menopausal, 82% were white, 69% of patients had dense breasts. NST consisted of chemotherapy in 76% and endocrine therapy in 24% of patients. Tumor subtype analyses included 29% Triple Negative disease ER-/HER2-, 38% Estrogen driven (ER+/HER2-), and 33% HER2 positive disease (HER2+/ER+ 24% and HER2+/ER- 9%), 90% of tumors were clinical T1 or T2, 66% had no axillary disease. 57% of patients ultimately underwent mastectomy and 43% underwent BCT. rCR was identified in 47% (159/338) of cases by CEM and 39% (121/313) by MR. Overall, 35% (160/452) achieved pCR. Comparing CEM versus MRI for assessment of residual disease,

the sensitivity was 74% versus 81%, specificity 83% versus 71%, PPV 88% versus 82%, and NPV 65% versus 69% respectively. Specificity was higher for CEM as compared to MRI (p=0.02). NPV was lowest for ER+/HER2- subgroups for both CEM (39%) and MRI (44%). Conclusions: Diagnostic accuracy of post-treatment response on CEM imaging is comparable to that of MRI in assessing residual malignancy amongst various breast cancer tumor subtypes. Specificity (defined as the ability to correctly identify patients achieving pCR after preoperative therapy) was increased with CEM compared to MR imaging. Sensitivity (defined as ability to correctly identify residual disease (non-pCR) after preoperative therapy) is slightly better with MR than CEM.

P2-09-16: A Phase 1b/2 study of palazestrant (OP-1250) in combination with ribociclib, in patients with estrogen receptor-positive, human epidermal growth factor receptor 2-negative (ER+/HER2-), advanced or metastatic breast cancer

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Background: Aromatase inhibitors (AIs) in combination with the cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) ribociclib improve outcomes in patients (pts) with ER+/HER2- advanced or metastatic breast cancer (mBC) and is a current standard of care for first-line treatment. However, resistance eventually develops, most commonly (~50%) due to mutations in ESR1.
Palazestrant (OP-1250) is a small molecule oral complete estrogen receptor (ER) antagonist (CERAN) and selective ER degrader (SERD) that binds the ligand binding domain of ER and completely blocks ER-driven transcriptional activity of both wild-type (ESR1-wt) and mutant (ESR1-mut) ER. In a phase 1/2 monotherapy study (NCT04505826) in pts with previously treated ER+/HER2- mBC, palazestrant was well tolerated with favorable pharmacokinetics (PK) supporting once a day (qd) dosing, and antitumor activity observed in both ESR1-wt and ESR1-mut disease at the recommended phase 2 dose (RP2D) of 120 mg. In preclinical studies, palazestrant in combination with ribociclib demonstrated activity in both ESR1-wt and ESR1-mut breast cancer models.
This study evaluates the safety, PK, and antitumor activity of palazestrant in combination with ribociclib in pts with ER-positive, HER2– mBC (NCT05508906).
Methods: Pts with evaluable ER+, HER2– mBC with ≤2 prior ETs (prior CDK4/6i allowed) and ≤1 prior line of chemotherapy were included. Pts received oral palazestrant at escalating doses of 30, 60, and 120 mg qd in combination with the approved dose of oral ribociclib (600 mg qd on a 3-weeks-on, 1-week-off schedule every 4 weeks). The RP2D dose of 120 mg of palazestrant was administered in dose expansion.
Results: As of May 14, 2024, 63 pts received 30 mg (n=3), 60 mg (n= 3) or 120 mg (n=57) of palazestrant in combination with ribociclib. Forty-two pts (67%) had measurable disease and 35 (56%) had visceral disease at baseline. Forty-six pts (73%) received prior CDK4/6i.

Among them, 34 (74%) pts received one prior treatment and 12 (26%) received two prior treatments with CDK4/6i ; 34 (74%) pts received prior palbociclib, 11 (24%) prior abemaciclib and 8 (17%) prior ribociclib. Fifteen pts (24%) did not receive any prior treatment in the metastatic setting[LNU1] [MB2] [MS3] . Mutations in ESR1 were present at baseline in 17 pts (29%).
At data cutoff, [MS4] [SK5] [MS6] [MS7] 39 pts (62%) remain on treatment. No dose-limiting toxicities were observed. The most common treatment-related AEs (TRAEs) across all dose levels were neutropenia (78%), nausea (65%), fatigue (46%), decreased WBC (40%), diarrhea (35%), and anemia (32%). The most common grade 3 and 4 TRAE was neutropenia (36% and 10%, respectively). Anti-tumor activity including partial responses and prolonged disease stabilization were observed with the longest treatment duration of 12 months and ongoing. Clinical benefit rates (CBR) were 79% among all CBR-eligible pts (n=29) and 78% among the CBR-eligible patients with prior CDK4/6i treatment (n=23). No meaningful effect of palazestrant on ribociclib PK or ribociclib on palazestrant PK were observed, consistent with previously reported data.
Conclusions: Palazestrant in combination with ribociclib was well tolerated and safety was consistent with the known profiles of each drug and similar to combinations of ribociclib with ET. Encouraging preliminary efficacy from the combined agents, including in pts with prior CDK4/6i treatment was observed. Updated data will be presented. Findings from this study support further clinical development of palazestrant in combination with ribociclib for the first line treatment of ER+/HER2–mBC.

P2-09-17: Precision medicine based on similarity network fusion (SNF) subtypes of multi-omics in CDK4/6 inhibitor failed HR+/ HER2- advanced breast cancer: the Linux trial

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Background: Endocrine therapy combined with CDK4/6 inhibitor represents the first-line standard treatment for HR+/HER2- metastatic breast cancer. However, there is no well-defined standard of care following resistance to CDK4/6 inhibitors. In previous study, we innovatively classified HR+/HER2- breast cancers into four subtypes using large-scale multi-omics data and similarity network fusion (SNF) (Nat Genet. 2023) : SNF1 for canonical luminal, SNF2 for immunogenic, SNF3 for proliferative and SNF4 for receptor tyrosine kinase (RTK)-driven. This platform trial aimed to evaluate the efficacy and safety of precision therapy based on SNF subtyping in patients with HR+/HER2- metastatic breast cancer who have failed CDK4/6 inhibitors treatment .

Methods: The Linux trial was an ongoing, multi-center, open-label, randomized controlled phase II platform trial utilizing a Bayesian Optimal Interval design. Eligible participants had histologically confirmed HR+/HER2- breast cancer that had progressed with a CDK4/6 inhibitors treatment for metastatic disease. Participants were categorized into four SNF subtypes and randomly assigned (2:1) to receive either subtyping-based precision therapy or physician's choice of chemotherapy (TPC) . The subtyping-based precision therapies included: everolimus (10 mg orally daily) + fulvestrant for SNF1, camrelizumab (200 mg intravenously on days 1 and 15) and famitinib (10 mg orally daily) + TPC for SNF2, fuzuloparib (100 mg orally bid) + TPC for SNF3, and apatinib (250mg orally daily) + TPC for SNF4. The primary endpoint was the objective response rate (ORR) for precision treatment group versus the control group in the intention-to-treat (ITT) population. Safety was analyzed in all patients who received at least one dose of the study drugs and had safety records.
Results: Between Jan 16, 2023 and May 23, 2024, 110 female participants were enrolled and randomly assigned to the subtyping-based group (n=70) or control group (n=40), with a median of two previous lines of therapy (range, 1-6). At the data cutoff, the median follow-up was 9.1 months. The ORR was 48.6% (34/70, 95% CI: 36.6%–60.6%) in the subtyping-based group compared to 17.5% (7/40, 95% CI: 5.2%–29.8%) in the control group in ITT patients. The subgroup ORRs of subtyping-based groups vs. control groups were 10.0% vs. 10.0% for SNF1, 70.0% vs. 30.0% for SNF2, 30.0% vs. 20.0% for SNF3, 65.0% vs. 10.0% for SNF4. Treatment-related adverse events were well managed. Grade 3-4 treatment-related adverse events occurred in 24 participants (34.3%) in the subtyping-based group, compared to 11 participants (27.5%) in the control group. No treatment-related deaths were reported in either group.
Conclusions: Our findings highlight the potential clinical benefits of SNF subtyping guided precision medicine in patients with HR+/HER2- breast cancer, suggesting a direction for further clinical investigation. Phase III randomized clinical trials for SNF2 and SNF4 assessing the efficacy of SNF subtyping-based strategies are now underway. (Linux, ClinicalTrials.gov, NCT05594095)

P2-09-18: Comparison of Suboptimal vs. Adequate Ovarian Suppression during Adjuvant Endocrine Therapy for Premenopausal Women with Breast Cancer: An Exploratory Analysis of the PREFER and GIM 23 Studies

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Background: The combination of LHRH analog (LHRHa) and aromatase inhibitors (AI) represents the standard adjuvant endocrine therapy (ET) for premenopausal women at intermediate and high risk of relapse. However, complete ovarian suppression may not be

achieved through LHRHa, and international guidelines provide different indications on how to monitor these patients and how often to evaluate hormonal status.

Methods: PREFER (NCT02895165) and GIM 23-POSTER (NCT05730647) are two Italian prospective, observational studies enrolling premenopausal women eligible to receive (neo)adjuvant chemotherapy and/or adjuvant ET. We conducted an exploratory analysis to investigate which factors were associated with suboptimal ovarian suppression during LHRHa treatment. We divided the enrolled patients into two groups: patients with suboptimal ovarian suppression (non-suppressed group) and patients with adequate ovarian suppression (suppressed group), based on estradiol levels (higher than 25.1 ng/L for non-suppressed) or resumption of menstruation at least 3 months after the start of ET plus LHRHa. Clinical features, treatment type and outcomes were compared between the two groups. Kaplan Meier method was used to estimate the percentage of patients with suboptimal suppression and Cox models were used to explore possible related factors.

Results: As of June 2024, out of 1863 patients registered (827 in the PREFER and 1036 in the GIM 23), all subjects enrolled in the coordinating center (n=545) were included in the present analysis. Median follow-up was 44.4 months (interquartile range [IQR] 20.6-84). Among the patients undergoing adjuvant ET (n=343), 202 received LHRHa plus AI, and 141 LHRHa plus tamoxifen: 315 have been included in the suppressed group, while 28 in the non-suppressed group. Median age at diagnosis was 38 years (IQR 33-44) in the non-suppressed group compared to 39 years (IQR 36-43) in the suppressed group. Clinical characteristics, including age, BMI, baseline FSH, and estradiol levels, did not correlate with suboptimal ovarian suppression. Regarding treatment, no significant differences in developing suboptimal ovarian suppression were found between those who received LHRHa during chemotherapy, those who did not receive LHRHa during chemotherapy, and those who were not administered chemotherapy. On the contrary, the use of AI was associated with an increased risk of suboptimal ovarian suppression: HR 12.83 (95% CI 3.01-54.65; p=0.001). The proportion of patients not-suppressed was 5.1% (95% CI 3.2-8.2) in the first year of LHRHa treatment and 10.5% (95% CI 7.1-15.4) after five years.

Conclusions: Among premenopausal women receiving LHRHa as part of adjuvant ET, approximately 10% did not achieve complete ovarian suppression. Since no baseline clinical or treatment features seem to be associated with suboptimal ovarian suppression, except for the combination with AI, all patients receiving this treatment should perform serial monitoring of hormonal profile throughout the years of adjuvant ET to identify those who are not adequately suppressed.

P2-09-19: Efficacy of cyclin-dependent kinase 4/6 inhibitor + endocrine therapy in patients w/ hormone receptor-positive, HER2-negative advanced or metastatic breast cancer w/ liver or lung only metastases: a US Food & Drug Administration pooled analysis

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Background: The standard of care treatment for patients (pts) with hormone receptor-positive (HR+), HER2-negative metastatic breast cancer is a CDK 4/6 inhibitor (CDKI, abemaciclib, palbociclib, ribociclib) added to endocrine therapy (ET). Patients with liver or lung metastases may have a worse prognosis and derive different amounts of benefit from the addition of a CDKI. This pooled analysis examines whether patients with only lung (LuOM) or liver (LiOM) metastases may respond differently to CDKI-based treatment.
Methodology: We pooled individual patient data from 7 randomized trials of CDKI+ET for patients with HR+, HER2-negative MBC submitted to the FDA before January 1, 2024, in support of marketing applications. The primary endpoint was investigator-assessed progression-free survival (PFS), and overall survival (OS) was a key secondary endpoint in all 7 trials. The analyses included all pts who received at least 1 dose of CDKI/placebo (PBO) with ET (aromatase inhibitor [AI] anastrozole or letrozole, or fulvestrant [F]). Using descriptive statistics, we performed a prespecified exploratory subgroup analyses in patients with LiOM or LuOM. Pts with bone-only disease, pleural lesions/effusion, ascites, other visceral metastasis, only nodal disease, and both lung and liver metastases were excluded. Analyses were performed in subgroups of pts treated with CDKI+AI only and pts with CDKI+F only. OS analyses were only performed on pts treated with CDKI+F based on data available at the time of this analysis. Median (med) PFS and OS were estimated using Kaplan-Meier (K-M) methods. Hazard ratios (HR) with 95% confidence intervals (CIs) for PFS and OS were estimated using Cox regression models. for PFS and OS were estimated using Cox regression models.
Findings: Seven trials were used for PFS analyses (n=4200) and 1582 pts met inclusion criteria for the LiOM (n=651) and LuOM (n=931). Key baseline demographic and prognostic factors appeared similar between the subgroups. Accuracy of site of metastases and treatment allocation were limited to information captured in trials and datasets.
LuOM PFS: 389 pts received CDKI+AI, 248 pts received PBO+AI, 197 pts received CDKI+F, and 97 pts received PBO+F. Med PFS was 25.3 mo (21.6-28.1) for CDKI+ET vs 15.2 mo (13.5-19.2) for PBO+ET (HR 0.64, 95% CI 0.53-0.78). Med PFS for CDKI+AI was 27.8 mo (23.1-31.1) vs 16.7 mo (13.8-24.2) for PBO+AI (HR 0.60, 95% CI 0.47-0.76). Med PFS for CDKI+F was 19.6 mo (13.8-not estimable [NE]) vs 14 mo (11.1-8.7) for PBO+F (HR 0.72, 95% CI 0.51-1.00).
LiOM PFS: 142 pts received CDKI+AI, 141 pts received PBO+AI, 234 pts received CDKI+F, and 134 pts received PBO+F. Med PFS was 11.2 mo (9.4-13.1) for CDKI+ET vs 5.6 mo (3.6-7.0) for PBO+ET (HR 0.59, 95% CI 0.49-0.72). Med PFS for CDKI+AI was 13.1 mo (10.4-14.9)

vs 9.4 mo (5.6-11.2) for PBO+AI (HR 0.63, 95% CI 0.47-0.84). Med PFS for CDKI+F was 9.4 mo (7.6 -12.9) vs 3.4 mo (2.0-5.4) for PBO+F (HR 0.51, 95% CI 0.40-0.65).
Three trials were used for OS analyses (n=1948). CDKI+F OS (n=662): 431 pts received CDKI+F (197 LuOM and 234 LiOM) and 231 pts received PBO+F (97 LuOM and 134 LiOM). In LiOM, med OS was 31.8 mo (28.0-36.5) for CDKI+F vs 25.3 mo (21.9-31.8) for PBO+F (HR 0.77, 95% CI 0.59-1.01). In LuOM, med OS was 48.2 mo (41.5- NE) for CDKI+F vs 51.6 mo (36.3-NE) for PBO+F (HR 0.90, 95% CI 0.61-1.31), although these results should be interpreted with caution due to the high level of censoring after 3 years and limited follow up. KM curve of LuOM suggested non-proportional hazards; a restricted mean survival time analysis showed a difference at 54.4 mo of 0.98 mo (SE 2.2).
Conclusion: Prior results show that pts with liver/lung metastases benefit from the addition of CDKI to ET. This pooled analysis suggests there may be differences in amount of benefit with adding CDKI to ET based on the site of the metastasis and the choice of ET. Further research is needed to understand which subgroup of pts with lung metastases may benefit more or less from adding CDKI to ET.

P2-09-20: Impact of body mass index (BMI) on the safety and efficacy of ribociclib (RIB) in patients (pts) with HR+/HER2- advanced breast cancer (ABC): pooled analysis of the MONALEESA (ML)-2, -3, and -7 trials

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Background: Studies evaluating the relationship between BMI and outcomes in breast cancer have had conflicting results. While some studies have found obesity to be associated with lower efficacy in early breast cancer, the same relationship has not been consistently found in ABC. To date, few studies have examined the effect of BMI on outcomes in pts receiving CDK4/6 inhibitors (CDK4/6i) + endocrine therapy (ET), which is the recommended standard of care in first-line HR+/HER2− ABC. The individual ML trials have shown consistent benefit with RIB + ET in pts with HR+/HER2− ABC. This pooled analysis examined the effect of BMI on the efficacy and safety of first-line RIB + ET in pts with HR+/HER2− ABC in the ML trials.
Methods: ML-2, -3 and -7 are randomized, phase 3, placebo (PBO)-controlled studies that investigated RIB (600 mg) + ET (letrozole, fulvestrant, or anastrozole) in pts with HR+/HER2− ABC. Pts who received tamoxifen in ML-7 or had early relapse (≤12 months after [neo]adjuvant ET) were excluded from this analysis. Given the similar efficacy across BMI subgroups (<25, 25 to <30, and ≥30) and the small number of pts who were underweight, pts were categorized into 2 BMI groups for this analysis: <25 (underweight + normal weight) and ≥25 (overweight + obese). Progression-free survival (PFS) and overall survival (OS) between treatment arms stratified by study and liver/lung metastases were evaluated by Kaplan-Meier methods. Safety and tolerability outcomes included the frequency of adverse events (AEs), dose reductions, and dose interruptions.

Results: Of the 1190 pts (RIB, n = 655; PBO, n = 535), 484 (41%) had a BMI <25 (underweight 6%; normal weight 94%), and 706 (59%) had a BMI ≥25 (overweight 55%; obese 45%). Median PFS was longer with RIB vs PBO in both BMI groups (BMI <25: 30.1 vs 15.0 mo; HR 0.56; 95% CI 0.44-0.70 and BMI ≥25: 35.7 vs 19.4 mo; HR 0.56; 95% CI 0.47-0.68). Longer median OS was observed with RIB vs PBO in both BMI groups (BMI <25: 63.4 vs 51.4 mo; HR 0.77; 95% CI 0.60-0.98 and BMI ≥25: 73.9 vs 57.6 mo; HR 0.70; 95% CI 0.57-0.87). In the RIB arm, fewer pts received subsequent chemotherapy in the BMI ≥25 group vs BMI <25 (26% vs 33%), while more pts received subsequent targeted therapy (including CDK4/6i) in the BMI ≥25 group vs BMI <25 (30% vs 25%). In the RIB arm, lower rates of all-grade (G) and G3/4 neutropenia were observed in pts with BMI ≥25 vs <25 (all grade 71% vs 85%; G3/4 58% vs 76%); however, the rates of hepatobiliary toxicity (all grade 30% vs 29%; G3/4 15% vs 12%) and QT interval prolongation (all grade 10% vs 11%; G3/4 4% vs 4%) were similar between BMI groups. In the RIB arm, dose reductions (41% vs 55%) and dose interruptions (71% vs 80%) due to AEs were less frequent in the BMI ≥25 group vs BMI <25, and a longer median time to first dose reduction (24 vs 9.5 mo) and time to drug discontinuation (25.9 vs 19.9 mo) were observed in pts in the BMI ≥25 group vs BMI <25.
Conclusion: This pooled analysis demonstrated that RIB + ET vs PBO + ET improved PFS and OS in pts with HR+/HER2– ABC, regardless of BMI. Pts with BMI ≥25 experienced a lower incidence and severity of neutropenia, less frequent dose reductions and interruptions, and longer time to first dose reduction and drug discontinuation vs pts with BMI <25. This study provides additional evidence for the benefit of RIB + ET in pts with HR+/HER2– ABC across BMI categories.

P2-09-21: Low-level Aurora kinase A (AURKA) amplification as a novel personalized biomarker of CDK4/6 inhibitor (CDK4/6i) resistance in patients with hormone-receptor positive (HR+) metastatic breast cancer

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Background: CDK4/6i have established roles in treating patients (pts) with high-risk early-stage and metastatic HR+/HER2- breast cancer (HR+ MBC). Understanding the genomic and molecular factors that dictate CDK4/6i response is a critical area of research. Low-level copy number gains in the mitotic spindle regulator AURKA have been implicated in promoting CDK4/6i resistance via whole-exome sequencing of tumor samples, and constitutive overexpression provokes resistance in vitro [Wander et al. Cancer Discov 2020]. Developing a clinical biomarker of AURKA copy number gain poses unique challenges given the generally low-level AURKA gain observed in resistant samples. Validation of a reliable assay to detect AURKA-dependent CDK4/6i resistance in the clinic may enable personalized use of emerging AURKA inhibitors such as alisertib, which is currently being evaluated for this indication (ClinicalTrials.gov identifier NCT06369285).
Methods: De-identified clinical and genomic records of pts with HR+ MBC in the Tempus

database whose tumors were sequenced using xT (DNA) and xR (RNA) were investigated. Tempus xT is a targeted, tumor–normal matched DNA panel that detects single-nucleotide variants, insertions and/or deletions, and copy number variants in 648 genes. Tempus xR is a whole-exome capture, next-generation sequencing assay that identifies transcriptional evidence of chromosomal rearrangements resulting in expression of fusion RNA species. Copy number analyses were performed with a proprietary algorithm assessing tumor purity, ploidy, and B-allele frequencies to estimate copy numbers and minor allele counts. RNA-based analyses classified low and high expressors based on median gene expression values. Real-world progression-free survival (rwPFS) was the time from start of CDK46/i to first recorded disease progression (determined by the primary treating oncologist), death from any cause, or initiation of a subsequent treatment line. Survival analyses were right-censored at 5 years.
Results: 848 pts with HR+ MBC with DNA sequencing prior to CDK4/6i were identified. Common pathogenic genomic alterations included PIK3CA (SNV: 43.7%; CNV: 2.4%) and ESR1 (SNV: 12.2%; CNV: 6%; fusion: 1.8%). 4.1% of biopsy samples harbored RB1 alterations. AURKA copy number gains were identified in 15.0% of pts, with most demonstrating low-level amplification (copy number 4–7: 13.8%; copy number >7: 1.2%). Of note, these low-level amplifications would not routinely be reported by available sequencing platforms in clinical settings. Linear regression modeling showed that AURKA copy number correlated with RNA expression (p-value <0.001). Overall, 713 pts with DNA sequencing prior to CDK4/6i exposure also had rwPFS information. Those whose tumors harbored high- or low-level AURKA amplifications (n=116) had inferior rwPFS on CDK4/6i (9.9 vs 17 months for no AURKA amplification (n=597; p=0.00032).
Conclusions: In this large, real-world translational research cohort of pts with HR+ MBC, low-level AURKA copy number gain was common. These data report the first evidence suggesting that low-level amplifications in AURKA, which conventional sequencing platforms miss, can provoke meaningful changes in gene expression as assessed via RNA transcriptome analysis. Further, low-level AURKA amplifications predict inferior outcomes on CDK4/6i for pts with HR+ MBC. Real-world clinical data for pts whose tumors harbored RB1 mutations, which have been associated with alisertib sensitivity in both the preclinical and clinical settings, trended with shorter rwPFS on CDK4/6i in this dataset and will be presented at the meeting. Efforts to refine these and other potential biomarkers that may guide clinical deployment of alisertib are ongoing.

P2-09-22:First-line inavolisib/placebo + palbociclib + fulvestrant in PIK3CA-mutated, hormone receptor+, HER2- locally advanced/metastatic breast cancer w/ relapse during/within 12 months of adjuvant endocrine therapy completion: INAVO120 long-term safety

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Background: INAVO120 (NCT03006172) previously demonstrated a statistically significant and clinically meaningful improvement in investigator-assessed progression-free survival (stratified hazard ratio 0.43; 95% CI, 0.32 to 0.59; P<0.0001) with inavolisib (inavo) + palbociclib (palbo) + fulvestrant (fulv) vs placebo + palbo + fulv, with manageable safety and good tolerability. Here we report long-term safety data from the inavo arm.
Methods: Safety and exposure analyses were conducted in all patients (pts) who received ≥1 dose of any study treatment (tx) (Safety Analysis Set; SAS). The current analysis includes all pts in the inavo arm on study tx for ≥1 year (yr) and those pts on study tx for ≥2yr. Demographic data are reported in the Full Analysis Set (FAS); defined as all pts who were randomized to receive study tx. NCI-CTCAE v5.0 was used to report safety data.
Results: The SAS and FAS populations included 162 and 161 pts in the inavo arm, respectively; in both the SAS and FAS populations, 69 pts were on study tx for ≥1yr and 27 were on study tx for ≥2yr (cut-off date: Feb 2, 2024).
The median ages were 53.0yr (range: 27–77) and 52.0yr (range: 27–69) for the 1yr and 2yr populations, respectively. The median tx durations for inavo were 21.6 months (range: 12.3–42.9) and 29.9 months (range: 25.5–42.9); the median relative dose intensities for inavo were 96.7% (range: 39.7–100.0) and 96.8% (range: 48.8–100.0).
All pts experienced an adverse event (AE). In the respective 1yr and 2yr populations, 88.4% and 88.9% of pts had a grade 3–4 AE. The most common grade 3–4 AEs were neutropenia, neutrophil count decreased, leukopenia, and white blood cell count decreased (55.1%, 31.9%, 8.7%, and 8.7%, respectively) in the 1yr population; and neutropenia, neutrophil count decreased, anemia, leukopenia, white blood cell count decreased, and anal fistula (51.9%, 29.6%, 7.4%, 7.4%, 7.4%, 7.4%, respectively) in the 2yr population. Grade 5 AEs were reported in 2.9% and 0% of pts; while serious AEs were reported in 27.5% and 44.4% of pts, respectively. All pts experienced an AE related to any study tx; 91.3% and 88.9%, respectively, experienced an AE related to inavo.
The incidence of key selected AEs (hyperglycemia, diarrhea, stomatitis/mucosal inflammation, and rash; grouped terms) were 63.8%, 56.5%, 55.1%, and 36.2% in the 1yr population; and 59.3%, 66.7%, 40.7%, and 44.4% in the 2yr population. Key selected AEs mainly occurred during earlier tx cycles.
Discontinuation from any study tx due to an AE was reported in 1.4% and 3.7% of pts, respectively; no pts discontinued inavo due to an AE in either long-term population. Inavo dose interruptions due to an AE were reported in 79.7% and 85.2% of pts; the most common AE leading to inavo dose interruption was hyperglycemia (30.4% and 25.9%) in both long-term populations. Inavo dose reductions due to an AE were reported in 18.8% and 14.8% of pts. The most common AEs leading to inavo dose reductions were hyperglycemia (2.9%) and stomatitis (2.9%) in the 1yr population, while in the 2yr population the AEs leading to inavo dose reductions were diarrhea, stomatitis, papulopustular rosacea, platelet count decreased, hyperglycemia, and myalgia (one pt each [3.7%]).
Conclusions: The long-term safety profile of inavo + palbo + fulv in the populations on study

tx for ≥1yr and ≥2yr was consistent with the INAVO120 SAS population reported previously, and similar to the long-term safety data of the phase 1 GO39374 study (NCT03006172). No new or unexpected AEs were reported.

P2-09-23: Differential outcomes of patients with HR-positive/HER2-negative metastatic breast cancer and a pathogenic BRCA1/2 or PALB2 variant under first line CDK4/6 inhibition

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Background: Patients (pts) with HR-positive/HER2-negative (HR+/HER2-) metastatic breast cancer (MBC) and pathogenic BRCA1/2 or PALB2 variants may derive less absolute benefit from CDK4/6 inhibitors (CDK4/6i) and endocrine therapy (ET).

Methods: The ESME MBC platform (NCT03275311) is a French real-world database including data from each newly diagnosed MBC patient having initiated at least one treatment from 2008 onwards in 18 comprehensive cancer centers. All women who initiated a first line treatment with CDK4/6i + ET between 2013 and 2023 were selected. Multivariable models including a Cox proportional hazard with a time-varying approach and landmark analyses at different timepoints (at the initiation of the first-line therapy and at 6 months after the initiation of the first line) assessed the association between BRCA and PALB2 status (categorized as BRCA/PALB2m (mutated), BRCA/PALB2wt (wild type), and untested), with progression-free (PFS) and overall survival (OS).

Results: Among 21696 pts with HR+/HER2- MBC, 4820 received 1st line ET + CDK4/6i and were eligible for this analysis (n=90 BRCA/PALB2m, n=523 BRCA/PALB2wt, n=4207 untested at the initiation of the first line). CDK4/6i included palbociclib (74%), ribociclib (17%), abemaciclib (13%) while ET partner included aromatase inhibitor (78,3%), fulvestrant (15,5%), or other (6,3%). BRCA/PALB2m carriers were younger and has less de novo MBC compared to untested patients. At the cut-off date of 2024/05/16, the median follow-up was 44.0m [43.1-45.1]. Median first line PFS was significantly shorter in BRCA/PALB2m pts compared with BRCA/PALB2wt and untested ones: 11.2m [8.9-16.1], 15.7m [14.2-17.3] and 18.9m [18.0-20.0] in BRCA/PALB2m, BRCA/PALB2wt and untested patients, respectively. In the multivariable analysis (including age, number of metastatic sites, presence of visceral metastases, de novo status, tumor grade and type of relapse (endocrine sensitive/resistant status), BRCA/PALB2m patients had a lower PFS compared to BRCA/PALB2wt patients (adjusted HR [95% CI] 1.48 [1.15; 1.90], p=0.003). Time-varying approach and landmark analysis at 6-month showed consistent results. This lower PFS did not translate into a lower adjusted OS with a median OS of 49.7m [42.2-not reached] and 50.1m [45.1; 57.2] in BRCA/PALB2m and BRCA/PALB2wt patients respectively (adjusted HR [95% CI] 0.93 [0.64; 1.36]).

Conclusion: In this large cohort of HR+/HER2− MBC patients treated with first line CDK4/6i+ ET, pathogenic variants identified in BRCA or PALB2 genes are associated with a significantly lower PFS. The role of PARPi as first line therapy for these patients is currently investigated (NCT06380751) and further research is needed to determine if similar differences are observed in early breast cancer.

P2-09-24: Correlation of TROP2 expression with outcomes of sacituzumab govitecan with or without pembrolizumab in patients with metastatic hormone receptor-positive/HER2-negative breast cancer: an exploratory analysis from the phase II SACI-IO HR+ trial

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Background: Sacituzumab govitecan (SG) is a TROP2-directed antibody drug conjugate (ADC) approved for previously treated triple negative and hormone receptor-positive/HER2-negative (HR+/HER2-) metastatic breast cancer (MBC) based on improvements in progression-free (PFS) and overall survival (OS) compared to chemotherapy (CT). Benefit with SG was observed regardless of TROP2 expression by immunohistochemistry (IHC), although the increase in response and survival rates with SG was greater in patients (pts) with higher TROP2 levels. SACI-IO HR+ is a randomized, open-label phase II study that compared SG with or without pembrolizumab in HR+/HER2- MBC (NCT04448886). Here we report the correlation of TROP2 expression by quantitative immunofluorescence (QIF) with clinical outcomes.

Methods: Pts with unresectable locally advanced or metastatic HR+ (ER≥1% and/or PR≥1%), HER2- breast cancer treated with ≥1 prior endocrine therapy and 0-1 chemotherapy for MBC were randomized 1:1 to Arm A (SG plus Pembrolizumab) or Arm B (SG). TROP2 expression was measured centrally by QIF. FFPE tissue (baseline research biopsy or most recent archival sample prior to study therapy) was stained using a Leica BOND Autostainer and imaged on a RareCyte CyteFinder II HT instrument. TROP2 protein was quantified in amol/mm2 with Qymia extension (v0.0.6) in Qupath (0.4.3) using a cell line standard curve calibrated by mass spectrometry. Association of TROP2 (as a continuous variable, median, and by quartiles) with PFS, OS, and G3 or higher treatment-emergent adverse events (TEAE) was evaluated using the Cox proportional hazards model, and the p-value from the log rank test was reported.

Results: Tumor samples from 82 pts (38 Arm A; 44 Arm B) who started study therapy were analyzed, of which 34 (41.5%) had PD-L1+ (defined as CPS ≥1) tumors. 72 (87.8%) pts had received prior CDK4/6 inhibitor therapy; 43 (52.4%) had no prior CT for MBC. Median

follow-up was 11.2 months (mo). Median TROP2 protein concentration was 4312.5 amol/mm2.
Among all pts, TROP2 was not significantly associated with PFS as a continuous variable (hazard ratio [HR] per 1-unit increase in log amol/mm2: 0.96, p=0.55) or by quartiles (≤25% [ref], median PFS 8.7 mo; >25%-50%, 4.5 mo, HR 1.86, p=0.09; >50%-75%, 6.7 mo, HR 1.14, p=0.74; >75%, 6.2 mo, HR 0.91, p=0.81). Median PFS was not significantly higher in pts with TROP2 levels ≥ vs < median (6.7 vs 5.9 mo; HR 0.77, p=0.31). TROP2 was not associated with PFS in either treatment arm (Arm A: HR 0.90, p=0.34; Arm B: HR 1.01, p=0.91) or by PD-L1 status (PD-L1+: HR 0.92, p=0.44; PD-L1-: HR 0.97, p=0.79).
Similarly, no significant associations between TROP2 and OS were observed among all pts (HR per 1-unit increment: 1.05, p=0.66), by treatment arm (Arm A: HR 1.05, p=0.79; Arm B: HR 1.03, p=0.85) or PD-L1 status (PD-L1+: HR 1.08, p=0.70; PD-L1-: HR 1.00, p=0.99).
Median OS did not significantly differ by TROP2 levels ≥ vs < median (17.3 vs 18.0 mo; HR 0.93, p=0.86). TROP2 by QIF was not associated with G3 or higher TEAE in all pts (HR per 1-unit increment: 1.00, p=0.99) or by treatment arm (Arm A: HR 0.81, p=0.42; Arm B: HR 1.16, p=0.41).

Conclusion: In this prespecified exploratory analysis of the SACI-IO HR+ trial, TROP2 expression by QIF was not associated with survival outcomes across all pts, by treatment arm or by PD-L1 status. Exploratory outcome analyses by TROP2 IHC expression and association with QIF will be reported.

P2-09-25: Subgroup Analyses From the Phase 3 EVER-132-002 Study of Asian Patients With HR+/HER2- Metastatic Breast Cancer

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Background: The EVER-132-002 randomized phase 3 study compared sacituzumab govitecan (SG) with chemotherapy treatment of physician's choice in Asian patients with hormone receptor–positive/human epidermal growth factor receptor 2–negative (HR+/HER2–) metastatic breast cancer (mBC). The primary end point of progression-free survival (PFS) per blinded independent central review (BICR) was met (HR, 0.67; 95% CI, 0.52-0.87; P = .0028). Overall survival (OS) was also improved with SG vs chemotherapy (HR, 0.64; 95% CI, 0.47-0.88; P = .0061), and the observed safety profile was manageable and consistent with other trials of SG. We present efficacy and safety by prior treatment with CDK4/6i and HER2 status from EVER-132-002.
Methods: Patients were enrolled from China, Korea, and Taiwan and were required to have HR+/HER2– mBC with 2-4 prior lines of systemic chemotherapy. Prior CDK4/6i treatment was not required for enrollment. Patients were randomized 1:1 to receive SG (10 mg/kg IV days 1 and 8 of 21-day cycle) or chemotherapy. The primary end point was PFS per BICR, and secondary end points included OS and safety. For the subgroup analysis, patients were

grouped by prior CDK4/6i treatment in the metastatic setting (yes/no) and by HER2 status (HER2 immunohistochemistry [IHC]0/HER2-low). HER2-low was defined as HER2 IHC1+ or HER2 IHC2+ and fluorescence in situ hybridization–negative.
Results: Of 331 patients (SG, n = 166; chemotherapy, n = 165), 161 (49%) received prior CDK4/6i (SG, n = 81; chemotherapy, n = 80) and 170 (51%) did not (SG, n = 85; chemotherapy, n = 85); 99 (30%) patients were HER2 IHC0 (SG, n = 53; chemotherapy, n = 46) and 232 (70%) were HER2-low (SG, n = 113; chemotherapy, n = 119). Age, baseline body mass index, and ECOG performance status were well balanced across subgroups and treatment groups. Patients in all subgroups and treatment groups had received a median of 2 prior systemic chemotherapy regimens in the metastatic setting.
PFS per BICR was improved with SG vs chemotherapy in patients with (HR, 0.56; 95% CI, 0.39-0.81) and without (HR, 0.79; 95% CI, 0.55-1.13) prior CDK4/6i treatment. OS was also improved with SG vs chemotherapy for patients with (HR, 0.50; 95% CI, 0.31-0.80) and without (HR, 0.77; 95% CI, 0.50-1.20) prior CDK4/6i treatment. PFS per BICR was improved with SG for patients who had HER2 IHC0 (HR, 0.59; 95% CI, 0.36-0.94) and HER2-low status (HR, 0.74; 95% CI, 0.55-1.00) compared to chemotherapy. OS was also improved with SG vs chemotherapy for patients who were HER2 IHC0 (HR, 0.65; 95% CI, 0.36-1.18) and HER2-low (HR, 0.61; 95% CI, 0.42-0.89).
Grade ≥ 3 treatment-emergent adverse events (TEAEs) were more common for SG than chemotherapy in patients with (80% vs 67%) and without (83% vs 72%) CDK4/6i, and for patients who were HER2 IHC0 (77% vs 71%) and HER2-low (84% vs 69%). The most common grade ≥ 3 TEAEs with SG were neutropenia (65%-71% across subgroups), leukopenia (37%-45% across subgroups), and anemia (16%-23% across subgroups) in all subgroups.
Conclusions: SG improved efficacy vs chemotherapy in Asian patients with HR+/HER2– mBC regardless of whether patients had received prior CDK4/6i in the metastatic setting and regardless of whether they had HER2 IHC0 or HER2-low status. The safety profile of SG was similar across the subgroups analyzed, and efficacy and safety in these subgroups were consistent with those observed in the ITT population. These results further support SG as an effective treatment option for patients with HR+/HER2– mBC irrespective of prior CDK4/6i treatment or HER2 status.

P2-09-26: Real world progression free survival in elderly vs. younger HR+/HER2- advanced breast cancer patients treated with first-line endocrine therapy plus CDK4/6i: a sub-analysis of the multicenter, PALMARES-2 study

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Background: Elderly (>75 years old) patients (pts) with Hormone Receptor-positive, Human Epidermal growth factor Receptor 2-negative advanced Breast Cancer (HR+/HER2- aBC) are a special clinical population characterized by more comorbidities, a higher number of concomitant therapies and globally higher frailty when compared to younger pts. The Cyclin-Dependent Kinase 4/6 inhibitors (CDK4/6i) palbociclib, ribociclib and abemaciclib in combination with endocrine therapy (ET) are the standard-of-care, first-line treatment for HR+/HER2- aBC pts regardless of their age. In the whole patient cohort of the PALMARES-2 study, the largest real-world study comparing the effectiveness of the three CDK4/6i, we recently showed that abemaciclib and ribociclib are more effective than palbociclib However, the real-world effectiveness of CDK4/6i in elderly vs. younger pts remains poorly investigated.
Methods: PALMARES-2 is a population-based, multicenter, real-world study that is evaluating the effectiveness of first-line line ET+CDK4/6i in consecutive HR+/HER2- aBC pts treated in 18 Italian cancer centers between 1st January 2016 and 1st September 2023. The primary study endpoint is real-world Progression-free Survival (rwPFS), defined as the time between CDK4/6i initiation and tumor progression, as assessed according to radiological, clinical and/or biochemical parameters. Here, we compared rwPFS in elderly (defined as >75 years old) vs. younger (≤75 years) pts. Multivariable Cox regression modelling was used to adjust the association between individual CDK4/6i and rwPFS for clinically relevant variables.
Results: Elderly pts accounted for 15.2% (n=302) of the whole PALMARES-2 study cohort (n=1982 pts). When compared to younger pts, elderly pts were less likely to have an ECOG PS of 0 (56% vs. 79%, p<0.001) or bone-only disease (17% vs. 25%, p=0.002), and more likely to have lobular tumor histology (25% vs. 17%, p=0.002), lung metastases (36% vs. 25%, p<0.001), and to have received palbociclib (57% vs. 37%, p<0.001). After adjusting for clinically-relevant covariates, elderly pts had independently better rwPFS when compared to younger pts in the whole study cohort (adjusted Hazard Ratio [aHR]: 0.77; 95% CI: 0.67-0.90, p<0.001), as well as in endocrine-sensitive (aHR: 0.76; 95% CI: 0.61-0.94, p=0.013) and endocrine-resistant (aHR: 0.80; 95% CI: 0.79-0.81, p<0.001) settings. We found a strong interaction between patient age and the type of CDK4/6i in affecting rwPFS (p inter<0.001), with ribociclib and abemaciclib being significantly more effective than palbociclib in younger pts (aHR: 0.77, 95% CI: 0.67-0.89, p<0.001) but not in elderly pts (aHR: 0.99; 95% CI: 0.92-1.06, p=0.73). Among 789 pts receiving ET plus palbociclib (n=616 younger; n=173 elderly), elderly pts had significantly better rwPFS at both univariate (median rwPFS in elderly vs. younger: 38.9 vs. 26.6 months; HR: 0.69, p val=0.006) and multivariable (aHR: 0.69; 95% CI: 0.57-0.84; p<0.001) analysis. We did not observe significant rwPFS differences in elderly vs. younger pts treated with ribociclib (aHR: 0.93; 95% CI: 0.76-1.13, p=0.45) or abemaciclib (aHR: 0.85; 95% CI: 0.59-1.22, p=0.37).
Conclusion: Elderly women are a numerically and clinically relevant subset of all HR+/HER2- aBC pts receiving first-line line ET plus CDK4/6i in a real-world setting. When compared to younger pts, elderly pts treated with palbociclib have significantly better rwPFS. Our findings, together with different safety profiles, manageability and drug-drug interactions of the three CDK4/6i, point to palbociclib as the preferred CDK4/6i in the

majority of elderly pts. Longer follow-up is required to compare overall survival in elderly vs. younger pts.

P2-09-27: Alpelisib (BYL719) with continued endocrine therapy following progression on endocrine therapy in hormone receptor–positive, HER2-negative, PIK3CA-mutant metastatic breast cancer: A Big Ten Cancer Research Consortium Study (BTCRC-BRE19-409)

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Background: Activating mutations in the PIK3CA gene are found in ~40% of patients with hormone receptor-positive (HR+) breast cancer (BC). The p110α-specific PI3K inhibitor alpelisib (ALP) is FDA-approved in combination with fulvestrant (FUL) for treatment of patients with HR+ HER2 negative (HER2-) PIK3CA mutated, advanced BC (ABC). The benefit of adding alpelisib to fulvestrant following progression on non-fulvestrant endocrine therapy (ET) was demonstrated in the SOLAR-1 study, but the majority of these patients were CDK4/6 inhibitor (CDKi) naïve. The BYLieve study established that alpelisib plus switch ET was effective after progression on prior CDKi/ET combination. We hypothesized that clinical benefit of both settings is driven by the addition of alpelisib, and therefore switch from CDKi to alpelisib without change in ET at time of progression on CDKi/ET combination could lead to similar benefit while preserving additional ET options for later lines of therapy. Methods: In this single arm, non-randomized phase II study, patients with PIK3CA-mutated HR+ HER2- ABC with progression on AI or FUL were continued on prior ET with addition of ALP at 300mg daily for 28-day cycles. Antihistamine rash prophylaxis was encouraged, and after enrollment of the first 13 patients, metformin hyperglycemia prophylaxis was encouraged. In contrast to the SOLAR-1 study, all patients received prior CDKi; no more than two prior lines of ET and no chemotherapy in the metastatic setting was allowed. Primary endpoint is progression free survival (PFS). Secondary objectives are overall response rate (ORR), clinical benefit rate (CBR), duration of response (DOR), overall survival (OS) and safety/tolerability. Results: 21 patients were enrolled, 4 (19%) with continued AI and 17 (81%) with continued FUL. Median age was 63 and all patients were female. 81% of patients identified as non-Hispanic white, 4.7% as African American, and 9.5% as Hispanic/Latino. 33% of patients had de novo metastatic disease and 85.7% had visceral involvement at time of study entry. 42.8% of patients had 2 prior lines of ET. 90.5% of patients had prior palbociclib, and 9.5% had prior ribociclib. 52.3% had PIK3CA exon 9 hotspot mutations and 23.8% had PIK3CA exon 20 hotspot mutations. At time of data cutoff, median follow up was 10.8 months, with 2 patients still on treatment. Patients received a median of 5 cycles of treatment (range 1-12), and 42.8% required ALP dose reductions, including 9.5% to 250mg daily and 33% to 200mg daily. No new safety signals were noted. Most common toxicities included hyperglycemia in 71% of patients (29% grade 3), fatigue in 62% (0% grade 3), nausea in 57% (5% grade 3), diarrhea in 52% (10% grade 3), and

rash in 38% (10% grade 3), comparable to reported rates in the SOLAR-1 and BYLieve cohorts. 67% of patients experienced grade 3 toxicities, similar to rates seen in SOLAR-1 and BYLieve trials, however in contrast to those studies, no grade 4 toxicities were seen here. Additionally, only 14.2% patients discontinued due to toxicities, lower than the rates reported in SOLAR-1 and BYLieve, possibly due to the encouraged use of prophylaxis with anti-histamine and metformin. 5 patients experienced SAEs at least possibly related to alpelisib, including hyperglycemia (1), diarrhea (2) and hypoxia (2). At 6 months, 6/21 (29%) of patients remained on combination therapy, 2 with AI and 4 with FUL. Progression free survival, clinical benefit rate, and duration of response will be presented. Conclusions: In HR+HER2- PIK3CA-mutant metastatic breast cancer progressing on ET/CDKi combination therapy, continued ET with switch from CDK4/6 inhibitor to alpelisib at time of disease progression is feasible and safe and clinical responses are seen. Planned correlative studies will assess circulating biomarkers of PI3K signaling that may identify which patients would benefit from this approach.

P2-09-28: Dalpiciclib plus endocrine therapy for visceral crisis in advanced breast cancer: a multicenter, prospective, external controlled phase 2 study

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Background: Patients with visceral crisis due to advanced breast cancer (ABC) have a poor prognosis, even with first-line chemotherapy. Visceral crisis has been a common exclusion criterion in clinical trials for patients with ABC. Thus, data on their efficacy in ABC patients with visceral crisis is limited. This study aims to explore the efficacy and safety of dalpiciclib plus ET in hormone receptor-positive, human epidermal growth factor receptor 2-negative (HR+/HER2−) ABC patients with visceral crisis.
Methods: This multi-center, prospective phase 2 study used a Simon two-stage design to evaluate HR+/HER2− ABC patients with visceral crisis, defined as pleural effusion; ascites; abdominal pain from liver or peritoneal metastasis; dyspnea from pleural effusion or lymphangitic spread in the lungs; elevated liver enzymes (> 2 × ULN); rapid bilirubin increase (> 1.5 × ULN) without Gilbert syndrome or biliary obstruction; pathologically confirmed bone marrow metastasis; and hemoglobin < 100 g/dL. Patients were treated with dalpiciclib (150 mg/day for 21 days, followed by 7 days off) plus the investigator's choice of ET (experimental group). If 9 or more subjects had survived at 6 months in the first stage, then 35 additional subjects would be enrolled. The null hypothesis would be rejected if 28 or more subjects out of a total of 53 had survived beyond 6 months. Results were compared to real-world data from an external control group receiving chemotherapy, which followed the same inclusion and exclusion criteria. Inverse probability of treatment weighting (IPTW) approach was used to estimate the average treatment effect on the treated (ATT). Variables in propensity-score-adjustment including age, ECOG, disease recurrence, number of metastatic sites, lines of prior treatment, and classification of visceral crisis. Primary endpoint was the 6-month survival rate. Secondary endpoints included but

not limited to OS, PFS, 3-month treatment failure rate (TFR), time to treatment failure (TTF), duration of disease control (DDC), and safety.
Results: Between February 2023 and May 2024, 17 of 18 patients in the experimental group survived 6 months of treatment in the first stage and proceeded to the next stage. Subsequently, an additional 35 patients were enrolled in the second stage. Of the 53 patients in total, 12 were not followed up for 6 months, while 37 survived 6 months, successfully rejecting the null hypothesis that the 6-month survival rate was ≤ 44%. In the external control group, 157 patients were included. After IPTW adjustment, the effective sample size was approximately 50 patients. Compared to the chemotherapy, dalpiciclib plus ET was associated with substantially longer PFS (median 9.03 months [95% CI 7.20-12.65] vs 4.63 months [95% CI 2.92, 5.65]; HR = 0.334 [95% CI 0.200-0.557], P < 0.001) and longer TTF (median 9.46 months [95% CI 6.41-12.48] vs 4.14 months [95% CI 2.53-5.13]; HR = 0.34 [95% CI 0.207-0.570], P < 0.001). The 3-month TFR in the experimental group (22.6% [95% CI 12.28-36.21]) was considerably lower than in the external control group (40.0% [95% CI 26.41-54.82], P = 0.0876). Median DDC was 8.67 months (95% CI 7.13-NE) in the experimental group, and 4.76 months (95% CI 3.65-6.05) in the external control group, respectively. Treatment-related adverse events occurred in 100% of patients in the experimental group and in 93.6% of patients in the external control group. Lower rates of abnormal liver function were observed in the dalpiciclib plus ET versus chemotherapy group (increased alanine aminotransferase: 17% vs. 28.7%; increased aspartate aminotransferase: 22.6% vs. 40.1%).
Conclusions: Compared with chemotherapy, dalpiciclib plus ET promotes better PFS for patients with HR+/HER2− advanced breast cancer experiencing a visceral crisis, with a manageable safety profile.
Clinical trial identification: NCT05431504

P2-09-29: Landscape analysis of breast cancer ovarian metastases reveals biology and potential therapeutic targets

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Background: Treatment resistance and metastases occur in 10-20% of patients with invasive lobular carcinoma (ILC), the most common special histological subtype of breast cancer. ILC often metastasize to the ovary, a site that is less common for the majority of no special type tumors (NST) also referred to as invasive ductal carcinoma (IDC). The hallmark of ILC is loss of E-cadherin (CDH1), which maps to chromosome 16q. There are limited studies on genomics and transcriptomics of ovarian metastases in patients with breast cancer.
Methods: Mutations and copy number changes were analyzed from 15,613 local breast cancer tissues (of which 1,350 were from patients with ILC), 22,010 non-ovarian

metastases, and 246 ovarian metastases, all of whom underwent tumor-only sequencing using the FoundationOne®CDx or FoundationOne® assays. A scar-based measure of HRD (HRDsig), incorporating copy number and indel features, was called using a machine learning-based algorithm. In addition, we analyzed a cohort of 27 breast cancer ovarian metastases from patients seen at UPMC, consisting of 13 ILCs, 8 NST, and 6 mixed Ductal Lobular Carcinoma (mDLC) using RNA seq and targeted DNA sequencing using the MammaSeq panel. Finally, functional studies including proliferation, migration, transwell chemotaxis and haptotaxis, and invasion assays as well as a series of stainings were utilized to study role of calcium sensing receptor (CaSR) in ovarian metastases.
Results: From a total of 246 ovarian metastases, 115 had CDH1 mutations. There were fewer CDH1 mutations in the ovarian metastases (47%) compared to the primary breast ILCs (81.3%). Consistent with this, there was a reduced frequency of 16q losses, the arm with CDH1, in ovarian metastases relative to local ILC (64% vs 84%, P=3.4X10^-9). These results highlight that while CDH1-mutations are enriched in ovarian metastases, they can also develop in non-ILC tumors. Comparing ovarian and non-ovarian metastases, we detected an enrichment of CDH1, PIK3CA and TBX3 mutations, and an attenuation of mutations in TP53, ESR1, MYC, and CDKN2B. There were no differences in TMB or MSI status, but HRDsig-positive cases were fewer in the ovarian metastases. Median age of patients with ovarian metastases was 52 years, non-ovarian metastases 60 years, local breast cancer (any subtype) 57 years, and local ILC 62 years. The younger age in patients with ovarian metastases was confirmed in the UPMC cohort, where median age at diagnosis of the primary tumor for patients with ovarian metastases was 43 years. In the UPMC cohort, the median disease-free survival of patients with ovarian metastases was 49.5 months, which is 14 months longer compared to that for all patients with breast cancer metastases. As expected, the majority of patients had received endocrine therapy (75%) and/or chemotherapy (56%). Like in the FMI cohort, the most frequent mutations were found in CDH1 and PI3K. Additional mutations were identified in KMT2C, FOXA1 and RUNX1, ERBB2, ERBB3, ATM, and JAK3. Transcriptomic analysis identified up-regulation of activated G protein coupled receptor (GPCR) pathways in ovarian metastasis compared to the primary tumor and normal ovarian tissue, including metabotropic glutamate receptor signaling. In functional studies, calcium sensing receptor (CaSR), a GPCR that was highly overexpressed in ovarian metastases, enhanced migration upon activation with calcium or calcimimetic, which could be blocked with the calcilytic NPS2143. Activated CaSR triggered the MEK/ERK pathway-dependent reorganization of F-actin fibers which was reversed upon treatment with MEK inhibitors. CaSR-induced migration was enhanced with estrogen stimulation and reduced with anti-estrogen receptor (ER) inhibitors.
Conclusions: Our study provides the largest comprehensive characterization of ovarian metastases in patients with breast cancer. It not only deepens our understanding of ILC ovarian
metastasis but provides the foundation for future studies aimed at improved prevention and treatment of breast cancer metastasis to the ovary.

P2-09-30: Real-world comparative efficacy of CDK4/6 inhibitors in first-line treatment of HR+/HER2- metastatic breast cancer

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Background: Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) combined with endocrine therapy (ET) is the most common first-line (1L) treatment for hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2−) metastatic breast cancer (mBC). Registrational studies for palbociclib (P), ribociclib (R), and abemaciclib (A) have shown consistent PFS benefit; nevertheless, overall survival (OS) results have been inconsistent. No head-to-head studies directly comparing CDK4/6i have been conducted, and although cross-trial comparisons can provide insights, they are limited by differences in trial design and patient population. We have used real-world evidence (RWE) data to directly compare real-world OS (rwOS) with different CDK4/6i when used as 1L treatment for patients with mBC treated in the USA.
Methods: This retrospective open-cohort study used data from two US de-identified electronic health record-derived data sets: primary analyses were conducted using data from the Flatiron Health database, which were then verified using data derived from the Tempus database. Eligible patients had HR+/HER2− mBC and received 1L metastatic treatment with CDK4/6i + ET between January 1, 2016, and April 30, 2023 (Flatiron Health), or December 31, 2022 (Tempus). Patients were followed from the first date of 1L treatment until death or last known activity. Median rwOS for each CDK4/6i was estimated using the Kaplan–Meier methodology. Treatment effects were analyzed using adjusted Cox proportional hazards models, including covariate and propensity score adjustments. Subgroup analyses were also conducted on patients treated with CDK4/6i + aromatase inhibitors (AI) as well as in patients who had a treatment-free interval (TFI) >12 months between last adjuvant ET and metastatic diagnosis date and were then treated with CDK4/6i + AI.
Results : 4,567 patients were selected from the Flatiron Health database (3,504, 575, and 488 treated with P, A, and R, respectively) and 612 from the Tempus database (494, 65, and 53, respectively). In the Flatiron Health database, median (95% CI) rwOS was 43.8 (41.7–46.6) months for P, 43.2 (34.5–48.8) months for A and 44.2 (37.3–51.1) months for R. Adjusted hazard ratios: 1.09 (95% CI: 0.89–1.33) for P versus A and 1.07 (95% CI: 0.90–1.28) for P versus R. Subgroup analyses also showed a similar risk of death between CDK4/6i for patients who received CDK4/6i + AI (2,334, 341, and 355 treated with P, A, and R, respectively. Adjusted hazard ratios 0.90 [95% CI: 0.66–1.23] for P versus A and 1.08 [95% CI: 0.88–1.33] for P versus R) and those with a TFI>12 months (2,226, 349 and 232 treated with P, A and R, respectively. Adjusted hazard ratios 0.91 [95% CI: 0.66–1.25] for P versus A and 1.11 [95% CI: 0.90–1.37] for P versus R). Findings were confirmed with Tempus data; adjusted hazard ratios indicated a similar risk of death between CDK4/6i: 0.92 (95% CI: 0.51–1.68) for P versus A and 1.14 (95% CI: 0.64– 2.05) for P versus R.
Conclusions: Our findings, using mature follow-up data, showed similar rwOS outcomes when different CDK4/6i are combined with ET as 1L treatment for patients with

HR+/HER2− mBC. Results were consistent across RWE databases. As 1L treatment evolves, these findings contribute to the ongoing development of combination strategies, such as novel oral selective estrogen receptor degraders, to improve clinical outcomes in patients with HR+/HER2− mBC and guide clinical decision-making.

P2-10-01: Real-world Experience with CDK4/6 Inhibitors in Hormone Receptor-Positive Metastatic and Recurrent Breast Cancer in Asian Population

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Purpose: Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) combined with endocrine therapy have demonstrated significant clinical benefits in progression-free and overall survival. This study investigates the outcomes associated with two kinds of CDK4/6i, palbociclib and ribociclib, in patients with hormone receptor (HR) positive metastatic and relapsed breast cancer to inform real-world evidence of treatment strategies.
Methods: This retrospective study included 340 Taiwanese patients with HR-positive advanced breast cancer from the Taipei Veterans General Hospital, between 2018 and 2023. We analyzed patient characteristics, treatment strategies and outcomes associated with two CDK4/6i. The efficacy of patients who experienced economic burden and interrupted CDK4/6i treatment after 2 years of national health insurance(NHI) reimbursement was also investigated.
Results: Patients receiving ribociclib and palbociclib showed no significant differences in age, histology, body mass index(BMI), or pathologic status. The distributions of disease status and endocrine therapy partners were comparable between the two groups. The dose reduction rate was similar, while patients with palbociclib tended to discontinue CDK4/6i usage, and those with ribociclib tended to switch to the other CDK4/6i or endocrine partners. Patients with palbociclib had a higher prevalence of prior chemotherapy for advanced diseases. There was no significant difference in progression-free survival (PFS) between the two CDK4/6i in the first-line setting. Adverse prognostic factors were increasing HER2 IHC score, higher Ki-67 levels, visceral and liver metastasis, prior chemotherapy, and endocrine therapy resistance while higher BMI, bone-only metastasis, and letrozole treatment were associated with a lower risk of progression. This limited follow-up time in our study was insufficient to assess the outcomes of patients treated with interrupted CDK4/6i for up to two years under the NHI reimbursement policy.
Conclusion: Treatment outcomes between the two types of CDK4/6i did not differ significantly, indicating the safety and efficacy of CDK4/6i for the Asian population. Ribociclib and palbociclib showed similar efficacy in PFS in the real-world setting.

P2-10-02: SACISUR, results of a real-world evidence observational study of the use of Sacituzumab-Govitecan (SG) in triple negative metastatic breast cancer (mTNBC) clinical practice in the south of Spain.

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Background: SG is a new antibody-drug conjugate against Trop-2 that has been approved for the treatment of mTNBC after progression on at least one previous line of chemotherapy (CTX) for advance disease. In the ASCENT trial, progression-free survival (PFS) and overall survival (OS) were longer with SG compared with CTX (5.6 vs 1.7 months PFS and 12.1 vs 6.7 months respectively).
Methods: This was an observational multicentre retrospective study.
We included patients with mTNBC treated at least with 1 cycle of SG between 1 January 2022 and 31 December 2023. This study has been supported by SAOM (Andalusian Society of medical oncologist). The aims of the study were analysing efficacy and safety of SG in our population. Special populations of patients with central nervous system (CNS) metastasis will be presented in a different poster.
Statistical analysis was made by IBM SPSS statistics, including descriptive results of the population and survival analysis by Kaplan-Meier.
Results: We report the data of 159 female patients treated in 18 different hospitals of Andalucía, Canarias and Extremadura (Spain). The median age was 53 years (46.5 % premenopausal, 35.2% Her2low). 75.5% of the patient had visceral disease, only 13.9% had CNS disease. 17.6% were de novo, stage I-III patients were treated with CTX in the early setting in 59.7% in neoadjuvant and 28.9% in the adjuvant setting. Pathological complete response was 17.5% in the case of neoadjuvant treated patients.
Previous line of therapy was 3 (1-8) with a medium of 7 cycles of SG. SG was received in second line in 41.8% patients, 3.8 % in first line and 54.4% patients third line or beyond. 17% received immunotherapy in first line.
With a median follow-up of 11.8 months, PFS was 5.2 months (95% CI, 4.05-6.27) and OS was 10.5 months (95% CI, 7.6-13.5) (fig 2). The percentage of patients with an objective response was 31.2% (68.9% with clinical benefit rate).
The incidence of diverse adverse events were neutropenia 59.4% (G3-4 30.4%), diarrhea 49% (G3-4 8.2%), nauseas 45.3% (G3-4 0.6%) and ALT/AST elevation 24.5% (G3-4 1.9%). The use of GCSF were 29.6 % as primary prophylaxis and 17.6 % as secondary prophylaxis. 5.7% of the patients finished SG due to adverse events and 43.4% had at least one dose reduction.

Conclusions: SG is a safe and promising option in mTNBC real-world patients. Our results were similar to that obtained in ASCENT trial and even better if we exclude patients with CNS metastasis.

P2-10-03: Real world outcomes of adjuvant chemotherapy in invasive lobular carcinoma of the breast: a retrospective cohort from a reference center in Brazil

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Background: Invasive lobular carcinoma is the second most common breast cancer histology and has lower response rate to chemotherapy (CT) compared to ductal/no special type (NST) tumors. In the adjuvant setting, many guidelines recommend CT based on the criteria used for NST. However, the benefit of CT is not necessarily the same. Here, we present survival outcomes of patients with invasive lobular carcinoma from a retrospective cohort.
Methods: This study has included patients seen at Instituto do Cancer do Estado de São Paulo, Brazil, between 2015 and 2020. Only patients with invasive lobular carcinoma in stages I-III have been enrolled.
Results: After screening, 153 records from patients were included to review, divided in stages I (22%), II (47%) , and III (31%). Mean of age at diagnosis was 62y (range: 28 - 91 years). Received chemotherapy as adjuvant, neoadjuvant and omitted in 76 (50%), 16 (10%), and 61 (40%) respectively. The median of disease-free survival in adjuvant, neoadjuvant and omitted were respectively 11y, 1.4y and 9.4y (p=0.0001); and median of overall survival (mOS) were 13.3y, 5.5y and 7.5y (p = 0.0395). Analyzed by staging, for stage I mOS was not raged among groups (p=0.011), stage II 13y, 5.5y and 7.2y (p=0.006), and stage III: 6.2y, 5.9y, and 4.6y (p=0.56). In a multivariable Cox proportional regression, the hazard ratio (HR) for age, staging and HER2 3+ were 1.07 (p=0.001), 4.2 (p= 0.0001), and 8.9 (p=0.001) respectively.
Conclusion: In our study, disease-free survival and overall survival were longer for adjuvant chemotherapy than neoadjuvant, specially for stage I and II. Although age, staging and HER2 3+ were independent risk factors for shorter overall survival.

P2-10-04: Effectiveness and toxicity profile of pertuzumab in combination with trastuzumab and taxane in patients with HER2 positive metastatic breast cancer treated in the Costa Rican Health Care System

Priyanka Khanna, Denis U. Landaverde

Background: The first line of treatment for HER2-positive metastatic breast cancer is the combination of pertuzumab, trastuzumab and taxanes. In Costa Rica, since 2014, this

combination began to be used in the national health care system, however its impact was not yet known since no local studies had been reported showing its e"ectiveness. The present study aimed to assess the performance of this treatment in our public health care system
Methods: From August 2015 to August 2021, a total of 148 patients were included in this study, with a histological diagnosis confirmed of HER2-positive metastatic breast cancer, who received pertuzumab, trastuzumab, and taxane (paclitaxel or docetaxel) as first-line treatment. Descriptive statistics were used for the demographic, clinical, and pathological characteristics. Statistical analysis of survival was performed using the log-rank test, and the Cox proportional hazards model. All the data were analyzed with the SPSS 20.0 program for Mac (Chicago, IL). Toxicities reported were recorded as established in the CTCAE
Results: The median age was 58 years, 81% of patients had performance status 0, 54% were hormone receptor positive, 90% received adjuvant or neoadjuvant chemotherapy, 42% had metastatic visceral disease including CNS. Paclitaxel was the most frequently taxane used with an average of 16 weekly cycles. The median duration of pertuzumab and trastuzumab together was 22.7 months. The most frequent reason for suspension was progression (56.1%) and only 3.4% due to toxicity; 3.4% of the patients abandoned treatment. The most frequent adverse events were peripheral neuropathy (34%), diarrhea (21%), febrile neutropenia (1)%, and grade 3 cardiotoxicity was seen in 2 patients. The most frequent site of progression during treatment was the brain (38.5%). With a median follow-up of 27.5 months, progression-free survival was 19 months (95% CI 15-25). Median overall survival of 73 months (95% CI 38-74). Conclusions This is the first study conducted in a Central American population confirming that the results obtained in the pivotal clinical trial were replicable in our population increasing the real-world data in our region.

P2-10-05: Survival in Older Women Receiving Adjuvant Radiation Monotherapy vs. Endocrine Monotherapy Following Lumpectomy

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Background: Breast cancer is increasingly prevalent in women over 65, and most are diagnosed with hormone receptor positive disease. While younger patients with early-stage breast cancer are treated with both adjuvant radiation therapy (RT) and endocrine therapy (ET), many clinical trials have shown that omitting radiation among older patients who take adjuvant ET does not decrease survival. However, ET is difficult for many older patients to tolerate, and some may prefer a short course of RT monotherapy instead of ET. While ET can reduce both local and distant recurrence risk, older women with favorable breast cancers may have a sufficiently low distant recurrence risk that omission of ET may be reasonable, if RT is given to reduce local recurrence. Therefore, the aim of this study was to determine whether treatment with adjuvant RT monotherapy results in comparable overall survival (OS) to treatment with adjuvant ET monotherapy in women age ≥65 diagnosed with favorable early-stage breast cancer.

Methods: Patients aged ≥65, diagnosed in 2010-2020 with ER+/HER2-, prognostic clinical stage I breast cancer (cT1-2, N0), who underwent lumpectomy, were selected from the National Cancer Database. Patients who received any chemotherapy and/or any neoadjuvant therapy were excluded. Differences across groups were tested. The Kaplan-Meier method was used to estimate OS, and log-rank tests were used to test for differences in OS between groups. Stratified analyses were conducted based on age group (65-69, 70-74, 75-79, 80+). A Cox Proportional Hazards model was used to estimate the association of the treatment group with overall survival after adjustment for available covariates.
Results: The final cohort included 91,505 patients, with 13.5% receiving no adjuvant therapy, 11.8% receiving RT alone, 29.5% receiving ET only, and 45.2% receiving both RT and ET. The median follow-up for the entire cohort was 67.6 months. Patients in the RT-only and ET-only groups were of similar age [median (IQR): RT 73yo (69-79) vs ET 75yo (71-80); p<0.001], although patients in the ET-only group were less likely to have a comorbidity score of 0 (ET 75.4% vs RT 80.8%; p<0.001). Patients in the ET-only group had minimally larger tumors [median tumor size (IQR): ET 1 (0.7-1.5) vs RT 0.9 (0.6-1.3); p<0.001], were less likely to have grade 3 tumors (ET 7.01% vs RT 8.38%; p<0.001), and were slightly more likely to have PR+ disease (ET 91.4% vs RT 89.6%; p<0.001).
In the unadjusted Kaplan-Meier analysis, patients receiving ET-only had a slightly lower 5-year OS compared to the RT-only group [ET 85.8% (85.3-86.3%) vs RT 88.9% (88.2-89.6%); log rank p<0.001]. A similar trend was observed when stratified based on age group, although notably less pronounced (all log rank p<0.05). In the adjusted multivariable analysis, RT alone remained associated with a slightly better OS than ET alone [ET ref, RT hazards ratio 0.91 (95% CI 0.85-0.97)].
Conclusions: For older patients with early-stage, ER+/HER2- breast cancer, survival outcomes were largely similar for those who received adjuvant RT monotherapy vs adjuvant ET monotherapy. While prior studies have evaluated de-escalation of radiotherapy, de-escalation of endocrine therapy has not been as extensively explored. Direct comparisons of RT alone vs ET alone may be warranted in this unique population with variable life expectancy and potentially different treatment preferences.

P2-10-06: Safety and efficacy of trastuzumab deruxtecan and concomitant radiation therapy in patients with metastatic breast cancer: a multicentre international retrospective cohort study

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Background. Trastuzumab deruxtecan (T-DXd) has emerged as the standard treatment for patients with metastatic HER2-positive (HER2+) breast cancer (BC) following disease progression on first-line therapy containing taxanes and trastuzumab, as demonstrated by

the findings of the DESTINY-Breast03 trial. Subsequently, results from the DESTINY-Breast04 have extended the approval of T-DXd to include HER2-low patients. Radiation therapy (RT) is often necessary in the metastatic setting, either for palliative purposes or with ablative intent in cases of oligometastatic or oligoprogressive disease. Our retrospective study aims to assess the safety of concurrent use of T-DXd and RT in a consecutive multicentre international cohort of BC patients.
Methods. We conducted a retrospective analysis of patients diagnosed with metastatic HER2+ or HER2-low BC, who were treated at four leading European institutions. Patients started treatment with T-DXd between May 2021 and March 2024, with some receiving concomitant RT with palliative or ablative intent and others not. We defined ablative RT based on biological dose with threshold of minimum 50Gy EQD2(10) delivered in a maximum of 12 fractions, according to the EORTC and European Society for Radiotherapy (ESTRO) OligoCare (EORTC-1822-RP) study. The primary objective of the study was to assess the association between RT administration and adverse events (AEs) exceeding grade (G) 2. In all cases RT was administered within one month before Cycle 1 of therapy with T-DXd or during systemic treatment without any drug interruption.
Results. Data of 118 consecutive patients treated with T-DXd with or without RT were retrospectively evaluated. Thirty-three patients received RT immediately before (within a month) or during T-DXd, for a total of 34 concomitant RT treatments, while 85 patients did not. Median age was 55 years old (range 30-88). At a median follow up of 18 months (range 1-33), 8 patients (24.2%) had died in the RT group and 17 patients (20.0%) in the no-RT group. Sixty-two patients (52.5%) received T-DXd as fourth or further line of systemic therapy, while 38 (32.2%) in third line and 15 (12.7%) in second line. Three patients (2.6%) with early metastatic disease relapse (<6 months after adjuvant anti-HER2 therapy completion) received T-DXd as first line treatment. Median total RT dose prescription was 34Gy (range 8-48) with a median number of fractions of 4 (range 1-15). Median EQD2 dose was 64Gy (range 12-104) and median BED 76Gy (14-125). The most frequently treated site was central nervous system (51.5%; n=17/33), followed by bone (33.3%; n=11/33). Fifteen patients received RT with ablative intent (45.5%), 18 patients RT with palliative intent (54.5%). A chi-square test of independence was performed to examine the relation between RT administration and the development of >G2 toxicity. The relation between these variables was not significant ($p=0.79$; significance level $p<0.05$). Regarding toxicities of special interest for T-DXd, 3 cases of G3 fatigue have been reported in no-RT group (3.5%) with 1 case in the RT group (3.0%). Overall, only 1 case of G3 nausea was observed (in the no-RT group). Grade 2 interstitial lung disease (IDL) or G2-3 pneumonitis were observed in 4 cases in RT group (12.1%) and in 8 cases in no-RT group (9.4%). No radionecrosis events were reported among the 17 patients treated with intracranial RT.
Conclusions. Our preliminary data are encouraging regarding the potential safety of this combination, showing that concurrent RT did not increase severe acute toxicity. Data from larger series are needed to confirm these results.

P2-10-07: Practice patterns and survival analysis of early-stage HER-negative breast cancers with low and intermediate levels of hormone receptor expression: a 2018-2020 US National Cancer Database analysis

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Background: Breast cancer (BC) with low hormone receptor (HR) expression (1-10%) is classified as HR-Low BC. We previously demonstrated that pathologic complete response (pCR) rates and biologic features of HR-Intermediate HER2-negative BC (11-30%), much like HR-Low BC, are similar to triple negative breast cancer (TNBC). Here, we report treatment patterns and overall survival (OS) for HER2-negative BC by level of HR expression from the US National Cancer Database (NCDB).

Methods: Patients (pts) with stage I-III HER2-negative BC diagnosed in 2018-2020 were categorized into four groups by estrogen receptor (ER) and progesterone receptor (PR) expression: ER<1% and PR<1% (HR-Neg), ER 1-10% and/or PR 1-10% (HR-Low), ER >11-30% and/or PR>11-30% (HR-Int), ER >30% and/or PR >30% (HR-High). Pts with undocumented HR status or without curative intent surgery were excluded. The primary outcome was OS by HR expression. Key secondary outcomes were treatment patterns and OS by neoadjuvant chemotherapy (NAC) response and endocrine therapy (ET) use. Categorical variables were compared between the groups using a Chi-square test. OS survival was explored using Kaplan Meier estimates, log-rank tests and univariate/multivariable cox proportional hazard models.

Results: 395,757 incident cases of early-stage HER2-negative breast cancer were identified, including 8857 (2.2%) HR-Low and 4375 (1.1%) HR-Int BC. HR-Low and HR-Int cohorts reported more advanced stage at diagnosis and were more likely to be node-positive with higher Ki-67 scores than HR-High BC (p<0.001 for all). HR-Low and HR-Int groups consisted of proportionally more Black patients than HR-High (20%, 19% vs 9%; p< 0.001). Oncotype DX results were more likely to be obtained with increasing levels of HR expression (HR-Neg 1%, HR-Low 11%, HR-Int 25%, HR-High 43%; p< 0.001). ET use was more frequent in HR-High than HR-Low or HR-Int BC (87%, 46% & 71%; p <0.001). The use of immunotherapy increased with lower expression of HR (HR-Neg 5%, HR-Low 4%, HR-Int 3%, HR-High 1%; p< 0.001). Additionally, NAC use was more frequent in HR-Low and HR-Int than HR-High (31%, 24% & 5%, p< 0.001). pCR rates were correlated with significantly higher OS at the 3 year (y) timepoint for all levels of HR expression (p< 0.001). NAC response had a significantly greater impact on 3y OS in the HR-Low (pCR 96.5%, no pCR [NR] 62.2%) and HR-Int (pCR 94.5%, NR 56.8%) groups compared to HR-High (pCR 96.7%, NR 91.5%).

Multivariable analyses showed significantly worse OS in HR-Neg (hazard ratio [HazR]: 2.73), HR-Low (HazR: 2.70), and HR-Int (HazR: 2.64) compared to HR-High tumors after

adjusting for age, tumor size, nodal status and grade (p< 0.001 for each). 5y OS in HR-Low, HR-Int, and HR-High groups were 78.5%, 81.4%, and 89.0% respectively. Among HR-positive BC, ET use was associated with longer OS in multivariable analyses (HazR: 0.85) with 5y OS for HR-Low (ET 82.3%, no ET 74.7%), HR-Int (ET 84.4%, no ET 73.1%), and HR-High (ET 90.0%, no ET 80.7%) groups (p< 0.001 for each).

Conclusions: HR-Low and HR-Int HER2-negative BC are rare subtypes with distinct biologic features compared to HR-High BC. This real-world analysis reveals significant differences in the survival outcomes, management, and treatment responses of HR-Low and HR-Int BCs. ET use is less common with lower HR expression yet associated with improved 5y OS. The use of immunotherapy is more frequent with lower HR expression; however, requires further investigation in HR-Low and HR-Int BC. The binary categorization of HR does not adequately address disease heterogeneity. This may contribute to outcome disparities in patients with HR-Low and HR-Int, HER2-negative BC.

P2-10-08: The Impact of Time-to-Surgery (TTS) for Breast Cancer Prognosis After Neoadjuvant Chemotherapy

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Background: Neoadjuvant chemotherapy (NAC) is a crucial component of comprehensive treatment for breast cancer, but the optimal timing for surgery post-neoadjuvant treatment remains contentious. Performing surgery too early may not allow sufficient recovery from bone marrow suppression, whereas delaying surgery might reduce the efficacy of the NAC. This study focuses on the effect of time to surgery (TTS) on survival outcomes and other clinical benefits, in an attempt to decipher an appropriate interval for breast cancer patients.
Methods: In this multi-institutional, retrospective study, patients receiving surgical resection post NAC between March 2010 and April 2022 from four medical centers in China were enrolled. Eligible patients were females aged 18 or older, diagnosed with stage II or III, or locally advanced breast cancer, who had initially been treated with neoadjuvant chemotherapy. The main exclusion criteria were bilateral breast cancer, stage IV disease, not having a full course of NAC, receiving neoadjuvant immune-related therapy or neoadjuvant endocrine therapy. Demographics, clinical characteristics, and treatment history were collected. Kaplan-Meier analysis was used to estimate overall survival (OS), disease-free survival (DFS), disease-specific survival (DSS) and local recurrence-free survival (LRFS). Univariate and multivariate Cox analyses were also conducted to discern the prognostic significance of TTS, alongside other factors including pCR status, Ki-67 index, etc.
Results: A final sample of 404 met criteria (39.9% hormone receptor-positive and 38.1% HER2-positive, 22.0% triple-negative breast cancer). Median age of diagnosis was 47, with median 45 months of follow-up. Patients were stratified into 2 groups based on the median

time interval between NAC and surgery: short-TTS group (TTS<25 days , n=194, 48.0%) and long-TTS group (TTS≥25 days, n=210, 52.0%). Patients in short-TTS group demonstrated improved OS (p=0.036) and DFS (p=0.011) compared to those with delayed surgeries beyond 25 days. However, the TTS did not significantly affect DSS (p=0.056) or LRFS (p=0.274). In univariate COX analysis, OS and DFS were significantly worse for long-TTS group, with a hazard ratio of 1.90 (95% CI, 1.04-3.48; p=0.036) and 1.72 (95% CI, 1.13-2.60; p=0.011) respectively. Multivariate analysis also verified that TTS stood as an independent prognostic factor for DFS (HR, 1.66; 95% CI, 1.08-2.55; p=0.022) rather than OS (HR, 1.87; 95% CI, 1.00-3.50; p=0.051). In subgroup analysis, we observed improved DFS in short-TTS group who did not achieve a pathologic complete response (pCR) (p=0.012) or expressed a Ki-67 index lower than 20% (p=0.040).
Conclusion: Our study indicated a better OS and DFS in all patients when surgery was performed within 25 days post neoadjuvant chemotherapy. Based on these findings, we suggest that patients might benefit from an interval of less than 25 days from the completion of NAC to surgery, especially for those with a Ki-67 index lower than 20% or those likely to have residual tumor based on imaging. For the first time, our study revealed that both pCR status and Ki-67 indices were important factors when determining the proper TTS for NAC patients. Future studies to confirm these results are warranted.
Keywords Breast cancer; Neoadjuvant chemotherapy; Time to surgery; pathologic complete response.

P2-10-09: Can an AI-based Clinical Decision Support System (CDSS) in the treatment decision-making process for breast cancer contribute to healthcare cost reduction ?

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Background: Reducing breast cancer treatment costs is vital for global patient care, especially in low-income countries, and alleviates the financial burden on healthcare systems, fostering better resource allocation for new therapies.
Material and methods :
The BreaCS consortium developed an AI-based CDSS for early breast cancer patients, targeting three questions at diagnosis : definitive tumor size (pT), nodal involvement and response to primary systemic therapy (PST). A multimodal fusion of the EUSOMA clinical data, biopsy pathology data and the preoperative MRI trained the AI based-model.
The economic impact was assessed using the real-world EUSOMA database from the consortium, comprising 5,000 early breast cancer patients. The impact on healthcare costs was calculated based on the treatment of 1,200 new early breast cancer patients annually by the consortium. Only the costs of the unnecessary surgical procedures were calculated according to the Belgian reimbursement system excluding non-surgical costs, hospital admission and other related expenses.

Results: Accurate prediction of the tumor size will refine decisions about breast-conserving surgery (BCS) versus mastectomy (ME), reducing the need for reconstructive surgeries. In our database, 42,5 % of patients had a superior pathological T stage compared to clinical staging. This led to 5% of patients requiring second breast surgery, with half undergoing a re-excision and the other half a ME. The annual cost for a second surgery for the consortium is estimated at 9000 euro.
Conversely, 40,5 % of patients had a smaller pathological T stage compared to the clinical estimation. 25 % of these patients had a pT1 while presenting with a cT2-3. Considering that the cT3 patients (approximately 3%) received unnecessary ME's, the cost for unnecessary reconstructions is estimated on a yearly basis to be 53,536 euro for the entire consortium.
Prediction of lymph node involvement will eliminate the need for any axillary surgery in pN0 patients. Of the entire population 71,7% underwent a sentinel lymph node biopsy (SLNB), of which 40,4 % had pN0. Approximately 350 SLNB could have been avoided, related to a cost of 70,000 euros on a yearly basis.
18% of patients receiving PST had a pCR. In these cases, predicting response might allow omitting all surgical procedures. For patients with a pCR, 66.4% received SLNB, 42,7 % axillary LN dissection, 38,6 % a ME and 56,8 % a BCS. All these surgeries have a combined total cost of 13,800 euro.
Conclusions: An AI-based CDSS that can predict tumor size, lymph node involvement and response to PST could reduce the surgical costs for early breast cancer treatment with approximately 150,000 euro on a yearly basis for 1200 patients. This excludes non-surgical costs, treatment of sequelae and impact on quality of life of the patients.

P2-10-10: Integration of Novel Antibody–Drug Conjugates in Advanced HER2-low, Hormone Receptor-positive and Triple-negative Breast Cancer

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Introduction: The emergence of sacituzumab govitecan (SG) and trastuzumab deruxtecan (T-DXd) into the armamentarium has changed the treatment landscape for patients with HER2-low, hormone receptor (HR)-positive and triple-negative advanced breast cancer (TNBC). SG is approved for specific patients with previously treated, advanced TNBC or HR-positive/HER2-negative breast cancer. T-DXd is approved for specific patients with previously treated advanced HER2-positive or HER2-low breast cancer, as determined by an FDA-approved test. Here, we report on an online educational program to understand current clinical knowledge about and use of SG and T-DXd by healthcare professionals (HCPs) using a small-group educational workshop experience to address challenges faced by HCPs who provide care for patients with breast cancer.
Methods: Between November 2023 and January 2024, we developed an educational program for oncology HCPs on newer antibody–drug conjugates (ADCs) in advanced breast cancer. A preactivity, case-based online survey was deployed to HCPs to assess current

clinical challenges and inform the development of specific topics for the educational workshop. A subset of HCPs was randomly selected and invited to participate in small-group workshops led by an expert in breast cancer management to discuss specific clinical challenges and provide personalized feedback on evidence-based implementation of SG and T-DXd in the treatment of patients with advanced HR-positive/HER2-negative breast cancer or TNBC. After the workshop, the learners were asked to retake the survey, and their responses were used to assess the efficacy of the educational intervention.
Results: Of 69 breast cancer oncologists who participated in the online preactivity survey, 30 were selected to participate in the workshop. Across key patient-case scenarios identified as topics of educational need with the preactivity survey, approximately 30% to 50% of workshop participants changed their treatment approach after the workshop. For example, when asked to select treatment for a woman with HR-positive metastatic breast cancer and a fluctuating HER2 immunohistochemistry (IHC) status over the course of her disease, 10% of participants selected T-DXd initially, but after the workshop, 63% opted to treat the patient with T-DXd. In another scenario, 57% opted to hold treatment and closely monitor a patient receiving T-DXd with scattered ground glass opacities in both lungs on a CT scan who is asymptomatic and has normal vital signs, including an oxygen saturation level of 98%. After the workshop, approximately 90% would hold rather than to permanently discontinue treatment. Finally, for a patient with HR-positive/HER2 IHC 0 metastatic breast cancer and progression in the liver, when asked to select a testing approach to determine the optimal next-line therapy, only 57% initially chose to repeat a tissue biopsy of the liver to assess HR/HER2 status, which increased to 90% after participation in the workshop.
Conclusions: These data highlight specific challenges that HCPs are encountering with the integration of SG and T-DXd into clinical practice. The simulation of real-life clinical situations and participation in workshops with breast cancer experts helped to inform treatment decision-making and improved HCPs application of evidence-based medicine. These results show the impact of comprehensive educational models—developed from insights from learner surveys and expert perspectives on key data—to equip HCPs with the knowledge to ensure their practice aligns with current recommendations and increase confidence in making evidence-based treatment decisions for patients.

P2-10-11: Utilization and impact of endocrine therapy in male patients with early breast cancer – results from a large German real-world claims data analysis

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Male breast cancer (BC) is a rare entity and presents a significant clinical challenge due to its late diagnosis and the resulting poorer prognosis compared to female BC. As shown by prior studies, approximately 99% of male BC patients are hormone receptor-positive (HR+).

Given the lack of large prospective trials on optimal therapy of male BC, treatment is analogous to that of female BC. The objective of this study was to analyze the use of endocrine treatment (ET) in male patients with early BC using claims data from a large German health insurance provider (AOK Baden-Wuerttemberg) and to assess the impact of ET on overall survival (OS).

Male patients who underwent surgery for early breast cancer between July 1st 2010 and December 31st 2019 were included into the analysis. As the data-set did not include information on the HR status, we assumed all patients to be HR+. Utilization of endocrine therapy was assessed based on filled prescriptions. Overall survival (OS) was defined as the period between first diagnosis of EBC until death.

In total, 144 male BC patients were included, as well as 432 age-matched controls. 21/144 patients (15%) did never use any ET. Most of the patients who received ET (93/107) were treated with tamoxifen (87%). Patients, treated with ET showed the same nodal status (N0: 72/107, 67%; N+: 35/107, 33%) as compared to patients without ET use (N0: 18/21, 86%; 3/21, 14%, p=0.091). However, patients treated with ET as compared to patients without ET were more likely to receive radiation therapy (with ET: 61/107, 57%; without ET: 2/21, 10%; $p < 0.001$) and to receive chemotherapy (with ET:54/107, 50%; without ET:1/21 (5%), $p < 0.001$). 5-year OS of patients who never started with ET was significantly reduced compared to patients with ET (without ET 37 % (95 % CI: 20 – 69 %) vs. with ET 74 % (95 % CI: 64 – 84 %), $p < 0.001$). Multivariate cox regression including age, nodal involvement, use of radiation therapy, and chemotherapy use revealed that age and intake of ET were independent prognostic factors for OS.

Although nearly all male breast cancer patients are HR+ a clinically relevant proportion never starts with ET. These patients have a significantly reduced OS as compared to patients who start with ET. Future research should focus on adherence to ET and factors that influence the utilization of ET.

P2-10-12: Pembrolizumab in Early Triple-negative breast cancer: Real world data in Hispano-American women (PETRHA) - Initial results

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Currently, the standard of care for women with stage II-III triple-negative breast cancer (TNBC) includes neoadjuvant chemotherapy combined with pembrolizumab, followed by adjuvant pembrolizumab. Notably, approximately 10% of the participants in the KEYNOTE-

522 trial were Hispanic or Latino, highlighting a gap in evidence regarding the benefit of pembrolizumab in this population. This study aims to evaluate the efficacy and safety of pembrolizumab in patients diagnosed with early TNBC treated in the region.
Hispano-American women diagnosed with early TNBC, including estrogen and progesterone receptors <10%, who received pembrolizumab as part of their systemic therapy were included in this multicenter retrospective cohort. Descriptive statistics were used to analyze sociodemographic and clinicopathological characteristics. Independent samples T-test, X2, and Fisher's exact tests were used to evaluate associations between variables, employing logistic regressions to calculate odds ratios (OR) when appropriate.
A total of 214 patients were included. 116 (54.2%) lived in Mexico, 53 (24.8%) in Argentina, 19 in Chile (8.9%), 18 in Colombia (8.4%), and 8 (3.7%) in Peru. Most women (58.2%) received cancer care in the private setting and the remaining in public/governmental settings. The median age at diagnosis was 44.0 years (IQR: 36.0 - 53.0 years), 57.7% were premenopausal, and 63.6% had node-positive disease.
Neoadjuvant pembrolizumab was administered 200 mg q3w in 83.7% and 400 mg q6w in 16.3% of patients. Seventy-three (34.1%) patients did not complete the planned number of neoadjuvant pembrolizumab cycles (8 cycles for q3w and 4 cycles for q6w). The reasons for not completing neoadjuvant pembrolizumab were immune-related adverse events (irAEs) (29/73; 39.7%), lack of access (26/73; 35.6%), disease progression (6/73; 8.2%), patient decision (1/73; 1.4%), and other (24/73; 32.9%). Patients treated in the private sector were more likely to complete neoadjuvant pembrolizumab than those in public or government-funded settings (66.7% vs 33.3%, $p = 0.002$).
The rate of pathological complete response (pCR) was 59.9% in the overall population, with a higher pCR rate among patients who completed the planned number of pembrolizumab cycles compared to those who received an incomplete number of cycles (69.8% vs. 42.5%, $p<0.01$, respectively). When considering patients who did not complete the standard number of neoadjuvant chemotherapy cycles, only an incomplete number of pembrolizumab cycles emerged as a predictive factor for non-PCR, with an OR of 0.32. irAEs occurred in 32.5% and 44.4% of those who completed and did not complete the planned number of pembrolizumab cycles, respectively. Overall, the most frequent any-grade irAEs were hypothyroidism (14.1%), rash (9.6%), and hepatitis (9.1%). No grade 5 irAEs were reported.
The efficacy and safety of neoadjuvant pembrolizumab in our population is consistent with data from the pivotal trial. However, our study highlights several key aspects regarding the treatment of early TNBC in Hispano-American women using neoadjuvant pembrolizumab. Disparities in access to pembrolizumab therapy were reflected as women with private insurance were more likely to complete pembrolizumab compared to those in public or governmental settings. Additionally, our study found a significantly lower pCR rate among women who did not complete the planned number of pembrolizumab cycles, underscoring the importance of treatment adherence. These findings emphasize the need for strategies to improve access to pembrolizumab, manage irAEs in a timely and appropriate manner, and support patients in completing their treatment regimens in our region.

This work was conducted on behalf of the Latin American Breast Cancer Association (LABCA).

P2-10-13: The Real World Practice of Prospectively Breast Cancer Screening Program for High Polygenic Risk Score Population in Taiwan

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Purpose: Breast cancer is the most common cancer among females in Taiwan, accounting for 28.5% of all newly diagnosed cancers in women. The risk of breast cancer can vary from person to person and is influenced by a combination of genetic, environmental, and lifestyle factors. Currently, mammography was the main modality of breast cancer screening in Taiwan.
A polygenic risk score (PRS) is a numerical score that is calculated based on an individual's genetic information, specifically their DNA sequence variants, in order to estimate their risk for developing a particular disease or condition. To calculate a PRS, genetic variants that have been identified as being associated with the disease or trait of interest are combined and weighted according to their effect sizes. These effect sizes represent the strength of the association between the genetic variant and the disease or trait. The weighted genetic variants are then summed to create an overall score that represents an individual's genetic risk for the disease or trait.
We designed a prospectively study to recall the patients with high PRS for receiving breast examination to determinate the correlation of PRS and breast cancer in Taiwan and demonstrate the real world practice.

Materials and Methods: The PRS of the study were estimated using genotyping data from the Taiwan Precision Medicine Initiative (registered number SF19153A). We supposed top 10% population (totally 1001 patients) for the definition of high PRS, and targeted the patient between 35 to 60 years old. We tried to call back these patients to receive breast examination since 1st April, 2023, and collected their baseline characteristics by structured questionnaire. Breast sonography is the main modality for examination, if the patient is older than 45 years old or 40 years old with family history of breast cancer, mammography is also performed.

Results: Total 599 patients between 35 to 60 years old were selected as high PRS. We contacted all of them, but only 140 patients (23.5%) came back to our hospital and received breast examination. The other patients refused to receive examination (n=69, 11.5%), visited other hospital (n=60, 10%), or had incomplete contact information (n=330, 55%). The median age of these 140 patients was 46.7 years old, and the youngest patient was 39 years old.
Eventually, 5 cases (3.6%) were diagnosed as breast cancer. Family history of cancer, menopause status, exposure to contraceptive agents, less times of pregnancy and delivery, lack of exercise, and alcohol consumption seemed like risk factors for breast cancer in this

screening program, but had no statistically significant difference.

Conclusion: In real world practice, it was still a predicament that the called-back rate of PRS breast cancer screen program was still low (23.5%). Nevertheless, the breast cancer detection rate for high PRS in our study was 3.6%, which is higher than the mammography-based screening program in Taiwan (0.5%). There is a long way to go to promote this program and make it a useful and effective tool for breast cancer screening.

P2-10-14: HER2 evolution in breast cancer: Evidence from an AI-assisted real-world data analysis of 3 million women in a diverse urban population

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Introduction: HER2 positive status gives breast cancer patients a wide range of treatment options. Previous HER2-targeted therapies did not show benefit in patients with low HER2 expression who were treated in the same way as HER2 negative cancers. Regulatory approval of trastuzumab deruxtecan for patients with low HER2 expression addresses a significant unmet medical need and impacts HER2 status reporting. As part of a qualitative exploratory analysis we utilized a novel AI-based approach to analyze a large unstructured real-world dataset, providing insights into biomarker testing and real-world prescribing practices. We found a number of patients with multiple HER2 statuses reported at different points in their patient journey.
Methods: A retrospective analysis of Real-World Data (RWD) was performed on 3 million females aged over 18, using data from a health information exchange (HIE) that includes six urban health systems in the US, varying significantly in size and activity profile. A total of 25 million clinical documents - principally visit and discharge summaries - were analyzed using Natural Language Processing (NLP) specifically optimized for medical language. This analysis generated a curated cohort of 17,125 female breast cancer patients diagnosed and actively treated between August 2021 and August 2023 at three of the six health systems, which contributed most of the cohort. Patients were included in the curated cohort if their data showed evidence of at least two relevant biomarker tests, such as ER/PR, HER2, BRCA1/2, PIK3CA, AKT1, or PTEN. Patient records demonstrating discordant results where the treating physician had recorded HER2 low, HER2 positive and HER2 negative findings either at the same time or at different times were manually examined. Patients who had been described as: “HER2 low”; “HER2: 1+”; “HER2: 2+ with no amplification by FISH”; “HER2: 2+, negative by FISH” or variations of these statements with the same meaning were considered HER2 low. Patients with “HER2: 0” or “HER2 negative” were considered HER2 negative. Patients with “HER2: 2+” but no qualifying reference to the result of a FISH test were considered HER2 positive.
Results: Distribution by age group and stage in the curated cohort of 17,125 patients closely matched national statistics. Evidence of biomarker testing was strongest for ER/PR, then

HER2, then BRCA1/2, and finally PIK3CA, AKT1, or PTEN in descending order of frequency, consistent with the evolution of breast cancer care. A total of 55 patients with discordant results showing all three possible values of HER2 status were found. Transitions from HER2 positive to either HER2 low then HER2 negative or to HER2 negative then HER2 low occurred at a similar rate to transitions to HER2 positive from either of the other statuses. Conclusions: AI-supported analyses of very large RWD datasets can be conducted rapidly, revealing patterns of evolving disease and practice across entire populations and within subgroups with complex inclusion/exclusion criteria. Our study shows that reporting of HER2 positive, HER2 low and HER2 negative status in tumor samples from the same patient is more common and dynamic than previously thought. Further research is needed to understand the evolution of HER2 status, including its relationship to disease progression and treatment with potentially significant implications for clinical practice.

P2-10-15: Reproducibility of the interactions of race on outcomes and toxicities associated with treatment of HER2+ Breast Cancer across databases

Britney Fitzgerald, Justin Petucci, Vasant Honavar, Monali Vasekar

Objective: Investigate the reproducibility of the interaction of race on outcomes and toxicities associated with HER2+BrCa treatment across multiple data sources.

Background: HER2+ breast cancer (BrCa) is an aggressive subtype, accounting for 20-30% of all BrCa cases. Trastuzumab, a monoclonal anti-HER2 antibody, remains the cornerstone of treatment. Previously, we identified race as a factor associated with increased toxicities and poorer outcomes in HER2+BrCa treatment using the TrinetX Database. Further investigation into the outcomes and toxicities in minority groups is necessary, emphasizing the importance of utilizing databases for such research.

Design/Methods: In this propensity score-matched cohort study we used the TriNetX Research Network to compare mortality of HER2+BrCa in non-Hispanic African American (NHAA) women to a corresponding non-Hispanic White cohort (NHW). Qualification into the two race based HER2+BrCa cohorts required the presence of a C50 ICD-10-CM diagnosis code and at least one proxy for HER2 positivity such as Trastuzumab (index event). Cohorts were matched for age, BMI, comorbidities, and lab values using 1:1 matching with a greedy nearest neighbor search. Toxicity outcomes were also compared between the cohorts. The associations of observed outcome frequencies in the two cohorts were tested for significance using the chi-square test. The odds ratios with 95% confidence intervals, are reported as an effect size and significance estimation. As a test of robustness of the results based on the TriNetX data, we compared the outcome of all time mortality after diagnosis across unmatched NHAA and NHW cohorts using the well-established SEER and NCDB patient-level databases.
Results: Using SEER, 68,697 patients met the inclusion criteria for HER2+BrCa (57,554

NHW, 11,143 NHAA). The median age at the index event was 60-64 years for NHW and 55-59 years for NHAA. In the unmatched HER2+BrCa cohorts, the odds of death for NHAA were 1.32 times higher than for NHW, with a P-value of < 0.00001. Using NCDB, 352,553 patients met the inclusion criteria (305,219 NHW, 47,334 NHAA). In these cohorts, the odds of death for NHAA were 1.61 times higher than for NHW, also with a P-value of < 0.00001. Using TriNetX, the odds of death at 5 years after diagnosis for NHAA were 1.1379 times higher than for NHW in the unmatched HER2+BrCa cohorts, with a 95% confidence interval. TriNetX data also showed that NHAA women had significantly increased odds of neuropathy and cardiomyopathy at 1, 3, and 5-year intervals after the HER2+BrCa index date, compared to NHW women. Additionally, the odds of an emergency room visit (for any reason) were up to 79% higher in NHAA women compared to their NHW counterparts. There was a lack of detailed toxicity data points, such as neuropathy and cardiomyopathy, in the NCDB and SEER databases, preventing cross-comparison.
Conclusion: We found increased odds of mortality for NHAA patients compared to NHW patients with HER2+BrCa undergoing Trastuzumab therapy across all three databases. Although toxicity data could not be directly compared across sources due to lack of granularity, the consistency of mortality findings highlights the reliability of our previous results from the TriNetX Database. In TriNetX, NHAA women with HER2+BrCa demonstrated higher odds of experiencing neuropathy, cardiomyopathy, and emergency room visits compared to their NHW counterparts. These results emphasize the importance of considering racial impact on HER2+BrCa outcomes and highlight the utility of real-world data sources like TriNetX for exploring BrCa outcomes and treatment toxicities. Our findings suggest the need for further research and targeted interventions to address disparities and improve treatment outcomes in diverse populations.

P2-10-16: A randomized phase III clinical trial of yoga for chemotherapy-induced peripheral neuropathy treatment (YCT)

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Background: Chemotherapy-induced peripheral neuropathy (CIPN) is a common and debilitating side effect among cancer survivors receiving neurotoxic chemotherapy that can cause functional disabilities and significantly increase the risk of falls. Currently, there are limited approaches to managing CIPN symptoms and related functional limitations. We conducted a pilot study that demonstrated yoga therapy's feasibility and preliminary efficacy in improving CIPN pain and functional outcomes. We then developed the YCT trial to assess the effect of yoga therapy on improving pain and balance symptoms among cancer survivors with CIPN pain.

Methods: YCT is a prospective phase III multicenter, parallel three-arm randomized clinical trial at Dana-Farber Cancer Institute (DFCI) and Memorial Sloan Kettering Cancer Center (MSK) (ClinicalTrials.gov Identifier: NCT05121558). YCT aims to determine the efficacy of

an eight-week yoga treatment vs. education control (EC) vs. usual care (UC) in improving CIPN pain and balance in cancer survivors. We plan to enroll and randomize 268 participants (2:1:1) to yoga, EC, and UC groups to detect an effect size of at least 0.48 for the primary pain outcome at eight weeks post-randomization between yoga vs. EC, with 80% power and a 2.5% Type I error rate, assuming 12% attrition by week 8 and 20% attrition by week 24. Major eligibility criteria include adults who 1) have no evidence of disease or stable diseases, 2) have completed neurotoxic chemotherapy such as platinum agents, taxanes, vinca alkaloids, and bortezomib at least three months before enrollment, 3) have a clinical diagnosis of CIPN with moderate to severe pain, defined as a score of at least four on the Brief Pain Inventory (BPI) average pain item, and 4) have experienced self-identified CIPN balance difficulty. Eligible subjects in the yoga arm will receive hourly gentle therapeutic yoga classes taught by protocol-trained oncology yoga instructors, twice weekly for eight weeks, and practice home-based yoga. Subjects in the EC arm will receive hourly education classes taught by protocol-trained healthcare instructors twice weekly for eight weeks. Subjects in the UC arm will continue usual care for eight weeks. We offer free yoga sessions for participants in control groups after study completion. Aside from patient-reported outcome measures (i.e., BPI, FACT/GOG-Ntx, QLQ-CIPN 20), we measured functional improvements by functional reach, timed up-and-go, and chair-to-stand tests. We also conducted quantitative sensory testing to assess changes in sensory function. Progress: 1) We are transitioning this trial to a multicenter trial, with DFCI as the coordinating center and MSK as a subsite; 2) As of July 9, 2024, 126 of the planned 268 participants have been enrolled. We anticipate initiating participant enrollment at DFCI in August 2024. The anticipated accrual completion is December 2025.

P2-10-17: A randomized phase III study of first-line saruparib (AZD5305) + camizestrant vs CDK4/6i plus physician's choice endocrine therapy or + camizestrant in patients w/ BRCA1/BRCA2/PALB2 mutations & HR+/HER2- advanced breast cancer (EvoPAR-Breast01)

Pedram Razavi, Judith Balmaña, Stephen J. Luen, Mario Campone, Laura Cortesi, Norikazu Masuda, Kyong Hwa Park, Qingyuan Zhang, Emily Nizialek, Cathy Qi, Karen Cui, Sibylle Loibl, Mark Robson, Filipa Lynce

Background:Emerging evidence indicates that homologous recombination deficiency (HRD) contributes to resistance to cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) plus endocrine therapy (ET). Patients with germline or somatic (g/s) mutations in BRCA1, BRCA2, and/or PALB2 genes (BRCA1m/BRCA2m/PALB2m) and hormone receptor (HR)-positive/human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer have poorer outcomes with first-line standard-of-care CDK4/6i plus ET than patients without these mutations. Clinical benefit with poly(ADP-ribose) polymerase inhibitors (PARPi) has been demonstrated in patients with HRD breast cancer, and PARPi are approved for the treatment of patients with gBRCA1/BRCA2m and HR-positive/HER2-negative early or advanced breast cancer. Clinical trials have shown that PARPi use in early

lines of therapy can result in a greater magnitude of benefit. PARPi use may also induce reversion mutations that restore homologous recombination proficiency, potentially sensitizing tumors to CDK4/6i. Saruparib (AZD5305) is a first-in-class highly selective PARP1 inhibitor that has increased potency and improved pharmacokinetic and pharmacodynamic properties compared with other approved PARPi. The phase III EvoPAR-Breast01 study (NCT06380751) is evaluating the efficacy and safety of saruparib plus camizestrant, a next-generation oral selective estrogen receptor degrader (SERD) and pure estrogen receptor antagonist, vs physician's choice of CDK4/6i plus ET or CDK4/6i plus camizestrant in participants with g/s BRCA1m/BRCA2m/PALB2m HR-positive/HER2-negative advanced breast cancer.
Trial design:EvoPAR-Breast01 is a randomized, open-label, 3-arm, multicenter, global study. This study will enroll pre-, peri-, and postmenopausal women, and men ≥18 years of age with histologically confirmed estrogen receptor-positive (expression in >1% of cells, irrespective of progesterone receptor status) and HER2-negative (immunohistochemistry score of 0, 1+, 2+/in situ hybridization non-amplified) advanced breast cancer. Patients must have an ECOG PS 0–1 and known BRCA1m/BRCA2m/PALB2m identified by local germline or central tumor testing. ET is permitted up to 28 days prior to randomization. Patients with disease progression ≤84 days from the last dose of (neo)adjuvant chemotherapy for early breast cancer or ≤365 days from the last dose of adjuvant CDK4/6i, PARPi, and/or platinum chemotherapy, or oral SERD for early breast cancer are excluded, as are patients who have received prior systemic treatment for locoregionally recurrent or metastatic breast cancer. Patients with uncontrolled cardiovascular disease and history of, or suspected, myelodysplastic syndrome/acute myeloid leukemia are not eligible for inclusion. Participants will be randomized 2:2:1 to receive saruparib plus camizestrant, physician's choice CDK4/6i (abemaciclib, ribociclib, or palbociclib) plus physician's choice ET (fulvestrant, letrozole, anastrozole, or exemestane), or physician's choice CDK4/6i plus camizestrant, respectively. Treatment will continue until disease progression per RECIST v1.1, unacceptable toxicity, or participant-initiated withdrawal. The primary endpoint is progression-free survival (PFS) by blinded independent review committee in the saruparib plus camizestrant vs CDK4/6i plus ET arms. Overall survival (OS) is a secondary endpoint. Planned statistical analyses of PFS and OS will be conducted using a stratified log-rank test. Participant enrollment is ongoing. Approximately 500 participants will be randomized across the three arms.

P2-10-18: A study to evaluate the safety and efficacy of vosilasarm (EP0062), an oral SARM, as monotherapy and in combination with standard of care regimens in patients with relapsed locally advanced or metastatic AR+/HER2-/ER+ breast cancer

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Ezquerra, Aidan Doherty, Mags McDonagh, Sital Patel, Geoff Fisher, Sue Brook, Hendrik-Tobias Arkenau, Hyo Han

Abstract: AR activation exerts potent antitumor activity across a number of ER+ /AR+ breast tumors, including those resistant to standard-of-care endocrine therapy and CDK4/6 inhibitors (Hickey et al, Nature Medicine 2021 27: 310-320). Agonist activation of AR alters the genomic distribution of ER and essential co-activators resulting in repression of ER-regulated cell cycle genes and upregulation of AR target genes, including known tumor suppressors. The clinical activity of SARMs has now been confirmed in several studies (LoRusso et al. Clinical Breast Cancer 2022 22;1 67-77, Palmieri et al Lancet Oncol 2024 25: 317-325).
Vosilasarm (EP0062) is an oral, non-steroidal, Selective Androgen Receptor Modulator (SARM) in development for the treatment of AR+/HER2-/ER+ locally advanced or metastatic breast cancer. This ongoing study (NCT05573126) is evaluating the safety and initial efficacy of vosilasarm in patients with AR+/HER2–/ER+ locally advanced or metastatic breast cancer as monotherapy and in combination.
Module A, which is ongoing, is comprised of dose optimisation cohorts in a 3+3 design, to investigate safety, tolerability, PK and PD and to define the optimum dose for planned combination expansions. 20 patients have been included to-date across 4 dose cohorts.
In Module B, vosilasarm is being evaluated in combination with standard-of-care therapies in patients with relapsed AR+/HER2-/ER+ locally advanced or metastatic breast cancer to confirm safety and explore efficacy. Approximately 20 patients will be included initially across 2 cohorts:

Cohort 1. Vosilasarm + elacestrant in patients with AR+/HER2-/ER+/ESR1mut + advanced/metastatic breast cancer that has progressed on one or two prior lines of endocrine therapy, including prior CDK4/6 inhibitor
Cohort 2. Vosilasarm + everolimus + exemestane in patients with AR+/HER2-/ER+ advanced/metastatic breast cancer that has progressed on a prior endocrine therapy + CDK4/6 inhibitor

Key inclusion criteria are as follows:

Post-menopausal women, ≥18 years
ECOG performance status of 0 to 1
Locally advanced or metastatic breast cancer
ER+, HER2- as per ASCO CAP guidelines
AR+, as defined as ≥ 10% AR nuclei staining by IHC
Endocrine-therapy-sensitive breast cancer defined as:

a) greater than 2 years of adjuvant endocrine therapy prior to development of advanced or metastatic disease,
OR
b) previous response (without disease progression for at least 6 months) to one of the

following treatments in the advanced/metastatic setting: SERD +/- CDK 4/6 inhibitor, AI +/- CDK 4/6 inhibitor

Measurable disease defined by RECIST version 1.1, or bone-only disease

The vosilasarm dose will be selected from the findings of the dose finding/optimisation cohorts in Module A. Combination agents will be initiated at approved doses. An initial 3-6 patients will be enrolled in order to assess safety and tolerability of vosilasarm + the combination agent and to establish the appropriate combination dose for vosilasarm, with subsequent expansion to 10 patients per cohort. Cohorts may be expanded to 25 patients dependent on safety and efficacy findings.
Endpoints include incidence and severity of AEs and SAEs, plasma PK parameters, Clinical Benefit Rate (CBR, complete response, partial response, or stable disease) at 24 weeks, ORR, duration of response, PFS, OS and quality of life.
Planned blood and tissue biomarkers in this study include prostate-specific antigen (PSA), cancer antigen (CA) 15-3, and circulating tumour DNA (ctDNA).
Recruitment of combination cohorts will be initiated in Q4 2024.

P2-10-19: A011801 (CompassHER2 RD): Postneoadjuvant T-DM1 + tucatinib/placebo in patients with residual HER2-positive invasive breast cancer

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Background: Patients with HER2+ early breast cancer (EBC) and invasive residual disease (RD) after neoadjuvant therapy (NAT) have a higher risk of relapse than patients who have a pathologic complete response (pCR). Escalation of therapy in patients with RD using post-neoadjuvant T-DM1 has become the new standard of care, leading to improved invasive disease-free survival (iDFS), but patients with estrogen receptor (ER)-negative or nodal RD have suboptimal outcomes, and central nervous system recurrences are a challenge. More effective treatment strategies are urgently needed. A011801 (CompassHER2 RD) is an escalation trial for patients with high-risk HER2+ RD after neoadjuvant systemic therapy, evaluating the addition of the HER2 selective tyrosine kinase inhibitor (TKI) tucatinib to post-neoadjuvant T-DM1. Methods: Eligibility and Intervention: Patients with high-risk HER2+ RD (i.e. ER-, node-positive, or both) after a predefined course of neoadjuvant HER2-directed treatment are randomized 1:1 to adjuvant T-DM1 + placebo, vs. T-DM1 and tucatinib with adjuvant RT +/- ET. Eligibility criteria include completion of ≥ 6 cycles of NAT, including ≥ 9 weeks of paclitaxel and trastuzumab +/- pertuzumab. All chemotherapy (CT) must be completed preoperatively unless participating in EA1181 (~15-30% will be participants in the CompassHER2 pCR de-escalation companion trial); these patients must receive postoperative CT to complete ≥ 6 cycles prior to enrollment on A011801. Pts who

received prior HER2-targeted TKIs or antibody-drug conjugates are ineligible. Objectives: The primary objective is to determine if iDFS is improved with addition of tucatinib to T-DM1 in patients with HER2+ EBC with RD after neoadjuvant systemic therapy; secondary endpoints include overall survival, breast cancer free survival, distant recurrence-free survival, brain metastases-free survival and disease-free survival. Correlative objectives include the association of i) tumor infiltrating lymphocyte (TILs) levels in the primary tumor and RD with iDFS, ii) TILs with tucatinib benefit, iii) iDFS and circulating tumor cells (CTC) at serial timepoints and iv) the magnitude of benefit of tucatinib (iDFS) in patients with/without detectable pretreatment CTCs. Quality of life and pharmacokinetic endpoints are also being evaluated. Statistics: A011801 is a prospective, double-blind, randomized, phase III superiority trial; stratified by i) receipt of postoperative CT (Y/N), ii) hormone receptor-status (+/-), and iii) pathologic lymph node status (+/-). The study targets an absolute difference of 5% in iDFS (control vs. experimental arm 82% & 87%, HR = 0.7), with a two-sided alpha of 0.05 and power of 80%. The sample size is 981; target accrual = 1031 pts; activation and estimated completion dates are 01/6/21 and ~ 01/2028. Accrual as of 7/1/2024: 741 patients. Support: U10CA180821, U10CA180882; Pfizer Inc; ClinicalTrials.gov Identifier: NCT04457596

P2-10-20: ACE-BREAST-03: A phase 2 trial evaluating ARX788, an anti-HER2 antibody drug conjugate (ADC), for the treatment of HER2+ metastatic breast cancer (mBC) in patients who have been previously treated with trastuzumab deruxtecan (T-DXd)

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Background: HER2-targeted ADCs revolutionized treatment for patients with HER2+ mBC. The HER2-targeted ADC, T-DXd, improved PFS and OS vs T-DM1 as second- line treatment for HER2+ mBC and is now the preferred treatment option in this setting. However, improved treatment options are needed for patients with disease progression on T-DXd. Novel technological advances in design of the antibody, linker, and payload of ADCs may further improve the safety/tolerability and efficacy, widening the therapeutic window to address resistance to other anti-HER2 therapies.
ARX788 is a next-generation anti-HER2 ADC stably conjugated to AS269 (a potent tubulin inhibitor). Proprietary site-specific oxime conjugation chemistry, enabled by incorporation of synthetic amino acids into the antibody construct, maximizes the delivery of the cytotoxic payload to tumor cells and minimizes systemic exposure to free payload. A phase 2/3 study in China demonstrated that ARX788 had significantly improved PFS compared with the combination of lapatinib and capecitabine (HR 0.64 [0.49, 0.82], p = 0.0006) in patients treated with prior trastuzumab and taxane (ASCO 2024 Abstract 1020). In addition, ARX788 has shown antitumor activity in heavily pretreated patients with HER2+ and HER2-low

mBC, including those with prior T-DXd exposure.
Methods: ACE-Breast-03 (NCT04829604) is a phase 2 trial evaluating ARX788 in patients previously treated with T-DXd. The primary efficacy endpoint is response rate by RECIST v1.1. Approximately 40 patients will be enrolled. Eligible patients have HER2+ mBC and up to 5 prior treatment regimens for mBC, including T-DXd. Patients with stable brain metastases who are off steroids are eligible. Treatment includes 1.5 mg/kg Q3W ARX788 until unacceptable toxicity or disease progression. Tumor status is assessed every 9 weeks through 27 weeks and every 12 weeks thereafter. The study is currently enrolling in the US.

P2-10-21: ADELA: A randomized, phase 3, double-blind, placebo-controlled trial of elacestrant plus everolimus versus elacestrant in ER+/HER2-advanced breast cancer (aBC) patients with ESR1-mutated tumors progressing on endocrine therapy (ET) plus CDK4/6i

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Background: ET+CDK4/6i is the standard-of-care (SOC) in 1L ER+/HER2- aBC; however, tumors eventually develop resistance. Constitutive activation of the PI3K/AKT/mTOR pathway can contribute to endocrine resistance in breast cancer. A common type of acquired resistance mechanism consists of alterations in the estrogen receptor 1 (ESR1) gene. ESR1 mutations occur in 40-50% of patients with breast cancer and predominantly emerge in the metastatic setting after prolonged exposure to AI regimens. There is an unmet need for novel therapeutic approaches to overcome different resistance mechanisms and improve clinical outcomes in patients with ER+/HER2- aBC with ESR1-mutated tumors who progress following ET+CDK4/6i. Elacestrant is a next-generation oral SERD that binds to the estrogen receptor alpha and induces its degradation. In the EMERALD study, single-agent elacestrant demonstrated a statistically significant and clinically meaningful improvement in PFS versus SOC ET in patients with ESR1-mutated tumors (HR = 0.55; 95% CI, 0.39-0.77; P = 0.0005) [Bidard 2022]. Differences were particularly notable among patients who received prior ET+CDK4/6i ≥12 months; median PFS with elacestrant was 8.6 months vs 1.9 months with SOC ET (HR = 0.41; 95% CI, 0.26-0.63) [Bardia, SABCS 2022]. The crosstalk between the ER and PI3K/AKT/mTOR pathways provides a rationale for evaluating the combination of elacestrant with specific PI3K/AKT/mTOR inhibitors. Everolimus, an mTORC1 inhibitor, is indicated for the treatment of postmenopausal women with HR+/HER2- aBC in combination with exemestane after progression on non-steroidal AIs. The ADELA study aims to compare elacestrant + everolimus vs elacestrant + placebo in ER+/HER2- aBC patients with ESR1-mutated tumors progressing on ET+CDK4/6i.
Methods: ADELA (NCT06382948) is an international, multicenter, phase 3, randomized, double-blind, placebo-controlled trial. Eligible patients are adult women or men with ER+ (≥10% positive stained cells), HER2- aBC with ESR1-mutated tumors, previously treated

with at least one and no more than two lines of ET for aBC, and evidence of disease progression on prior treatment with a CDK4/6i in combination with ET for aBC after ≥ 6 months. Patients receiving CDK4/6i-based therapy in the adjuvant setting are also eligible, provided that disease progression is confirmed after ≥ 12 months of treatment but <12 months following CDK4/6i treatment completion in this scenario. Other criteria include measurable (RECIST v.1.1) or evaluable disease, adequate bone marrow and organ function, and ECOG PS 0-1. Exclusion criteria include prior chemotherapy for aBC, active uncontrolled or symptomatic brain metastasis, and/or leptomeningeal disease. Patients will be randomized 1:1 to 28-day cycles of oral elacestrant 345 mg + everolimus 7.5 mg QD or elacestrant 345 mg + placebo QD until disease progression or unacceptable toxicity, based on the dose determined in the ongoing ELEVATE trial (NCT05563220). Patients will receive dexamethasone mouthwashes four times daily during the first 8 weeks. Stratification factors are presence of visceral metastases (yes vs no) and duration of prior CDK4/6i-based therapy (≥12 vs <12 months). The primary endpoint is blinded independent review committee (BIRC)-assessed PFS. Secondary endpoints include investigator-assessed PFS and OS, BIRC- and investigator-assessed ORR, CBR, DoR, TTR, and best percentage change in target lesions, as well as safety and quality of life. PFS will be estimated using the Kaplan-Meier method. Stratified log-rank tests will be used to assess treatment-group differences. The planned enrollment is 240 patients.

P2-10-22: APOBEC3 as a Biomarker of Response to Neoadjuvant Chemotherapy in Breast Cancer: A Pilot Study

Enrico Moiso, Aaron Cheng, April Risinger, Shraddha Dalwadi, Robert Marciniak, Reuben Harris, Kate Lathrop

Introduction: It is widely accepted that invasive breast cancer is comprised of multiple molecular subtypes that can be predictive of benefit to specific therapeutic interventions and prognostic as to risk of recurrence and development of metastatic disease. However, the molecular profiling of invasive breast cancer is fluid and an area of rapid discovery. The APOBEC3 family of proteins, particularly APOBEC3A (A3A) and APOBEC3B (A3B), are DNA deaminases that have been linked to mutational signatures in breast cancer. Retrospective studies have shown A3B mRNA expression is associated with decreased overall survival and resistance to tamoxifen and worse outcomes in patients on hormonal therapy and a CDK4/6 inhibitor. However, prospective trials evaluating the association between A3A and A3B expression with response to current neoadjuvant therapies are lacking.
Methods: We are conducting a non-randomized, single institution window trial to examine the association between A3A and A3B expression and pathologic response to standard of care neoadjuvant chemotherapy regimens. The eligible study population includes: all subtypes of breast cancer, stage II or III, available diagnostic tissue for APOBEC3 expression testing, anticipated neoadjuvant therapy followed by surgery. APOBEC3A/A3B expression, both at mRNA and protein levels, will be defined on the initial diagnostic tissue and the post neoadjuvant therapy tissue in patients with residual disease and compared to pathological

complete response rate. APOBEC3A/3B expression will also be correlated to the different major histology groups: hormone receptor positive, HER2 positive, and triple negative. This trial opened July 2023 and has accrued at the time of submission 35 patients. Target accrual is 100 patients. All patients are recruited in the breast medical oncology clinic at the Mays Cancer Center.
Anticipated Results and Conclusions: At the time of presentation, we anticipate having 50% of our targeted patient accrual on trial with 30% of patients with pre and post neoadjuvant clinical data with correlation to pathological response. Based on preclinical data, we expect altered sensitivity to neoadjuvant treatment in APOBEC3 expressing tumors. Understanding the role of APOBEC3 enzymes in early phase of breast cancer treatment response will identify patients at risk for treatment resistance and thus present an opportunity to target this patient population with novel therapeutic approaches.

P2-10-23: ASSESSING CLINICAL FEATURES AND OUTCOME OF BREAST CANCER IN PALB2 MUTATION CARRIERS: AN EUROPEAN MULTICENTRIC RETROSPECTIVE HOSPITAL-BASED COHORT CONTROL STUDY (PALBREAST)

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Background: PALB2 is a breast cancer susceptibility gene that encodes the BRCA2-interacting protein. Mono-allelic mutations of PALB2 are associated with an increased risk for breast and ovarian cancer in women, prostate cancer in men, and pancreatic cancer in both genders. Women with no family history of breast cancer have a cumulative risk of 33%, compared to 58% in women with two or more family members with breast cancer. Several studies aimed to describe breast cancer phenotypic characteristics in PALB2 mutation carriers. Some of these studies suggested an association with triple-negative phenotype, older age at diagnosis (>60 years), tumor size > 2 cm, negative HER2 status, lymph nodes positive and bilaterality. Nevertheless, results among different studies are contradictory and no data on prognosis of these patients are reported. Furthermore, the clinical potential of PARP inhibition beyond currently approved indications to additional patients whose tumors have (epi)genetic changes affecting homologous recombination repair (HRR) raises new interest in PALB2 mutations as molecular target. So far, the study is aimed to collect, in an European dataset, a large number of PALB2 related BC characteristics starting from 2010 until now and to evaluate the outcome of those patients. This database will be useful for establishing the clincal needs for PALB2 patients, particularly in order to design new interventional studies with target therapies
Trial design: PALBREAST is a multicenter hospital-based retrospective cohort control study conducted across several Institutions with known expertise in managing hereditary breast cancer. It is planned to enroll at least 300 PALB2-related BC diagnosed at the different Units

and 300 BC as control group tested for multigene panel and resulted negative for mutations. It is expected to complete the accrual in one year; furthermore, a minimum follow-up of one more year is required for the final analysis of primary and secondary endpoints. Each participant with PALB2 mutations and BC will be registered in the database where age and modality at diagnosis, type of surgery (quadrantectomy vs. mastectomy vs bilateral mastectomy), tumor characteristics (grading, histology, carcinoma in situ vs invasive, multifocal vs multicentric, proliferation rate, hormonal receptors, nodal status) stage, age at relapse, treatment (neoadjuvant vs adjuvant, chemotherapy vs hormonal treatment), follow-up status will be inserted. A CT/PET for the last follow-up will be performed at the end of the study. All participants will be followed by telephone for overall survival until the end of the study.
The primary end-points are incidence and mortality rates of gPALB2-BC. Secondary end-points are to evaluate the modalities of diagnosis and the imaging characteristics, the type of surgery (bilateral vs. monolateral), the somatic PALB2 mutations rates evaluated by centralized RAD51 assay in the control group, the Disease Free Survival (DFS), and Overall Survival (OS) in comparison with patients with somatic mutations and wild-type PALB2 breast cancer, adjusted for age, stage and period of diagnosis, matched for age, stage, subtype and diagnosis period. Also, the type of therapy (chemotherapy, hormonal treatments and target therapies) and the rate of second BC and other tumors in the patients and in the family will be evaluated. Planned statistical analyses of DFS and OS will be conducted using a stratified log-rank test. Participant enrolment is ongoing. Ninety-seven patients have been already registered in three different centers in Italy.
Clinical trial identification: NCT06403904
Funding: This study was supported by AstraZeneca.

P2-10-24: ASTRO-VAC CNS : Bria-IMT in the Management of Tumor Agnostic Metastatic CNS Lesions

Sailaja Kamaraju, Jonathan Thompson, Deepak Kilari, Ben George, Blaise Bayer, Tamar Aghajanian, William Williams, Charles Wiseman, Giuseppe Del Priore

Background: The Bria-IMT regimen has demonstrated the ability to induce a robust immune response and achieve disease control in heavily pre-treated advanced breast cancer (BC) patients. Bria-IMT is an irradiated allogeneic BC cell line that has been engineered to secrete GM-CSF and may function as antigen-presenting cells. Bria-IMT antigens are taken up by dendritic cells and presented to T cells, generating a tumor-directed immune response. Prior presentations report that Bria-IMT is able to induce regression of intracranial metastases. In the ongoing randomized phase 2 and (NCT03328026) and previously completed trials, a reduction in the size and/or number of intracranial lesions was observed in 5 out of 6 evaluable patients with a median maximal decrease of 60% (range -42% to -100% decrease) in the sum of the diameters in multiple BC subtypes. Bria-IMT is well tolerated with no concerning adverse events (AEs). Disease control after prior antibody-drug conjugates (ADCs) has been observed in 40% of patients, and clinical benefit has been

seen in 5/7 patients with untreated intracranial metastases. CD8+ Immuno PET shows an increase in CD8+ tumor infiltrating lymphocytes suggesting systemic activation and infiltration into lymphoid organs and metastatic sites. Sequencing of CPI with BC and the latest phase 3 formulation are associated with improved clinical outcomes, including OS (median 13.4 months), PFS, and CBR, which are reflected in the design of this ongoing pivotal registration enabling Phase 3 study.
[https://clinicaltrials.gov/study/NCT06072612]. Methods: This is a single site, open label, single-arm, phase 1, multi disease basket trial to evaluate the feasibility and efficacy of the Bria-IMT regimen in combination with pembrolizumab (CPI) in patients with CNS metastases who progress on SOC therapy. Eligibility encompasses all solid tumors with Life expectancy of ≥ 12 weeks and ECOG ≤ 2. Prior surgical resection and/or radiation therapy to CNS lesions are permitted following recovery from these procedures prior to enrollment. Prior immune therapy is allowed if administered more than 6 weeks before the first cycle of Bria-IMT. The Bria-IMT regimen includes: Day -2/-3 Cyclophosphamide 300mg/m2, Day 0; 20 million irradiated SV-BR-1-GM cells intradermally in 4 sites, and 0.6 mcg peg α interferon subcutaneously in the inoculation sites. CPI will be administered every cycle q3wks on day -2/-3. The primary endpoint is to evaluate the safety and feasibility of Bria-IMT in combination with pembrolizumab in patients with solid tumor CNS metastasis. Safety of the treatment will be measured by the frequency and severity of adverse events (AEs). Incidence of dose-limiting toxicities (DLTs), including immune-related toxicities and treatment-related toxicities. Key secondary endpoints will be intracranial disease control rate (measured as ORR, SD, PR, CR, PFS), extracranial disease control (measured as ORR, SD, PR, CR, PFS), and radiographic progression-free survival. Follow-up will be continued for 3 months after treatment termination. Each subject is anticipated to participate in the trial for approximately 6 months unless withdrawn from study or is lost to follow-up before the end of the study. Clinical trial identification Short Title: ASTRO-VAC., Sponsor: Bria Cell

P2-10-25: AXSANA: Status of the international prospective multicenter cohort study evaluating different surgical methods of axillary staging in clinically node-positive breast cancer patients treated with neoadjuvant chemotherapy (NCT04373655)

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Background: The optimal surgical staging procedure of the axilla in patients who convert from a clinically positive (cN+) to a clinically negative node status (ycN0) after neoadjuvant chemotherapy (NACT) is still controversial. Diverse techniques such as Axillary Lymph Node Dissection (ALND), Targeted Axillary Dissection (TAD), Target Lymph Node Biopsy (TLNB), and Sentinel Lymph Node Biopsy (SLNB) alone are given preference in different international guidelines. So far, no prospective comparative data on the oncological outcome or the morbidity by procedure are available. Further research is needed to de-escalate axillary surgery in this patient group safely.

Trial design

AXSANA is an international prospective cohort study including initially cN+-patients converting to ycN0-status after NACT and treated with different axillary staging techniques according to the standard at their treating institution. AXSANA was initiated by the EUBREAST (European Breast Cancer Research Association of Surgical Trialists) network and includes patients with cT1-4c tumors, who present initially with axillary lymph node metastasis scheduled for neoadjuvant chemotherapy. According to an amendment in 2020, the inclusion of patients with highly suspicious nodes without confirmation using a minimally invasive biopsy is allowed. All patients converting to ycN0 status undergo follow-up for 5 years regardless of the ypN status.

Primary endpoints: Invasive disease-free survival, axillary recurrence rate, and health-related quality of life (HRQoL). HRQoL is evaluated using four standardized questionnaires (EORTC QLQ-C 30, EORTC QLQ BR 23, Lymph ICF, and SOC-13) at baseline and 1, 3, and 5 years after surgery.

Secondary endpoints: Feasibility and performance of different axillary staging techniques (detection rate, number of removed lymph nodes and association with complications, operating time, and use of clinical and economic resources); impact of learning curve, and the detailed mapping of surgical and oncological treatment standards in different countries. The effect on different regional treatment strategies (radiotherapy, ALND) in patients with ypN0(i+), ypN1(mi), and ypN1 is assessed.

Current status of the study: 5,499 patients from 290 study sites and 27 countries were enrolled in the study between June 2020 and June 2024. Among 3,382 ycN0-patients with a defined surgical concept 909 (26.8%) women were scheduled for ALND, 1,817 (53.6%) for TAD, 547 (16.2%) for SLNB, 19 for TLNB (0.7%) and 90 (2.7%) for other procedures. A target lymph node was marked in 3,099 patients, most frequently using clips/coils (2,418, 78.0%), followed by magnetic seeds (n=320, 10.3%), carbon ink (n=228, 7.4%), and radar markers (n=138, 4.5%).

Funding: AGO-B, Claudia-von Schilling Foundation, Ehmann Foundation, Eugen und Irmgard Hahn Foundation, AWOgyn, Merit Medical, Endomagnetics, Mammotome

Target accrual: unlimited

P2-10-26: Adjuvant pembrolizumab and chemotherapy or surveillance in Early Triple Negative breAst cancer with high stromal tumor-infiltrating lymphocytes (sTILs) score (ETNA)

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Background: Several studies have shown that patients with stage I TNBC derive limited, if any, benefit from chemotherapy. sTILs, a surrogate marker for an active adaptive antitumor immune response, are strongly prognostic and predictive in patients with TNBC across all treatment settings. A recent individual patient-data meta-analysis has shown optimal survival outcomes in patients with stage I TNBC and sTIL ≥ 50% who did not undergo (neo)adjuvant systemic therapy, with a linear association between sTILs and prognosis [Leon Ferré et al, JAMA 2024].
Trial Design: ETNA trial (NCT06078384) is a phase II, open-label, international, multicenter, biomarker-driven study designed to evaluate chemotherapy de-escalation in patients with T1b/c N0M0 TNBC (ER/PgR < 10% by IHC) and sTILs ≥ 30% in resected primary tumors. Eligible patients who have undergone adequate breast cancer surgery will be included in one of two cohorts based on their sTILs levels, as determined by a central review of the surgical specimen. Cohort 1 will include patients aged > 40 years with 30% ≤ sTILs < 50% and those aged ≤ 40 years with 30% ≤ sTILs < 75%. Patients will receive 9 cycles of adjuvant pembrolizumab 200 mg every three weeks and Paclitaxel 80 mg/m^2 weekly for 12 doses. Cohort 2 will include patients aged > 40 years with sTILs ≥ 50% and those aged ≤ 40 years with sTILs ≥ 75%, who will undergo standard surveillance without adjuvant systemic treatment. A total of 1728 pT1b/cN0M0 TNBC patients will be screened for inclusion. Based on the TNBC pooled analysis [Leon Ferré et al, JAMA 2024] and the prevalence of young patients < 40 years in the stage I TNBC population of CANTO (10%), we expect 12.5% of screened patients to be eligible for cohort 1 and 8% for cohort 2. The study will enroll 216 patients in cohort 1 and 138 patients in cohort 2. The primary endpoint is 5-year distant disease-free survival in the 2 cohorts. Secondary endpoints include 5-year invasive disease-free survival, distant recurrence-free survival and overall survival, safety analysis, and quality of life evaluations. Cohorts 1 and 2 will be analyzed independently. Ancillary studies will be performed on the surgical and blood samples to explore prognostic biomarkers and obtain a comprehensive description of TILs in patients with early-stage TNBC. Site activation is currently ongoing.

P2-10-27: An Open Label Study of BTX-A51 in Patients with ER+/HER2- GATA3 Mutant and Wild Type Metastatic Breast Cancer

Zung Thai, Alexander Philipovskiy, Judy Wang, Denise Yardley, David Cook, Senthil Damodaran

Background: GATA3, a transcription factor, plays a critical role in the development of breast tissue. Mutations in GATA3 are observed in approximately 15% of hormone receptor positive (HR+) breast cancers (BC) and are associated with shorter progression free (PFS) and overall survival (OS). In BC these mutations tend to be exclusive of TP53 mutations. Preclinical studies have demonstrated that targeting MDM2 can be synthetic lethal in HR-positive BC GATA3 somatic mutations. BTX-A51 is a multi-specific kinase inhibitor of casein kinase 1 alpha (CK1α) and cyclin-dependent kinases 7 and 9 (CDK7 and CDK9), that synergistically targets master regulators of cancer to activate apoptosis. In preclinical models, BTX- A51 treatment increased p53 levels while decreasing expression of MDM2 and myeloid cell leukemia-1 (MCL-1), resulting in programmed cell death. In Phase 1 studies in patients with advanced acute myeloid leukemia and solid tumors, BTX-A51 showed encouraging clinical activity including objective clinical responses at dose levels that are safe and well-tolerated. Consequently, the current study will test the hypothesis that MDM2 inhibition with BTX-A51 will improve clinical outcomes in patients with GATA3 mutant HR+/HER2-negative metastatic BC.

Methods: This is phase 2, open-label, multi-institutional study evaluating the efficacy, safety, and pharmacokinetics (PK) of BTX-A51 in patients with ER+/HER2- GATA3 mutant and wild type metastatic BC. Key inclusion criteria include pre- and postmenopausal women and men aged ≥18 years; have histologically confirmed HR+/HER2-negative advanced / metastatic BC not amenable to resection or radiotherapy of curative intent; have received at least 2 lines of endocrine therapy and no more than 3 lines of chemotherapy in the adjuvant or advanced setting; and have RECIST v1.1 evaluable disease. Patients will receive BTX-A51 orally at 21 mg 3 times a week on a 28-day cycle and will undergo RECIST v1.1 evaluation at baseline and every 8 weeks. Treatment will continue until progression, death, unacceptable toxicity, or withdrawal from the study. The primary endpoint is objective response rate (ORR) per Investigator assessment. Secondary endpoints include tolerability, disease control rate at 24 weeks, duration of response, progression free survival, overall survival, and PK of BTX-A51. Blood samples for circulating tumor DNA will be collected at baseline, prior to C2, and prior to C3. The study employs a Simon- 2 stage design with a total of 40 patients and 22 patients enrolled to Stage 1. If ≥2 patients respond in the first stage, the study will proceed to stage 2. If ≥ 5 responses are observed among the total of 40 patients, the treatment will be regarded as promising. This two-stage design yields 90% power under the alternative hypothesis of ORR 20% (null ORR 5%) while controlling the one-sided type I error at 5%. Of the first 22 patients enrolled, ≥ 13 should have a pathogenic GATA3 mutations with ≥ 24 patients with GATA3 mutations enrolled in total. The study is currently recruiting patients with ER+/HER2- negative metastatic breast cancer (NCT04872166).

P2-10-28: COMPASS-TNBC - A phase Ib/II, open-label, modular, dose-finding and dose-expansion study to explore safety and anti-tumor activity of novel therapeutics in patients with early relapsed mTNBC. Module 1: Dato-DXd & Module 2: Dato-DXd + durvalumab.

Thomas Grinda, Rasha Cheikh-Hussin, Magali Lacroix-Triki, Kamar Serhal, Chayma Bousrih, Elie Rassy, Francoise Farace, Marianne Oulhen, Jean-Luc Perfettini, Awatef Allouch, Guillaume Montagnac, Fernanda Mosele, Ines Vaz-Luis, Joana M Ribeiro, Alessandro Viansone, Benjamin Verret, Suzette Delaloge, Jacqueline Deneuve, Claude Coutier, Juliette Gouenard, Fabrice André, Stefan Michiels, Barbara Pistilli

Background: Approximately half of all patients (pts) who develop metastatic triple negative breast cancer (mTNBC) after standard neo/adjuvant systemic treatment (NAST) experience a relapse occurring during or within 12 months following the completion of curative treatments, defined as early relapse. Early relapsed mTNBC is a distinct aggressive entity, often resistant to standard therapies and with a poor prognosis (median overall survival, [OS] < 10 months). Given the limited effectiveness of currently available options, new treatment strategies are hardly awaited in this situation. COMPASS-TNBC has been co-designed with a patients' association and wishes to broaden inclusion criteria to meet the important medical need in this situation. It assesses the efficacy of new treatment strategies and conducts translational analyses across different modules, in patients with early relapse mTNBC. The first 2 phase II modules are: Module 1 - Datopotamab deruxtecan (Dato-DXd, an anti-TROP2 antibody-drug conjugate (ADC) with a cleavable linker, and a topoisomerase I inhibitory payload) as monotherapy; Module 2 - Dato-DXd + durvalumab, an anti–PD-L1 monoclonal antibody (mAb).
Methods: Eligible pts are aged ≥18 years, have untreated unresectable or metastatic TNBC (ER and PR < 10%, HER2 non 3+ and non-amplified), have received NAST at the localized stage and have radiological (RECIST) and histological evidence of early relapse (≤12 months from the end of curative treatments). Pts must have an ECOG PS of 0-2, adequate organ function, and ≥1 nonirradiated measurable lesion. Stable CNS metastases (including leptomeningeal disease) are allowed. Pts are excluded if they have clinically significant corneal disease, a history of interstitial lung disease/pneumonitis, active immune disease, or prior treatment with an anti-TROP2-ADC. Pts are randomized in a 1:1 ratio to receive either Dato-DXd (6 mg/kg) (Module 1) or Dato-DXd (6 mg/kg) combined with durvalumab (1120 mg) (Module 2), both administered intravenously every 3 weeks until disease progression or unacceptable toxicity. Each arm will include 25 pts and is designed using the Bayesian optimal phase 2 (BOP2) design. An intermediate analysis will be performed after 12 pts in each module. The primary endpoint is the objective response rate (ORR) at six months, determined and assessed every six weeks using RECIST v1.1 by the investigator. Secondary endpoints include investigator-assessed duration of response (DOR), progression-free survival (PFS), clinical benefit rate (CBR), OS, safety, patient-reported outcomes (EORTC QLQ-C30 and QLQ-BR45), and treatment-emergent antidrug antibodies (ADA). Tumor and blood samples are longitudinally collected at baseline, on treatment

(C1D3 or C2D3), and at the end of treatment. Planned exploratory analyses include treatment-specific biomarkers/mechanisms of response and resistance using high-throughput imaging mass cytometry, RNA sequencing, and whole exome sequencing, on tumor samples, circulating tumor DNA (ctDNA), and circulating tumor cells (CTCs). Additionally, patient-derived organoids will be generated to test and develop novel treatment strategies. In modules 1 and 2, saliva and mouth swab will also longitudinally collected to determine predictors of oral mucositis in patients treated with Dato-DXd. Study enrollment started in June 2024 and is currently ongoing.
Clinical trial information: EU CT Number: 2023-503606-36-00

P2-10-29: Cascade Testing in Racially/Ethnically Diverse Relatives of Patients with Breast or Ovarian Cancer (CASSIE)

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Background: About 5-10% of breast cancers and 15% of ovarian cancers are attributed to germline pathogenic variants (PVs) in genes such as BRCA1 or BRCA2. Cascade testing, the process of informing at-risk family members of a PV detected in the proband and offering genetic counseling/testing, is a significant public health intervention which can reduce the burden of disease through targeted early detection and prevention strategies. Several studies have demonstrated that uptake of cascade testing is low, ranging from 15-57%, with testing more commonly pursued when the proband is White. Barriers include time constraints during clinical encounters, lack of contact and emotionally distant relationships with family members, geographic barriers, fear of a positive result and medical mistrust among racially/ethnically diverse patients. To address these barriers, we have developed a patient-facing web-based decision aid (DA), RealRisks, which is available in English and Spanish and has been rigorously tested in a randomized controlled trial (RCT) of genetic testing uptake among diverse women with a family history of breast or ovarian cancer.
Study Design: We are conducting a pilot cluster-RCT of the RealRisks DA and direct access to genetic counseling/testing services to increase cascade testing. We will enroll 30 probands diagnosed with breast or ovarian cancer and a known PV in a hereditary breast and ovarian cancer (HBOC) gene. They will invite 1-4 female first-degree relatives (FDRs) (60 total) who have not undergone germline genetic testing to participate. Cluster randomization will occur at the proband level with stratification based upon U.S. or foreign-born. Female FDRs will be assigned to a standard of care (SOC) notification letter alone or in combination with RealRisks and access to genetic counseling/testing services based upon randomization assignment of the proband. RealRisks consists of interactive modules to collect family history and elicit personal preferences on genetic testing. Probands and FDRs will complete surveys at baseline and 3 months. A subset of probands and FDRs will participate in semi-structured interviews to better understand barriers and facilitators to cascade testing.
Eligibility criteria: Eligible probands include: 1) men or women diagnosed with Stage 0-IV

breast or ovarian cancer; 2) PV in a high or moderate-penetrance HBOC predisposition gene (ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, TP53) identified within the past year; 3) at least 1 female FDR who has not undergone germline genetic testing. Eligible FDRs include: 1) women, age ≥18 years; 2) FDR of patient with breast or ovarian cancer and known PV; 3) English or Spanish-speaking; 4) able to provide consent.
Outcomes: Our primary endpoint is genetic testing uptake at 3 months among FDRs. Secondary endpoints include genetic testing knowledge, genetic testing intention/decision, informed choice and decision conflict among FDRs at baseline and 3 months. Through qualitative analysis, we will assess barriers and facilitators to communication of genetic test results to family members, experiences with RealRisks, and access to genetic counseling/testing and downstream cancer screening/preventive services.
Statistical methods: We will have >80% power to detect a difference in cascade testing uptake of 20% in the control arm and 60% in the intervention with a 1-sided alpha of 0.05, assuming an intraclass correlation coefficient (ICC) of under 0.20 to account for clustering and 10% dropout.
Current/target accrual: The trial will be activated in August 2024.
Discussion: Results from this pilot trial will inform a larger multicenter RCT to reduce disparities in cascade testing uptake for HBOC syndrome.
Funding: Conquer Cancer YIA, P50MD017341

P2-10-30: Classifying for HER2 Dependence to De-Escalate Neoadjuvant Chemotherapy in Patients with HER2+ Early Breast Cancer Undergoing HER2 Double-Blockade - CHERRY-PICK Trial (LACOG 0721)

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Background: HER2-positive breast cancer (BC) represents 20% of BC subtypes and is marked by high proliferation and aggressive behavior due to HER2 gene amplification. Chemotherapy combined with anti-HER2 blockade is the standard of care treatment neo/adjuvant setting for HER2+ early BC. The relevance of HER2 as a therapeutic target underscores the importance of accurate HER2 testing. HER2 heterogeneity is associated with resistance to HER2-targeted neoadjuvant therapy, and thus de-escalation of standard therapeutic regimens will depend on accurate tests and HER2 enriched selected tumors. More recently, 18F-FDG-PET identified patients with HER2-positive, eBC who benefited from chemotherapy-free dual HER2 blockade with trastuzumab and pertuzumab, and in which a PET-based, pCR-adapted strategy was associated with an excellent 3-year iDFS. The CHERRY-PICK clinical trial aims to evaluate an anti-HER2 chemo-free neoadjuvant regimen using a fixed-dose combination of pertuzumab and trastuzumab (PHESGO),

targeting a HER2 enriched population, selected by standard biomarkers (HR status and HER2 score) using a PET/CT response based strategy for pCR.
Trial Design: CHERRY-PICK (LACOG 0721) is a nonrandomized, phase II, single-arm study assessing the efficacy of neoadjuvant pertuzumab and trastuzumab (PHESGO) and de-escalating chemotherapy in patients with HER2+ enriched tumors through a PET/CT response based pCR-adapted strategy. The study will enroll women aged 18 to 80 years who have an ECOG performance status of 0 or 1. Eligible participants must present with clinical stage T1c-T2 N0-N1 BC. Tumors must be classified by immunohistochemistry at a central pathology review before enrollment as HER2 3+ score and estrogen receptor (ER) negative, or low (≤10%), and progesterone receptor (PR) negative (<1%). At baseline for image evaluation, patients must have a PET/CT SUVMax of ≥ 2.5 at primary tumor and tumor size of at least 10mm.
Initial dose of PHESGO is 1,200 mg pertuzumab, 600 mg trastuzumab, and 30,000 units hyaluronidase administered subcutaneously over approximately 8 minutes followed every 3 weeks by a dose of 600 mg pertuzumab, 600 mg trastuzumab, and 20,000 units hyaluronidase administered subcutaneously over approximately 5 minutes. After 3 cycles of PHESGO patients will perform PET/CT for response evaluation.
Patients achieving PET/CT response (≥40% reduction in the baseline SUVMax) will continue treatment for 5 additional cycles, completing a total of 8 cycles of neoadjuvant PHESGO therapy. After surgery, patients with pCR will complete 1 year of PHESGO and those with non-pCR will receive adjuvant T-DM1 or standard of care chemotherapy plus PHESGO, as per physician choice. Patients without PET/CT response after cycle 3 will be discontinued from the study and will receive treatment according to institutional standard of care.
The primary objective is to evaluate the pathological complete response (pCR) rate in patients who demonstrate a PET-CT response after 3 cycles. Approximately 63 participants will be enrolled to achieve a final sample size of 44 participants evaluable for the primary endpoint, considering a 20%-30% PET-CT non-responder rate. Secondary objectives include PET-CT response rate, objective response rate by PERCIST and RECIST 1.1, invasive disease-free survival (iDFS), event-free survival (EFS), overall survival (OS), safety, cardiotoxicity, and health-related quality of life (HRQoL). Baseline characteristics will be analyzed using descriptive statistics. The pCR rate will be reported as percentage, with a 95% confidence interval calculated using the Clopper-Pearson method. Time-to-event outcomes will be estimated using the Kaplan-Meier method. Comparisons of these outcomes will be performed using stratified log-rank tests. The trial is open for accrual, with the first site activated in June 2024 and a total of 11 centers planned to participate in Brazil.
Trial Registration: NCT06068985

P2-11-01: Comprehensive spatially resolved single-cell analysis of immunotypes in triple-negative breast cancer revealed the central role of intratumoral MHC class II expressions

Yi Liu

Background: Several studies demonstrated the central role of preexisting immune responses and outcomes in triple-negative breast cancer (TNBC). Emerging studies described 3 distinct tumor-immune microenvironments, termed immunotypes, based on the amount and locations of tumor-infiltrating lymphocytes (TILs), namely immune inflamed (IN), immune excluded (IE), and immune desert (ID). We previously demonstrated that patients with IE tumors had poor outcomes despite having high sTIL. Here, we further evaluated tumor and immune cell characteristics associated with each immunotype.
Methods: NanoString Digital Spatial Profiling (DSP) and CosMx, a spatial multi-omics single-cell imaging platform, were performed in 75 samples from the Mayo Clinic (MC) TNBC cohort (Leon-Ferre BCRT 2018). Firstly, tumors with sTIL quantified by H&E ≤ 30% were classified as ID. The rest of the tumors with high sTIL > 30% were categorized according to the intratumoral CD8 protein expression by DSP, with IE having intratumoral CD8 in the lower median and IN having intratumoral CD8 in the upper median. Chi-square test, gene set enrichment, Cox regression, and Kaplan-Meier analysis were used. Differential expression listed as log 2-fold change (FC) was estimated from the linear mixed model with significance defined as two-sided $p< 0.05$.
Results: In the proteomic DSP-multiplex analysis, intratumoral CD34, GZMB, PTEN, and SMA were significantly more abundant in ID, whereas only B2M and PD-L1 were more abundant in IE. As expected, T cell markers (CD3, CD8, CD4) and their activation markers (ICOS, GZMB) were significantly higher in the intratumoral compartment of IN. Immune checkpoint proteins, including PD-L1, STING, Tim-3, and VISTA, but not CTLA4, were higher in IN samples. IDO1, CD20 (B-cells), and CD68 (macrophage) were more abundant in IN, whereas CD34 (stem cells and/or mast cells) and CD56 (a potential NK cell marker) were not differentially expressed in the intratumoral compartment of IN. Only fibronectin (FN) and smooth muscle actin (SMA) were more abundant in ID. In the CosMxTM single-cell spatial analysis, GSEA analysis demonstrated significant enrichment of the GOBP termed Antigen Processing and Presentation in IN (FDR < 0.001). We further identified 19 genes that were differentially expressed in tumor cells at BY.adj $p<0.05$ across the three immunotypes. 13 out of 19 transcripts were involved in antigen presentation, including transcription factors CIITA and NLRC5, the MHC components HLA-A, HLA-B, HLA-C, HLA-DRA, HLA-DRB1, HLA-DPA1, HLA-DPB1, and HLA-E, the peptide antigen transporters TAP1 and TAP2, and the MHC II invariant subunit protein CD74. Using Kaplan-Meier analysis, all MHC II components HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRB1, and HLA-DRB5 were associated with outcome at log-rank $p<0.05$, and only HLA-B was associated with outcomes (p 0.028). However, HLA-DRA expressions in immune cells, including B cells, macrophages, total dendritic cells (mDC and pDC), and total T-cells (T CD4, T CD8, and Treg), were not associated with outcome.
Conclusions: Using comprehensive spatially resolved single-cell analysis, we demonstrated the central role of intratumoral MHC class II expression associated with inflamed phenotype. Intratumoral MHC class II expressions were associated with significantly improved outcomes but not the MHC expressions in immune cells. Strategies to increase intratumoral MHC expression may improve outcomes for this group of patients in the future.

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P2-11-02: Is Adjuvant Chemotherapy Needed in Small Node-Negative Triple Negative Breast Cancer?

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Background: The efficacy of adjuvant chemotherapy in small node-negative triple-negative breast cancer (TNBC), specifically T1a/bN0 remains unclear. However, recent studies have highlighted that tumor-infiltrating lymphocytes (TILs) are associated with better survival outcomes in TNBC patients. As a result, a few TIL-based clinical trials are now being conducted. This study aims to evaluate the need of adjuvant chemotherapy in patients with T1a/bN0 TNBC according to TIL levels.
Methods: Patients who underwent primary surgery for T1a/bN0 TNBC between 1995 and 2020 at two tertiary hospitals (Asan Medical Center, Samsung Medical Center) were retrospectively reviewed. Clinicopathological features were compared according to receipt of adjuvant therapy. Kaplan-Meier survival analysis and Cox proportional hazard models were applied to determine the impact of adjuvant chemotherapy on recurrence-free survival (RFS). Subgroup analysis was performed according to tumor size, TIL level and chemotherapeutic regimen.
Results: A total of 712 patients were included in this study. Among them, 371 (52.1%) patients underwent adjuvant chemotherapy. Patients who underwent adjuvant chemotherapy were significantly younger compared to patients who did not undergo chemotherapy (mean age 50.7 years vs 55 years, t-test p-value <0.001). Patients with a high-grade tumor, larger tumor, and high Ki-67 were more likely to have undergone chemotherapy. Whereas patients with a low TIL (≤ 30%) tumors were more likely to not have chemotherapy. Most of the patients had only adriamycin-included chemotherapeutic regimens (N=256, 69%), and only 9 (2.4%) patients underwent a taxane-included regimen. Other chemotherapeutic regimens include CMF and Didox. During a median follow-up of 72 months, 58 (8.1%) patients had a locoregional recurrence or distant metastasis. Adjuvant chemotherapy was found to have no significant impact on RFS (5-year survival rate 93.9% no chemotherapy vs 93.0% chemotherapy, log-rank test p-value 0.59). After adjusting for age, histologic grade, tumor size, Ki-67, and TIL level, patients who underwent chemotherapy had no significant difference in RFS compared to patients who did not receive chemotherapy (hazard ratio 1.12, 95% confidence interval 0.564-2.226, p-value 0.746). Subgroup analysis according to tumor size (T1a, T1b), TIL (≤ 30%, >30%), and chemotherapeutic regimens (only adriamycin and/or taxane) also showed that adjuvant chemotherapy had no significant impact on recurrence.

Conclusion: In the overall small node-negative TNBC patient population, adjuvant chemotherapy did not significantly impact recurrence. Avoidance of chemotherapy in T1a/bN0 TNBC patients can be considered. Further clinical studies are needed to evaluate the efficacy of adjuvant chemotherapy in patients with small node-negative TNBC.

P2-11-03: Quantification of Breast Cancer 1 gene (BRCA1) promoter methylation in individuals with and without cancer using cell-free DNA

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Introduction: BRCA1 promoter hypermethylation (BRCA1meth), which is a driver of homologous recombination deficiency (HRD), is observed in up to 25% of triple negative breast cancers (TNBCs) and ovarian carcinomas (OvCas). BRCA1meth is associated with worse outcome compared to BRCA1 mutations (Menghi et al., PMID: 35857626), and, when found in individuals without cancer (i.e. constitutional BRCA1meth), it is associated with increased risk of breast and ovarian cancer (Lonning, et al., PMID: 36074460). Current methodologies to determine the presence of BRCA1meth in a cancer sample require tissue biopsy and have not been adopted for standard clinical assessment. The GRAIL cell-free DNA (cfDNA)-based targeted methylation platform is a robust, biopsy-free, scalable assay that was optimized to distinguish cancer methylation patterns between different cancer signal origins. Here, we describe the GRAIL platform's ability to detect and quantify BRCA1meth.

Methods: As part of the CCGA study (NCT02889978), clinical data were recorded and plasma samples were collected and processed on GRAIL's targeted methylation platform from 2,958 patients with cancer prior to any treatment, and from 2,790 individuals without cancer. Samples were evaluated from patients with a variety of cancers: breast, ovarian, cervical, uterine, lung, prostate, head and neck, kidney, bladder, stomach, esophageal, colorectal, and hematological malignancies. A dilution series of contrived samples was analyzed to establish analytical characteristics of BRCA1meth detection using the GRAIL assay.

Results: When applied to contrived samples, the GRAIL platform was able to detect BRCA1meth at an LoD95 of <1% (as measured by BRCA1meth fraction = methylated molecules / total molecules).

When applied to a cohort of 2,790 individuals without cancer, we found 4% of individuals harbored evidence of BRCA1meth in the fractional range of 0.4% to 37%, with the majority of individuals (81%) having <5% BRCA1meth fraction, similar to surveys using peripheral blood cell DNA (Lonning et al., PMID: 36074460). Consistent with recent data of constitutional BRCA1meth prevalence in umbilical cord blood of newborns (Nikolaienko, et al., PMID: 38053165), we see lower incidence of BRCA1meth in males without cancer (3%)

compared to females without cancer (5%, p=0.01).

BRCA1meth was significantly enriched in the cfDNA of patients with TNBC and OvCa compared to female individuals without cancer (17/112 (15%), adjusted p=0.001 for TNBC; 14/101 (14%), adjusted p=0.01 for OvCa), in concordance with previous assessments of BRCA1meth prevalence across different tumor types (Menghi et al., PMID: 35857626). In TNBC and OvCa, the per-sample BRCA1meth fraction ranged from 0% to 54% and was correlated with the methylation-based estimate of tumor fraction in both TNBC (R=0.68, p=0.0052) and OvCa (R=0.65, p=0.03). High BRCA1meth fractions (up to 16-21%) were observed in four individuals with uterine, colorectal, bladder, or kidney cancer. This suggests that BRCA1 deficiency may be implicated in these rare cases, which would imply HRD, making them candidates for specific treatments.

Finally, across tumor types that affect both sexes, we found consistently lower percentages of BRCA1meth in male vs. female patients, reflecting the differential rates of constitutional BRCA1meth across the two sexes. This suggests that the dynamics of BRCA1 promoter methylation and its consequences for the development of cancer may differ by sex.

Conclusions: This work shows that the GRAIL platform, as a single blood-based assay, can sensitively detect and quantify BRCA1meth from cfDNA, which may inform cancer susceptibility in non-cancer bearing individuals and may also have potential treatment selection implications for cancer patients.

P2-11-04: Incidence and pattern of central nervous system recurrence in TNBC according to response after neoadjuvant treatments

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Background: Response to neoadjuvant treatment (NAT), assessed by pathological complete response (pCR) or the Residual Cancer Burden Index (RCB), is significantly associated with recurrence-free and overall survival in patients (pts) with early-stage triple-negative breast cancer (TNBC). However, distant recurrence sites may vary based on NAT response, with a predominance of CNS recurrences in pts with pCR observed in the KEYNOTE522 trial. This has not yet been confirmed in real-world data (RWD).
Methods: In this retrospective RWD study, we describe the CNS recurrence rate at metastatic diagnosis (dg) in Stage I-III TNBC (defined as ER <1%, PR <1%, and HER2 negative by ASCO-CAP guidelines) pts treated with NAT according to pCR and RCB. Patients with bilateral or multifocal cancer were excluded. We used a prospectively maintained institutional database of patients at a single institution supplemented by review of electronic health records. Time to CNS recurrence was calculated from the time of breast surgery to CNS recurrence defined as diagnosis of CNS metastases with or without non-CNS recurrence within 3 months of initial diagnosis of recurrence. CNS metastases include brain

metastases (BM) and/or leptomeningeal disease (LMD). Medians with ranges; cumulative incidences were from Kaplan-Meier comparisons used Fisher's test and log-rank test.
Results: From a total of 630 pts with TNBC who received NAT followed by breast surgery between 2015 and 2022, 37 were excluded with bilateral or multifocal cancer and 593 were included. The median age at initial diagnosis was 51.2 years (yrs) (21.0 - 89.5). Of these pts, 477 (80%) received anthracyclines, 523 (88%) taxanes, 176 (30%) platinum, and 124 (21%) immunotherapy as part of their NAT.
Among them, 214/593 (36%) achieved pCR or RCB0, 78/593 (13%) were classified as RCB1, 207/593 (34%) as RCB2, and 71/593 (12%) as RCB3; 23 pts who did not achieve pCR had an unknown RCB score.
With a median follow-up of 2.8 years (95% CI: 2.6 – 3.1 years), 102 patients experienced distant or local recurrence, with 24 (23%) cases occurring in the CNS. Among pts who achieved pCR, 6/9 (67%) recurrences occurred in the CNS, compared to 18/93 (19%) patients without pCR who had CNS recurrence (p = 0.005). Based on the RCB score, proportion of CNS recurrence were 6/9 (67%) for RCB0, 3/9 (33%) for RCB1, 8/37 (28%) for RCB2, and 7/40 (17%) for RCB3.
Among patients with CNS recurrence, 4/6 (67%) with pCR versus 18/18 (100%; p= 0.054) without pCR had parenchymal BM; 4/6 (67%) versus 2/18 (11%; p=0.018) had LMD. Isolated CNS recurrence was more common in the pCR group; 5/6 (83%) versus 3/18 (17%; p=0.007). Additionally, 3/4 (75%) patients with pCR and 5/18 (28%; p=0.12) without pCR had a single BM as the CNS recurrence. Also, among patients with CNS recurrence, those with pCR were numerically more clinical stage III: 4/6 (67%) vs 6/16 (33%; p=0.19).
The median time to CNS recurrence was 1.62 yrs (0.57-4.66) for patients with pCR and 1.03 yrs (0.35-1.72) for those without pCR. According to RCB 1, 2 and 3 score, median time to CNS recurrence were 0.88 yrs (0.35 - 1.02), 1.12 yrs (0.53 - 1.71), 0.93 yrs (0.38 - 1.72). Cumulative incidence of CNS recurrence at 1 and 2 yrs for pts with pCR was 0.97% (CI95% 0.0 - 2.3) and 1.52% (CI95% 0.0 - 3.22); for pts without pCR was 2.26% (CI95% 0.70- 3.80) and 5.49% (CI95% 2.98 - 7.93), respectively (log-rank: p= 0.2).
Conclusion: Although uncommon, the proportion of pts with relapsed disease with CNS as the first site of recurrence was significantly higher in pts achieving pCR compared to those with residual disease following NAT for TNBC, though there was no statistically significant difference in the cumulative incidence of CNS recurrence by pCR status. Isolated brain relapse was more common among patients with pCR. These findings highlight the critical need for strategies to identify pts at risk for CNS events and to develop effective CNS prevention approaches in high-risk pts.

P2-11-05: Accurate and cost-effective reconstruction of TNBC heterogeneity through 3D digitization of core needle biopsies

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Objective: Increasing evidence suggests that the tumor microenvironment (TME) of patients with triple negative breast cancer (TNBC) is hallmarked by extensive inter and intra-tumor heterogeneity. Deeper insights into the biological basis of this heterogeneity are needed to elucidate the aggressive nature of TNBC. Early attempts to stratify TNBC patients using bulk or single-cell transcriptomics have shown promise to understand differences in clinical outcomes and provide a personalized medicine approach. However, most examples limit themselves to a single 2D tissue slice per patient. Unfortunately, such an approach represents only a fraction of a 3D core needle biopsy, which is the only available tissue source to examine the treatment naïve TNBC microenvironment.
Recently, spatial omics technologies demonstrated superior results for determining heterogeneity as these tools simultaneously profile the intact TME architecture and spatially resolved transcriptomes. Constructing 3D datasets by sectioning tissue and profiling each slice ($n > 40$) is feasible, but cost-prohibitive and impractical. An innovative digitization workflow that infers transcript localization across 3D datasets without probing each tissue slice would reduce costs and maximize tissue usage, whilst subsequently enhancing our understanding of tissue composition in the treatment naïve TNBC biopsy. Our goal is to facilitate building a comprehensive and large-scale map depicting TNBC heterogeneity from difficult-to-access tissue sources that could shed the much-needed light on TNBC.
Method: We sectioned and imaged a treatment-naive core needle biopsy of TNBC into 48 serial slices, with 8 for spatial transcriptomics (Visium, 10X Genomics) equally spaced and 40 for H&E staining. Image coloration was normalized in a semi-automatic manner, and sections were registered on the associated H&E images using VALIS to create a 3D representation of the tissue. Transcripts from spatial transcriptomic sections were enhanced at super-resolution and mapped across serial sections using iSTAR. 3D spatial transcriptomic data analysis and quantitative comparisons with in-silico 2D slices was performed using the Giotto Suite ecosystem.
Results: We generated a fully digital and super-enhanced (20 μm pixels) 3D spatial transcriptomics dataset from a single core needle TNBC biopsy by integrating image registration with spatial transcriptomics. Transcripts were projected across tissue sections using spatial interpolation and deep learning methods that learn the spatial and histology relationships for genes, thereby providing a comprehensive and super-resolved 3D representation. We observed that specific 3D structures, such as stromal plasma infiltrates and tertiary lymphoid structures, can be more accurately identified from the full 3D digital biopsy. We also illustrate how spatial gene expression patterns, key signaling pathways, and cellular architecture are organized in three dimensions, which cannot be easily inferred from 2D datasets alone. Importantly, systematic analysis comparing 3D versus 2D in-silico generated slices provides a quantitative framework that can be used to determine the limitations and power of spatial transcriptomic data analysis in 2D.
Conclusion: This study demonstrates the feasibility of incorporating spatial transcriptomics and 3D registration methods into standard spatial analyses, enabling a detailed understanding of the spatial organization within a core needle TNBC biopsy and leading to informed insights on tissue architecture and function. Our approach facilitates a data-driven

approach to better understand the strengths and limitations of profiling tumor heterogeneity in core needle biopsies of breast cancer. Finally, our approach enables researchers to make more informed research designs for large-scale projects, maximizing the usage of precious and often difficult-to-obtain patient samples.

P2-11-06: Neoadjuvant chemotherapy plus camrelizumab vs. chemotherapy in patients with locally advanced immunomodulatory triple-negative breast cancer: a prospective, randomized, open-label, phase 2 trial

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Background: Blockade of the PD-L1/programmed cell death protein 1 checkpoint improves the efficacy of classical chemotherapy in the neoadjuvant treatment of triple-negative early breast cancer (TNBC) but results in adverse events. Treatment approaches for immune-sensitive diseases and the efficacy of adding camrelizumab to chemotherapy regimens is unclear, especially in TNBC patients with a high tumor burden.
Methods: This open-label, randomized, phase 2 study randomly assigned (1:1) 90 patients ≥ 18 years with stage II–III histologically documented immunomodulatory TNBC (CD8 ≥10%) were randomly assigned (1:1) to receive chemotherapy with or without intravenous 200 mg camrelizumab every 2 weeks. Chemotherapy comprised nab-paclitaxel (100 mg/m^2)and carboplatin at a dose based on an area under the concentration–time curve of 1.5 mg per milliliter per minute once every week for 3 weeks for a 28 days per cycle within the first 12 weeks, followed by epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2) every 2 weeks for 8 weeks, followed by surgery. The primary endpoint (pathological complete response, pCR) was evaluated using an intention-to-treat approach. This study is registered with ClinicalTrials.gov (NCT05582499).
Findings: Between October 2022 and September 2023, 156 patients were recruited and assessed for eligibility. Of the 90 eligible patients, 45 were randomly assigned to receive camrelizumab plus chemotherapy, and 45 was assigned to receive chemotherapy, with 76.7% having stage III disease. At the data cutoff (March 30, 2024), the pCR rate was 62.2% (28/45 patients) in the camrelizumab–chemotherapy group and 42.2% (19/45 patients) in the chemotherapy group (rate difference: 20.0% [95% CI, -0.8 to 39.2]; P=0.058). Treatment-related adverse events of grade 3 or higher occurred in 33 patients (73.3%) in the camrelizumab-chemotherapy group and in 32 patients (71.1%) in the chemotherapy group. Bulk transcriptomic analysis indicated that patients who received immunotherapy and achieved pCR had significantly greater baseline levels of CD8+ T cells than did those who did not achieve pCR(non-pCR).
Interpretation: In immunomodulatory TNBC patinets with a high tumor burden, neoadjuvant treatment with camrelizumab in combination with nab-paclitaxel plus

carboplatin and anthracycline-based chemotherapy significantly improved pCR rates with an acceptable safety profile.

P2-11-08: Spatial transcriptomic analysis for tumor infiltrating immune microenvironment modulation by neoadjuvant pembrolizumab with chemotherapy for patients with triple negative breast cancer

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Introduction: Adding pembrolizumab to conventional neoadjuvant cytotoxic chemotherapy (NAC) improved pathologic complete response rate (pCR) and event free survival (EFS), which has become practice changing treatment. Furthermore, EFS benefit has been maintained even in non-pCR patients. Here, we are to explore the role of pembrolizumab in terms of tumor microenvironment using a spatial transcriptomic analysis.
Methods: We examined a prospective cohort of patients for residual TNBC after NAC with Visium Spatial Gene Expression (Visium V4 Slide - FFPE v2, 10X Genomics). Data was preprocessed with Spacer Ranger, aligned to GRCh38, and analyzed using the Seurat R package. Expression data was normalized and batch effect corrected with SCTransform and RunHarmony. Cell types and Hallmark pathway scores were determined with AddModuleScore. Differential gene expression and gene set enrichment analysis was performed using FindMarker in Seurat and ClusterProfiler. Broader cell types were deconvoluted using a reference with the spacexr R package. Tumor borders were assessed with the SpaCET R package.
Results: In total, 20 cases of residual TNBCs were analyzed. Of 20 cases, sixteen TNBCs had been treated with pembrolizumab (PEM) plus NAC and four with only NAC. In residual cancer burden (RCB) class, four were class I (three in PEM+NAC and one in NAC), fifteen in class II (twelve in PEM+NAC and three in NAC) and one in class III (PEM). Four patients have experienced distant metastasis after standard adjuvant treatment (three in PEM+NAC and one in NAC group).
In DEG analysis, we found CXCL9, CXCL10 and CXCL13 increased in PEM+NAC whereas CCL18 and CD36 in NAC group (FDR <0.01, respectively). GSEA result was shown that interleukin 18(IL18) and interferon gamma (IFNG) response pathway was significantly up-regulated in PEM+NAC group compared to those in NAC group. Cell type scores of CD4+T cell, CD8+T cell, M1 macrophage (Mac) and cancer cell were higher in PEM+NAC group, whereas M2 Mac, myCAF as well as iCAF were higher in NAC group (FDRs < 0.01). The distance between cancer and cytokines including CXCL9 and CXCL10 were closer in PEM+NAC group compared to the that in NAC group (FDRs <0.005). Otherwise, the distance from cancer cell to IFNG, IFNA and CCL5 were not different between PEM+NAC and NAC groups.
Furthermore, we analyzed the interaction between cancer cells and immune cells. In tumor associated Macs, CXCL9 and CXCL10 expressed Mac were nearer by cancer cells in

PEM+NAC group than NAC group even though SPP1 Mac also closer to cancer cells in PEM+NAC group compared to NAC group (Ps <0.05). Moreover, CD69 and PDCD1 expressed exhausted CD8+ T cells were farther from cancer cell in PEM+NAC group compared to distance in NAC group. However, there was no different distance from cancer cells to IFNG and IFN alpha expressed CD8+ T cells between two groups.
Conclusion: After PEM+NAC, residual TNBC more closely interacted CXCL9+/CXCL10+ macrophages even though SPP1+ macrophages with hypoxia also be near to tumor cells compared to those in NAC only. In addition, PEM+NAC expelled exhausted CD8 T cells from cancer in this spatial transcriptomic analysis. Based on these finding, we suggested that adding pembrolizumab to NAC would modulate tumor infiltrating immune cells, which may contribute better survival outcomes.

P2-11-09: Adding Carboplatin to neoadjuvant chemotherapy (NACT) in early triple-negative breast cancer (eTNBC): a systematic review and meta-analysis

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Background: Triple negative breast cancer (TNBC) is characterized by a poor prognosis and a high risk of early relapse. The inclusion of platinum agents in neoadjuvant regimens has been associated with an improvement in pathological complete response rate (pCR) in this subset of patients. However, the use of carboplatin (CBDCA) is still challenging due to inconsistent long-term outcome improvements and the risk of its chronic toxicity. This meta-analysis attempts to assess the impact of the addition of carboplatin to chemotherapy regimens in the neoadjuvant setting in eTNBC.
Methods: A systematic review and meta-analysis was conducted by searching MEDLINE, PubMed and the principal oncology meetings articles and abstracts from 2014 to 2024 with no restriction of language. The research was performed through MeSH terms. Phase II-III randomized studies evaluating the addition of carboplatin to neoadjuvant chemotherapy (NACT) in early Triple-Negative Breast Cancer (eTNBC) reporting response rate and survival outcomes were included. Case reports, reviews and observational studies were excluded. Pathological complete response (pCR) was the primary endopoint; Disease-free survival (DFS) was the secondary endpoint of our investigation. For the primary endpoint (pCR) a random-effects model was used for data analysis, whereas Odds Ratio (OR) was extracted and converted into logOR as outcome measure. Heterogeneity using I2 statistics and publication bias using Fail-Safe N test were assessed. For what concerns the secondary endpoint, a mixed-effects model was used for data analysis, whereas Hazard ratios (HR) and 95% confidence interval (CI) were extracted and converted into logHR and corresponding standard error to obtain the Summary HR (SHR). Heterogeneity was assessed using I2 statistics and publication bias using Egger's regression test. The data analyses were performed with Jamovi 2.4.11 software.
Results: Overall, 30 studies were retrieved of which 9 clinical trials were eligible for this

meta-analysis. Five studies (BrighTNess, GeparSixto, GS5-01, BR 15 1 PEARLY, NACATRINE) completely satisfied the inclusion criteria and were included, with a total of 2956 patients. The addition of CBDCA to NACT was associated with a significant improvement in pCR rate: OR 1,63 [95% CI: 1.3-2.0, p<0.01]; a low heterogeneity (I2=20.4%) with a possible publication bias was detected (Fail-Safe N=45.000, p<0.001). Platinum-based NACT significantly increased DFS with a SHR 0,74 [95%CI: 0.6-0.9, p<0.001], (I2 =24.74%, no publication bias p=0.567).
Conclusions: NACT has a major role in the treatment of EBC. The addition of CBDCA to NACT resulted into a significant improvement in pCR rate and DFS. Given the well-known higher platinum-sensitivity of TNBC and its impact on long-term outcomes, carboplatin should be a standard component of NACT in high risk eTNBC.

P2-11-10: Assessing the Differential Response to Immunotherapy in BRCA1/2 Mutation Carriers with Triple-Negative Breast Cancer

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Background: The addition of pembrolizumab to standard neoadjuvant chemotherapy (NAC+P) for patients with triple-negative breast cancer (TNBC) has been shown to result in higher rates of pathologic complete response (pCR) and longer event-free survival compared to neoadjuvant chemotherapy alone (NAC). Alterations in the DNA damage response and homologous recombination pathways may differentially affect the tumor-immune microenvironment and therefore influence response to immunotherapy. It is not known whether BRCA1/2 mutation carriers with TNBC have a differential response to immunotherapy compared to noncarriers. Our objective was to compare pCR rates between BRCA1/2 carriers and noncarriers with TNBC undergoing NAC compared to NAC+P.
Methods: 341 women with stage II-III TNBC treated with NAC+P based on the KEYNOTE 522 (KN522) regimen from 8/2021-5/2024 were compared to 433 consecutive patients who received NAC prior to 7/2021. Demographic and clinicopathological characteristics, and BRCA1/2 mutational status were collected. pCR rates, defined as no disease in the breast and axilla (ypT0/is pN0), were compared between BRCA1/2 carriers and noncarriers across treatment regimens using Chi square tests. Logistic regression was used to evaluate the association between BRCA1/2 status and pCR.
Results: Of non-KN522 patients, 76 (18%) were BRCA1/2 carriers and of KN522 patients, 56 (16%) were BRCA1/2 carriers. Overall, BRCA1/2 carriers were younger, had a lower BMI), and were more likely to be White. There was no difference in histology, grade, presence of lymphovascular invasion, clinical T or N stage, or chemotherapy regimen between carriers and noncarriers. The pCR rates in the non-KN522 group were 50% for BRCA1/2 carriers compared to 36% for noncarriers (p=0.021). In the KN522 group, pCR rates were 68% for BRCA1/2 carriers compared to 55% for noncarriers (p=0.073). Among mutation carriers treated with KN522, the pCR rate was 69% (31/45) for BRCA1 and 60% (6/10) for BRCA2 carriers. On multivariable analysis for the entire cohort, BRCA1/2 status

(OR 1.55, 95% CI 1.03-2.34, p=0.036), receipt of KN522 (OR 2.13, 95% CI 1.71-3.12, p<0.001), younger age at diagnosis (OR 0.98, 95% CI 0.97-0.99, p=0.002), and high grade (OR 9.42, 95% CI 4.01-27.6, p<0.001) were associated with pCR. Among patients receiving KN522, only high grade was associated with pCR (OR 17.4, 95% CI 5.03-110, p<0.001); BRCA1/2 status, age, BMI, race, and stage were not associated with pCR.
Conclusion: BRCA1/2 carriers with TNBC had better response to NAC compared to noncarriers. This was also observed in patients who received NAC+P, although this did not reach statistical significance. Additional work with a larger sample size of carriers may further delineate the differential response to NAC+P. This will be increasingly important for BRCA1/2 carriers as we investigate optimal escalation and de-escalation strategies, particularly when immunotherapy is combined with alternative therapies (i.e., PARP inhibitors).

P2-11-11: Clinicopathological features predictive of neoadjuvant immune checkpoint inhibitor benefit in triple-negative breast cancer

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Background: The KEYNOTE-522 (KN-522) regimen [chemotherapy (CT) + immune checkpoint inhibitor (ICI)] is the treatment of choice for patients with triple-negative breast cancer (TNBC) in the neoadjuvant setting. Addition of ICI could be associated with life-threatening immune related adverse events (irAEs). There is an unmet need to identify a subgroup of TNBC patients who would benefit from ICI, while sparing its use in the majority where CT alone may be sufficient. Our study aimed to compare the pathological complete response (pCR) rate between two matched cohorts (CT + ICI vs. CT alone), identify clinicopathologic variables that would predict benefit from ICI and study association of irAE with pCR.
Methods: Patients treated with CT + ICI (n=128) were 1:1 matched to patients treated with CT alone (n=128) on age range (10 years), race, clinical-AJCC stage, and histologic type [metaplastic vs. no special type] from four US academic institutions. Additionally, data on Nottingham grade, degree of necrosis, and percentage of tumor infiltrating lymphocytes (TILs) was collected from pre-treatment core needle biopsy (CNB). To identify the clinicopathologic features of patients who could benefit from ICI, multivariable logistic regression models were used. Data on incidence of any grade irAEs was collected and its association with pCR was also analyzed using multivariable logistic regression models.
Results: pCR was achieved in 64/128 (50%) patients treated with CT + ICI vs. 46/128 (35.9%) treated with CT alone (p = 0.02). The optimal threshold to predict pCR for TILs was 30% for both CT + ICI and CT alone groups, with AUC of 0.78 and 0.73, respectively (p<0.001 for both). In the CT group, lower TILs in CNB, non-Black race, metaplastic histology and higher degree of necrosis were associated with non-pCR; where the corresponding ROC curve had an AUC of 0.83. Patients who received CT were categorized into quartiles (Q) based on the predicted risk of non-pCR (Q1 representing the lowest risk,

Q4 representing the highest risk of non-pCR (34/128 or 26.6%), and Q2 and Q3 representing intermediate levels of risk). In the Q4, the pCR rate with CT alone was only 2.9% (1/46) while it was 20% (6/64) in the CT + IT cohort (p=0.044). The following variables were associated with Q4 vs. Q1-3: non-Black race (96.9% vs. 88%, p=0.04), metaplastic histology (29.7% vs. 1.6%, p<0.01), lymph node (LN) positive disease (54.6% vs. 50%, p<0.01), Nottingham grade 2 (32.8% vs. 6.3%, p<0.01), and lower mean TILs (12.8 +/- 15.0 vs. 36.7 +/- 26.5, p<0.01). The incidence of any grade irAEs was 32% (41/128) in patients treated with CT + ICI. Among patients with an irAE, 63.4% achieved pCR vs. 43.7% without irAE who achieved pCR (p=0.04). There was a higher likelihood of pCR in patients treated with CT + ICI who experienced irAE (OR=3.34, 95% CI 1.17-9.58) while controlling for age, AJCC stage, nottingham grade, race, TILs, and number of ICI doses.
Conclusions: In our real-world multicenter study, the pCR rate with neoadjuvant CT + ICI was lower than that reported in KN-522, with a similar irAE rate. We identified a subgroup of patients with TNBC likely to benefit from addition of neoadjuvant ICI to CT: those with metaplastic histology, non-Black race, LN positive disease, nottingham grade 2, or low TILs (<30%). Occurrence of any irAE was associated with increased likelihood of achieving pCR. Our results warrant validation in larger cohorts.

P2-11-12: Use of the prognostic H&E stromal tumor-infiltrating lymphocytes score and the predictive 27-gene IO score to identify patients with stage I TNBC who may benefit from immune checkpoint inhibitor-based, chemo de-escalated curative-intent therapy.

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Background: Chemotherapy plus immune checkpoint inhibitor (ICI) is the standard curative therapy for stage II/III TNBC, whereas chemotherapy without ICI remains the standard for stage I (T1c) TNBC. Stage I TNBCs may be immunogenic and have excellent outcomes with less/no chemo, highlighting a potential opportunity for a less toxic, ICI-based curative approach. In a Dutch registry, stage I TNBCs with high H&E stromal tumor-infiltrating lymphocytes [sTILs-hi; ≥75%] had excellent (2%) 3-year distant recurrence or death incidence despite not receiving chemo, whereas stage I TNBCs with intermediate [sTILs-int; 30-75%] or low [sTILs-lo; <30%] had 11% and 27% 3-year distant recurrence (de Jong et al, J Clin Oncol 2022; 40:2361-2374). The sTIL score is not consistently predictive of ICI benefit, whereas the DetermaIOTM 27-gene IO score RT-PCR assay has been shown to predict ICI benefit in the neoTRIP randomized trial and in other datasets. We hypothesize that the prognostic sTIL score could be harmonized with the predictive 27-gene IO score to identify patients with stage I TNBC who would most likely benefit from chemo de-escalation and an ICI-based curative approach.
Methods: We evaluated outcomes, H&E sTILs (scored by a trained pathologist using the International TILs Working Group method), and IO score using samples from stage I (T1c) TNBCs (n=57) treated at our institution from 2010-2022. sTILs and IO score measurements

were repeated in matched biopsies v. surgical excisions when available (n=34 with no intervening therapy). Cumulative incidence of distant recurrence or death was calculated from the time of resection. sTILs and IO scores were described using the above thresholds reported by de Jong, et al. For matched biopsy/surgery specimens, averaged scores were reported.
Results: The 3-year distant recurrence or death incidence was: 7% (n=4/55) for the entire cohort; 20% (n=3/15) for no chemo & 5% for chemo (n=2/42); and 0% (n=0/2) for sTILs-hi, 0% (n=0/15) for sTILs-int & 10% (n=4/38) for sTILs-low. The proportion of IO positive scores in this stage I TNBC cohort was lower (IO+ 18%, n=10/57; mean: -0.28, stdev 0.29) compared to IO scores from previously reported stage II/III TNBC cohorts (neoTRIP: IO+ 42% [Dugo et al., 2022], neoPACT: IO+ 52% [Sharma et al., 2023]). IO scores and sTIL scores across matched biopsy versus surgical excisions were concordant [ICC(3,k): 0.88 & 0.9]. IO scores were moderately correlated with sTILs (r2=0.67), however IO+ tumors were found across all sTILs strata (TILs-lo: n=1/38, 2%; TILs-int: n=7/17, 41%; TILs-hi: n=2/2, 100%).
Conclusions: This dataset reaffirms excellent outcomes for T1c, chemo-treated TNBC, and highlights a potential role for chemo-deescalated, ICI-based therapy for patients with IO+ and sTIL-lo/int tumors who are predicted to benefit from ICI but who still are at high risk of recurrence without systemic therapy. A clinical trial is warranted to evaluate efficacy of ICI in T1c TNBC, and to test the utility of IO score for determining ICI benefit in this setting.

P2-11-13: Retrospective validation of digital twin-based prediction of personalized triple negative breast cancer response to neoadjuvant therapy regimens

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Introduction: Neoadjuvant therapy is standard of care for locally advanced triple-negative breast cancer (TNBC), yet only 50-65% achieve a pathological complete response (pCR). Personalizing therapeutic regimens remains a challenge. We have developed MRI-guided digital twins (mathematical models predicting patient responses to neoadjuvant chemotherapy (NAC)) to address this need [1,2]. This study aims to validate digital twins by virtually replicating results from previous clinical trials investigating the efficacy of various chemotherapy regimens.

Methods: This study employed 105 TNBC patients from the ARTEMIS trial (NCT02276433) [3] who received 4 cycles (4×) of Adriamycin/Cytoxan (A/C) every 2-3 weeks, followed by 12 cycles (12×) of weekly Taxol (T). Each patient had multi-parametric MRI to monitor tumor anatomy, perfusion, and cellularity. Personalized digital twins were created by calibrating a biology-based mathematical model to each patient's MRI data collected before, after 2 cycles, and at the end of A/C [1]. The calibrated digital twin has been shown to predict the response of patient's tumor to both the actual and alternative NAC regimens [2].

The accuracy of predicted response to the actual regimen was validated in prior work [1]. To further validate the accuracy of predicted response to alternative NAC regimens, we simulated the response of the individual patients in our cohort to the regimens investigated in three landmark clinical trials (INT C9741 [4], ECOG 1199 [5-6], and SWOG S0221 [7]) that compared A/C and T administrative schedules in locally advanced breast cancer, and determine if our digital twin-based methodology can recapitulate the trial observations.
INT C9741 compared two A/C-T regimens: 1) tri-weekly A/C-T: 4× A/C per 3 weeks → 4× T per 3 weeks, and 2) dose-dense A/C-T: 4× A/C per 2 weeks → 4× T per 2 weeks. The trial found dose-dense A/C-T significantly improved the disease-free and overall survival (DFS/OS) compared to tri-weekly A/C-T.
ECOG 1199, along with recent meta-analyses [8], compared three T regimens combined with tri-weekly A/C: 1) tri-weekly Taxol: 4× A/C per 3 weeks → 4× T per 3 weeks, 2) bi-weekly Taxol: 4× A/C per 3 weeks → 4× T per 2 weeks, and 3) weekly Taxol: 4× A/C per 3 weeks → 12× T weekly. Weekly and bi-weekly Taxol provided similar DFS/OS superior to tri-weekly Taxol, especially for TNBC [6].
SWOG S0221 compared four different A/C-T regimens: 1) Arm 1: 6× A/C per 2 weeks → 6× T per 2 weeks, 2) Arm 2: 15× A/C weekly → 6× T per 2 weeks, 3) Arm 3: 6× A/C per 2 weeks → 12× T weekly, 4) Arm 4: 15× A/C weekly → 12× T weekly. All regimens showed similar DFS over the population, with a non-significant DFS/OS improvement in TNBC patients with bi-weekly A/C-T (Arm 1).

Results: For INT C9741, our digital twin predictions yielded a pCR rate of 49.52% and 73.33% for tri-weekly and dose-dense A/C-T, respectively, with a significant difference ($P < 0.001$) via $\chi 2$ test. This result was consistent with the trial observation on dose-dense A/C-T.
For ECOG 1199, our digital twin predictions yielded pCR rates of 49.52%, 60.00%, and 55.24%, respectively, for the tri-weekly, bi-weekly, and weekly T regimens, with no significant difference ($P > 0.1$). The weekly and bi-weekly T led to higher pCR rates than the tri-week T, consistent with the trial observations.
For SWOG S0221, our digital twin predictions yielded pCR rates of 79.05%, 72.38%, 73.33%, and 69.52% for the four regimens, with no significant difference. Arm 1 had the highest pCR rate, again consistent with the trial outcome.

Conclusion: Our digital twin predictions matched previous clinical trial observations on various NAC regimens, supporting their use in tailoring NAC for TNBC patients. This method can also be beneficially used for designing adaptive clinical trials.

[1] Wu et al., Cancer Res, 2022. [2] Wu et al., SABCS, 2023. [3] Yam et al., Clin Cancer Res, 2021. [4] Citron et al., J Clin Oncol, 2003. [5] Sparano et al., NEJM, 2008. [6] Sparano et al., J Clin Oncol, 2015. [7] Budd et al., J Clin Oncol, 2015. [8] Khan et al., J Clin Oncol, 2020.

P2-11-14: Treatment Patterns and Safety of Adjuvant Therapy After Chemoimmunotherapy for Early-Stage Triple-Negative Breast Cancer in a Real-World Scenario: The Neo-Real Study

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Background: The KEYNOTE-522 trial established neoadjuvant pembrolizumab plus chemotherapy (P+CT) followed by adjuvant pembrolizumab as the standard of care for stage II-III triple-negative breast cancer (TNBC). Nevertheless, numerous doubts were raised in clinical practice on how to integrate this regimen with other treatment standards such as adjuvant capecitabine (C) or olaparib (O). This study aims to evaluate treatment patterns and safety outcomes of adjuvant therapies following neoadjuvant P+CT using real-world data (RWD).

Methods: The multicentric Neo-Real study evaluates patients with TNBC treated with neoadjuvant P+CT since July 2020 across ten cancer centers. The objective of this analysis is to describe the treatment patterns in the real-world and to analyze the safety associated with these treatments, focusing on grade ≥ 3 adverse events (AE) and rates of drug discontinuation due to AE.

Results: To date, 410 patients have been included in the Neo-Real study, with 349 having a pathologic report following surgery and 185 concluding all the adjuvant therapy phase. Median age was 43 years; 69.5% had stage II and 25.8% stage III disease. Pathologic complete (pCR) response was achieved in 62.5% (n = 218) of patients and residual disease was found in 37.5% (n = 131). Among patients with pCR, 14% (n = 31) did not receive any adjuvant therapy, and 86% (n = 187) received Pembrolizumab alone (P). Among those with residual disease and a BRCA wild-type/unknown status (n=114), 26.3% (n = 30) received adjuvant P alone, 54.4% (n = 62) received P plus capecitabine (P+C), 10.5% (n = 12) capecitabine alone, 4.4% (n = 5) other regimens (including clinical trials), and 4.4% (n = 5) received no adjuvant therapy. Among those with a BRCA mutation and residual disease (n=12), 75% (n = 9) received pembrolizumab plus olaparib (P+O), 16.7% (n = 2) P+C, and 8.3% (n = 1) olaparib alone.
Rates of grade ≥ 3 AE were 6.3% with P, 16.3% with P+C, and 14.3% with P+O (P=0.057). The most common grade ≥ 3 AE were immune-related AE (6.3%) with P, diarrhea (6.1%) and hand-foot syndrome (8.2%) with P+C, and anemia (14.3%) with P+O. Drug discontinuation due to toxicity during adjuvant therapy occurred in 5.4%, 12.7%, and 10% of the cases, respectively (P=105).
The immune-related grade ≥ 3 AE with adjuvant P consisted of endocrine disorders (2.8%), hepatitis (1.4%), pneumonitis (0.7%), colitis (0.7%), arthritis (0.7%), and skin toxicity (0.7%). Notably, considering all the course of pre- and post-surgery therapy, 23.7% of the

immune-related AE occurred during the adjuvant phase.

Conclusions: In a real-world scenario, most patients treated with neoadjuvant chemoimmunotherapy continued adjuvant pembrolizumab after achieving pCR, while adjuvant capecitabine and adjuvant olaparib were frequently used in combination with pembrolizumab for those of residual disease. Combined adjuvant strategies showed higher rates of grade ≥ 3 AE and drug discontinuation. The efficacy of the combined adjuvant strategies remains to be determined.

P2-11-15: Axillary Pathologic Complete Response in Triple-Negative Breast Cancer Patients with Mammary Pathologic Complete Response: A Systematic Review

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Background: Neoadjuvant chemotherapy plays a crucial role in breast cancer by providing prognostic information through assessment of systemic treatment response. Response patterns can indicate which patients may have better survival outcomes and may benefit from less invasive therapies. Considering mammary and axillary responses, patients with HER2-positive and triple-negative subtypes exhibit higher rates of pathologic complete response (pCR). This study aimed to evaluate the rate of axillary pCR in triple-negative breast cancer patients achieving mammary pCR.
Methods: This systematic review was conducted following the PRISMA protocol and was registered in PROSPERO (ID: 498121). PubMed, Embase, and Web of Science databases were searched. MESH Terms used were: ((((triple negative breast cancer) and (neoadjuvant chemotherapy)) and (lymph node)) and (axilla response)) and (breast response). Article screening was performed independently by two evaluators using the Rayyan platform. Pathologic complete response (pCR) was defined as the absence of invasive disease in the breast and axilla, with or without residual in situ disease after neoadjuvant chemotherapy (ypT0/ypTis/ypN0). Axillary pCR also included the absence of isolated tumor cells and micrometastases. Studies evaluating mammary and axillary pCR after neoadjuvant treatment in breast cancer patients, including triple-negative tumors, were included without restrictions on chemotherapy type or surgical procedures. Methodological quality assessment was conducted using the Newcastle-Ottawa Scale for cohort studies.
Results: As of November 2023, 180 studies were retrieved, with ten cohort studies meeting the inclusion criteria, totaling 7,206 patients, of whom 30% had triple-negative tumors. Each of the 10 pre-selected articles, all cohort studies, underwent bias assessment using the Newcastle-Ottawa Scale. All 10 selected articles scored 6 or higher, indicating acceptable quality in their methodology and enabling inclusion in this systematic review. Among these, 42% had clinically positive axilla (cN+) prior to neoadjuvant chemotherapy, confirmed by cytologic examination in 419 cases and clinical or pathological examination in 2,575 cases.

Axillary pCR rates were consistently higher than mammary pCR rates across the studies. In 9 out of the 10 selected articles, the rate of axillary pCR was higher than the rate of mammary pCR; in one of them, the rate was similar. In none of the evaluated articles was the mammary response rate higher than the axillary. The overall rates of pCR were 33.4% in the breast (ranging from 19 to 48%), 49.3% in the axilla (ranging from 25 to 92%), and 26.9% (ranging from 14 to 38%) for both sites combined in the triple-negative population. Conclusion: Despite high chemosensitivity in triple-negative breast cancer, rates of mammary pCR are consistently lower than the rates of axillary pCR. Factors beyond tumor subtype likely influence response patterns, indicating the need for further research to identify predictive biomarkers and optimize treatment strategies.

P2-11-16 Characterization of disseminated tumor cells (DTCs) in patients with triple-negative breast cancer (TNBC)

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Background: Despite successful treatment of the primary tumor, recurrence occurs in about 30% of breast cancer patients. One possible reason might be micro-metastases, so-called disseminated tumor cells (DTC) in the bone marrow (BM), which split off from the primary tumor in early stages of the disease. The most aggressive subtype is triple negative breast cancer (TNBC), which is hormone-receptor negative (<1% ER/ PR) and HER2 negative (IHC Score 0, 1 or 2 and CISH-). The majority of TNBCs are of high grade and show a high proliferation rate. Patients usually receive chemotherapy but have an increased risk of recurrence and poor prognosis. Particularly patients that do not achieve pathological complete remission (pCR) after chemotherapy might require targeted treatment approaches. Little is known concerning the characteristics of DTCs and their role in TNBC patients.
Methods: BM aspirates were collected from the anterior iliac crest of 80 patients with primary (n=67) or recurrent (n=13) TNBC during surgery. After density gradient centrifugation, cell suspensions were transferred onto glass slides and subjected to a sequential multi-parameter immunofluorescence staining procedure, detecting pan-cytokeratin (CK), vimentin (vim), and for exclusion of hematopoietic cells CD45 in a first panel. Cells were digitalized and detected using the Zeiss Axioscan 7 scannig microscope. After enzymatic release of the fluorochromes, a second antibody panel including HER2, Ki67 and PD-L1 was applied to the same slides, followed by scanning and detection. Nuclei were identified using DAPI within the mounting media in both staining steps. We analyzed 2 x 106 cells per patient.
Results: The DTC positive rate was 56% (n=45) among the cohort. In total, we detected 253 DTCs resulting in a median number of 4 DTCs per patient. Of the 64 patients treated with NACT, 35 were DTC positive and had a decreased pCR rate (54%) compared to DTC negative patients (n=29; pCR rate: 69%). Concerning epithelial-to-mesenchymal transition, phenotype characterization revealed that 14 patients showed epithelial DTCs (CK+/Vim-)

only, 45 cases had epithelial (CK+/Vim-) as well as mesenchymal (CK-/vim+) DTCs and DTCs with mixed characteristics (CK+/vim+). One patient displayed mesenchymal DTCs only (CK-). Based on the phenotypical (panel 1) and therapeutic (panel 2) marker expressions, we found 20 different DTC subpopulations. The majority of epithelial DTCs (CK+/vim-) revealed no expression of PD-L1, Ki67 or HER2 (68%). However, DTCs with hybrid characteristics (CK+/vim+) displayed increased numbers of Ki67+ (85%) and PD-L1+/Ki67+ DTCs (30%). The most frequently occurring profile was CK+/vim+/PD-L1-/ Ki67+/ HER2- (n=75 cells). Interestingly, the mesenchymal DTCs (CK-/vim+) revealed elevated DTC numbers with PD-L1+/Ki67+/HER2± (45%) which increased up to 80% in the CK-/vim- DTCs. HER2 positive cells were predominantly found in non-epithelial DTCs (CK-). Conclusion: Our data indicate that the process of EMT might be linked to the occurrence of DTC subpopulations positive for Ki67, PD-L1 and HER2 which could be therapeutically relevant. Further, our results rise the question whether DTCs are dormant in TNBC patients.

P2-11-17: Atezolizumab in combination w/ polychemotherapy w/ or without a pretherapeutic immune therapy window in patients w/ early triple negative breast cancer (eTNBC) & low tumor burden – prospective data from the randomized neoadjuvant neoMono trial

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Background: Neoadjuvant chemotherapy in combination with an immune checkpoint inhibitor (ICI) is standard of care in cases of high-risk (tumor size ≥ 2 cm and/or N+) early triple negative breast cancer (eTNBC). In contrast, node negative patients with tumors < 2 cm are currently treated with chemotherapy alone. In fact, based on a large series of real-world data the benefit of chemotherapy in TNBC smaller than 10 mm is in question. Overall, the optimal management of node negative patients with T1c tumors is still an unanswered question.
The neoMono trial prospectively analyzed whether the addition of a preceding Atezolizumab monotherapy window prior to Atezolizumab and neoadjuvant chemotherapy (CTX) improves pCR rates among patients with early TNBC (eTNBC). The results of the primary endpoint analysis have been reported last year at this meeting. While the trial did not demonstrate significant superiority of the immune therapy window regarding pCR rates, impressive pCR rates in the PD-L1 positive population (91.5% in the experimental arm A and 82.2% in the control arm B) as well as in the PD-L1 negative population (56.1% in arm A and 64.5% in arm B) were observed.
Here we present a subgroup analysis including only patients with eTNBC and low tumor burden (defined as tumors smaller <= 2 cm (i. e. cT1c) and cN0).

Methods: NeoMono is a phase 2 randomized multicenter trial planned to recruit a maximum

of 458 female and male pts with primary TNBC (defined as ER/PR < 10% and HER2 negative) with tumor stages cT1c – cT4d (cN0 and cN+) in 34 study sites. PD-L1 status had to be identifiable by central pathology by means of the VENTANA PD-L1 (SP142) assay and was defined by PD-L1 expression on immune cells (IC). Neoadjuvant treatment in both study arms consisted of Atezolizumab 1200 mg every 3 weeks in addition to neoadjuvant CTX (12 x Carboplatin/Paclitaxel q1w followed by 4x Epirubicin/Cyclophosphamide q3w), in arm A preceded by an Atezolizumab monotherapy window of 840 mg once two weeks prior to initiation of combination therapy. The neoMono statistical design uses Bayesian posterior probabilities (uniform prior distribution) and logistic regression. In this subgroup analysis we included only patients with tumors smaller than 2 cm and node negative disease.

Results: 57 pts in arm A and 51 in arm B were included in this subgroup analysis. Demographics and baseline characteristics were balanced between both study arms. Among patients with PD-L1 (IC) tumors (PD-L1 (IC) positive defined as ≥ 1%) pCR rates were 100% in arm A and 90% in arm B. The corresponding pCR rates for PD-L1 (IC) negative tumors (PD-L1 (IC) negative defined as <1%) were 65.9% in arm A and 76.3% in arm. In multivariate analysis (including therapy arm, PD-L1 status, grade 3 and age) only PD-L1 (IC) status (OR 8.46; p <0.044) interacted significantly with pCR.

Conclusion: To our knowledge our analysis is the largest prospective dataset regarding patients with eTNBC and low tumor burden treated with neoadjuvant chemotherapy and an immune checkpoint inhibitor. No significant differences were seen between treatment arms although numerically PD-L1 positive patients showed increased benefit from the immune therapy window. pCR rates were higher compared to the overall study population which may be attributed particularly to smaller tumor size.
We acknowledge that the neoMono trial did not include a chemotherapy only arm, therefore, no conclusions regarding a benefit of the addition of an ICI in general to neoadjuvant chemotherapy can be drawn.
Our result underscore the need to further evaluate the clinical efficacy of combined neoadjuvant chemo- and ICI-therapy in patients with eTNBC and low tumor burden.

P2-11-18: Patterns of Treatment in Older Patients with Early-Stage Triple Negative Breast Cancer

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Background: Triple negative breast cancer (TNBC) comprises approximately 15-20% of breast cancer diagnoses in patients over 70 years of age. Chemotherapy has been the mainstay of treatment for both early and advanced stages of TNBC and is associated with a clear survival advantage for older patients with this aggressive subtype. Despite an aging population and an increase in the incidence of breast cancer in older patients, there remains

a knowledge gap in the global treatment patterns of older patients with TNBC.
Methods: A retrospective cohort study was conducted at two academic cancer centers in Canada. Adult women with stage I-III triple negative breast cancer were included from 2005 to 2023. Patients were stratified by age < 70 years (younger cohort), and ≥70 years (older cohort). Demographic, clinical, and histopathologic data were extracted from the institutional databases. Descriptive statistics were used to summarize the data and the Kaplan-Meier method was used to provide survival estimates.
Results: A total of 731 patients were included with a median follow-up time of 49 months (IQR 24-79). The median age was 57 years (IQR 47-67); 20.8% of patients (n=152) were 70 years and older, whereas 79.2% (n=579) were < 70 years. Median tumor size was 22 mm (IQR 16-34) with 36.0% (n=263) having positive lymph nodes at diagnosis. Neither of these characteristics was significantly different between age groups (p=0.99, p=0.17 respectively). In terms of baseline health status, 87.5% (n=133) of the older patients had an Eastern Cooperative Oncology Group score of 0 or 1. Of the older patients, 95.4% (n=145) underwent breast surgery compared to 99.5% (n=576) of younger patients (p < 0.001). Similarly, 69.1% (n=105) of older patients underwent chemotherapy compared to 94.0% (n=544) of younger patients (p < 0.001). Immunotherapy in the early-stage setting was also differentially administered based on age group, with 5.3% (n=8) of older patients receiving it compared to 16.8% (n=97) of younger patients (p < 0.001). Chemotherapy use amongst patients ≥70 years increased over time with 41.7% receiving chemotherapy in the 2005-2010 time period as compared to 76.5% in 2015-2022 (p=0.01). Additionally, use of neoadjuvant chemotherapy (NAC) increased throughout the time period of interest with no older patient receiving NAC in the 2005-2010 interval versus 49.0% receiving NAC in 2015-2022 (p<0.001). The 5-year breast cancer-specific survival (BCSS) in the entire cohort was 89.7% (95%CI 87.2-92.3%). Five-year BCSS in older patients was 87.6% and 90.2% for the younger subgroup (p=0.64). Five-year overall survival was 77.2% in the older subgroup versus 87.5% in the younger subgroup (p<0.0001).
Conclusion: In the largest Canadian cohort of patients with early-stage TNBC to date, our analysis showed that older patients continue to be undertreated compared to their younger counterparts. However, despite the age-related differences in treatment patterns, 5-year BCSS did not significantly differ by age and was higher than 85% in women ≥70 years. This excellent outcome is likely driven by the increased use of chemotherapy and preference for neoadjuvant sequencing over time in this patient population highlighting the importance of systemic therapy in older patients with TNBC.

P2-11-19: Unique kinetics of myeloid derived suppressor cell (MDSC) accumulation associated with immune related adverse events (irAEs) in patients with early stage triple negative breast cancer (eTNBC) receiving neoadjuvant chemo-immunotherapy

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Background: The KEYNOTE (KN)-522 trial established pembrolizumab in combination with chemotherapy as the standard of care for the treatment of eTNBC (clinical stage II-III). This trial demonstrated that the addition of pembrolizumab to standard anthracycline-platinum-taxane neoadjuvant chemotherapy was associated with a clinically and statistically significant improvement in pathological complete response (pCR) rates and event-free survival compared to chemotherapy alone. Pembrolizumab is a programmed cell death protein 1 monoclonal antibody, known to cause irAEs in a significant subset of patients— in the KN-522 trial the rate of any grade and >3 grade irAEs were approximately 34% and 13%, respectively. The addition of pembrolizumab does add significant toxicity to standard chemotherapy, which can lead to early discontinuation of curative intent treatment. Therefore, identification of early predictors of irAEs and ways to mitigate them is very important. We hypothesized that an increase in circulating MDSC levels is an early marker of irAEs associated and that MDSCs differ phenotypically and functionally from tumor driven MDSCs. Accumulation of MDSCs has been previously reported to correlate with stage and inversely correlate with response to systemic therapy.

Methods: PBMCs were isolated at baseline, Cycle 1 Day 1, Cycle 1 Day 8, Day 1 Cycles 2-4, and after surgery from n=40 eTNBC patients treated with the KN-522 chemotherapy regimen. Phenotypic characterization of MDSCs was performed by high parameter flow cytometry. Data was concatenated and visualized in UMAPs using FlowJo. Kinetics of MDSC accumulation during treatment was assessed and associated with irAEs defined as: adverse events that associated with exposure to an immune checkpoint inhibitor (ICI) and consistent with an immune phenomenon. Phenotypic characteristics of MDSCs from metastatic TNBC patients at baseline and MDSCs from non-metastatic TNBC at the time of irAEs were compared.

Results: Twelve out of 40 patients (30%) on study had one of the following documented iRAEs: hypothyroidism (n=4), hepatitis (n=3), adrenal insufficiency (n=2), colitis (n=1), myocarditis (n=1), and hemophagocytic lymphohistiocytosis (n=1). Levels of MDSCs increased during treatment among non-metastatic patients undergoing ICI, and this surge was more prominent among patients experiencing irAEs. In eTNBC patients with irAE associated MDSCs, we observed marked differences in the PMN-MDSC phenotype when compared to MDSCs from metastatic TNBC patients (tumor derived MDSCs). One key finding was significantly higher CX3CR1 expression, the receptor for fractalkine, among irAE associated MDSCs vs. tumor derived MDSCs. Interestingly, accumulation of CX3CR1+ PMN-MDSCs was also found to be the prominent MDSC subtype in the patient who developed severe grade 4 hemophagocytic lymphohistiocytosis.

Conclusions: Our results highlight a potential contribution of a unique subset of PMN-MDSCs to the pathogenesis of irAEs that could involve signaling by fractalkine and provide a potential target of therapeutic intervention.

P2-11-20: Discovering Co-driver Genes and Pathways of Mutant TP53 in Breast Cancer by CRISPR Screening and Multi-omics Approaches

Lilian Nwachukwu, Anasuya Pal, Yining Zheng, Laura Gonzalez-Malerva, Duatin Grief, Lydia Sakala, Jin Park, Joshua LaBaer

In the United States, breast cancer is one of the leading causes of cancer death in women. Triple-negative breast cancer (TNBC) is the most aggressive of all molecular subtypes of breast cancer. It is highly heterogeneous and difficult to target and treat due to its lack of targetable receptors. Our aim is to identify new molecular targets that could aid the treatment of TNBC cases and reduce cytotoxicity of the current therapeutic regimen. The TP53 gene, a known tumor suppressor is the most frequently mutated gene in triple-negative breast cancer occurring in >80 % of TNBC cases.

Statistical pathway analysis from RNA-Seq and ChIP-Seq done on the MCF10A mammary epithelial cell lines expressing the 10 most clinically prevalent p53 mutants identified the Hippo pathway to be dysregulated when associated with aggressive cellular phenotypes. Upon suppression of the upstream Hippo tumor suppressor pathway, the activation of a series of genes promoting cancer hallmark phenotypes ensues. A cell-based TEAD reporter assay showed transcriptional activity of TEAD to be higher in MCF10A cells expressing more aggressive p53 mutants compared to the cell lines with less aggressive mutants. Knockdown of the TAZ protein decreased the invasiveness of the invasive p53 mutant expressing cell lines.

We hypothesized that, in addition to neo-morphic activities of different p53 mutants, the heterogeneity in clinical phenotypes observed in TNBC is due to functional interactions of the driver mutation in p53 with other functionally important co-existing mutations and dysregulated pathways, called ‘co-drivers’.

To identify potential functionally important ‘co-driver’ mutations that cause cancer progression and heterogeneity in clinical phenotypes, a genome-wide screen was performed using lentiviral CRISPR screen library on the invasive MCF10A-p53-R273C-MycOE cell line. In vivo experiments showed that injection of the library-transduced cells yielded tumor in mice. PCR and whole-exome sequencing of the tumor showed frameshift mutations in the enriched EVC2 (EvC Ciliary Complex Subunit 2), NR1D1 (Nuclear Receptor 1D1), and ARAF (a Ras-activating RAF family kinase) genes. Confirming the hits from screening, knockout of these genes in R273C-MycOE cells also showed increased cell invasion.

In parallel, in vitro screens carried out using CRISR knock-out library on the least aggressive MCF10A-p53-Y234C cells identified potential co-drivers. Knockout of GCNT1 and FGD6 genes in MCF10A-p53-Y234C cells increased cell invasion and induced dephosphorylation and activation of TAZ. TEAD activity was also found to be higher in these knock-out cells compared to the Y234C cells. Knockdown of the TAZ protein showed decreased TEAD

activity in the FGD6 and GCNT1 knock-out cells and substantially decreased cell invasion.

We have carried out functional characterization of the TP53 mutations in TNBC and confirmed the cellular phenotypes for specific p53 mutants obtained from the in vitro and in vivo screens. We are validating these identified 'co-driver' genes and targets to discover the molecular mechanisms affecting phenotypic heterogeneity of the p53 mutants and investigating the mechanisms through which Hippo pathway drives the progression of TNBC, as well as assessing the pathway as a target for potential therapy for TNBC with specific TP53 mutations.

P2-11-21: Characterisation of treatment and outcomes for adenoid cystic carcinoma as a distinct clinical sub-type of triple negative breast cancer

Fiona Britton, Steven Churchill, Paul Bishop, Guy Betts, Joseph Haigh, Emily Heathcote, Anne Armstrong, Robert Metcalf

Background: Adenoid cystic carcinoma (ACC) most commonly arises within the salivary glands but can also occur in other glandular tissue including the breast. ACC of the breast (ACCB) represents c.0.1% of breast cancer diagnoses and typically has triple negative receptor status; however the biology and clinical course are very different from triple negative invasive ductal/lobular carcinomas. ACC is typically a slow growing disease with high rates of recurrence many years after diagnosis. Furthermore, ACC has very low response rates to cytotoxic chemotherapy. We report a single institution case series of patients with ACCB, over 26 years and present their management and outcomes.
Methods: Search of an institutional pathology database for diagnoses of ACCB from 1990 to 2020 was conducted to identify patients. A retrospective review of their patient records was undertaken, including a summary of clinical and pathological characteristics, description of curative-intent management and outcomes.
Results: Sixteen ACCB patients were identified who all presented with early breast cancer; T1 (n=7), T2 (n=6), T3 (n=3). Two patients had nodal disease (N1) at diagnosis. Median age at diagnosis was 65 years (range 34 to 91 years). All patients underwent upfront surgical resection with either mastectomy (6/16) or wide local excision (WLE) (10/16), and both patients with known lymph node involvement had axillary lymph node clearance. Six patients received adjuvant chemotherapy with anthracycline and taxane based regimens. Eleven patients went on to receive adjuvant radiotherapy, including 9 WLE patients and 2 mastectomy patients.
Two-year survival was 100%, with no patients experiencing local or distant recurrence. Metastatic recurrence was identified in 7 (44%) patients, including both patients with lymph node involvement at diagnosis. Median time to first recurrence was 74 months (range 27 to 127 months), while median overall survival from recurrence was only 9 months (range 3 to 15 months). Only two patients received systemic anti-cancer therapy for the treatment of metastatic disease, and one patient had known metastatic disease monitored for 15 months with surveillance imaging. One patient had a mastectomy for local

recurrence and a solitary lung metastasis resected, remaining on surveillance with no evidence of disease 33 months later. The other six patients have all died from their metastatic disease. Survival data is based on a minimum follow-up of five years. Five-year survival was 87.5% (14/16), with a median overall survival of 89.5 months.
Conclusions: ACCB is a rare breast cancer subtype and follows a different clinical course to IDC/ILC; ACCB specific management and follow-up should be considered to reflect this. These patients experienced disease recurrence at later timepoints. Previous case studies report better outcomes for ACCB patients when compared to typical triple negative breast cancer, however the majority of ACCB patients recur more than 5 years after diagnosis. Clinicians should be aware of a greater propensity to late follow up for ACCB patients.

P2-11-22: Tolerance of Neoadjuvant Chemotherapy in Combination with Immunotherapy in Older Patients with Triple Negative Breast Carcinoma: The MSKCC Experience

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Background: Triple-negative breast cancer (TNBC) comprises 20% of breast cancer subtypes and has elevated risk of recurrence and mortality. In patients with early-stage TNBC, neoadjuvant chemotherapy (NAC) is standard of care. Among patients treated with NAC, pathologic complete response (pCR) at surgery portends a favorable prognosis. The KEYNOTE 522 (KN-522) trial reported improved pCR, event free survival, and overall survival using immunotherapy with an anthracycline, taxane, and carboplatin-based regimen. However, the median age in KN-522 was 49 years and 90% of the enrolled population was ≤65 years of age. Due to persistent underrepresentation of patients ≥65 years in clinical trials, tolerance and toxicity patterns of the standard KN-522 regimen are not well understood in this subgroup. In this study, we aim to assess real-world feasibility of the KN-522 regimen in patients ≥65 years by studying the impact of treatment modification on clinical outcomes. Methods: We conducted a retrospective analysis of patients age ≥65 with non-metastatic TNBC treated at Memorial Sloan Kettering Cancer Center (MSKCC). Patients were excluded if they received a portion of their care elsewhere or were found to have metastatic disease prior to initiation of NAC. Percent completion of drugs was summarized using mean and interquartile range and its association with pCR, defined as absence of invasive carcinoma on surgical pathology (ypT0N0 or ypTisN0), was assessed using Wilcoxon rank sum test. Fisher's exact test was used to compare the pCR rate between patients who completed ≤85% and >85% of the KN-522 regimen, a threshold that prior studies suggest may affect clinical outcomes. Results: We identified 55 patients ≥65 who began treatment with the KN-522 regimen. Ages ranged from 65 to 85. Of these 55 patients, 2 (4%) had stage I, 40 (73%) had stage II, and 13 (24%) had stage III disease. Only 27 (49%) patients completed >85% of the planned treatment, while only 5 (9.1%) completed the full KN-522 regimen. In 12 (22%) patients, one or more drugs were not administered (cyclophosphamide 10 [18%], anthracycline 9 [16%], carboplatin 3 [5.5%], taxane 1 [1.8%],

pembrolizumab 1 [1.8%]). Of 52 patients who began the carboplatin and taxane portion of the regimen, 29 (56%) received carboplatin weekly while 22 (42%) received it every 3 weeks. Toxicity required dose reduction in 7 (13%) and treatment discontinuation in 27 (52%) of one or both drugs. Of 45 patients who began the anthracycline and cyclophosphamide portion of the regimen, toxicity required dose reduction in 4 (9%) and treatment discontinuation in 10 (22%) of one or both agents. pCR was observed in 24 (44%) patients. The pCR rate did not differ significantly between those who received ≤85% and >85% of the treatment regimen (13 [46%] vs 11 [41%], p=0.8). Grade 3 or higher anemia, neutropenia, and thrombocytopenia were present in 22 (40%), 28 (64%), and 10 (25.5%) patients, respectively. Six (10.9%) had febrile neutropenia. Twelve (21.8%) had immunotherapy-related toxicity, the most common of which were thyroid dysfunction (4, 7.3%), hepatotoxicity (3, 5.5%), and pneumonitis (3, 5.5%). Toxicity required hospitalization in 16 (29%) patients. There were no treatment-related deaths. Conclusion: Treatment completion rate for the standard KN-522 regimen in patients ≥65 years of age was less than 10%, with less than half of patients completing >85% of the planned treatment regimen. The pCR rate observed in this population appeared to be lower than previously reported with this regimen but was not significantly different between those who received ≤85% and >85% of the planned treatment. There is a need for better tolerated chemotherapy and immunotherapy combination regimens in older patients.

P2-11-23: Impact of age on neoadjuvant chemo-immunotherapy (IO) administration and outcomes in patients with early-stage triple-negative breast cancer (TNBC)

Alexis LeVee, Bethania Santos, Megan Wong, Nora Ruel, Heather McArthur, Joanne Mortimer

Introduction: Although patients diagnosed with early-stage triple negative breast cancer (TNBC) are commonly diagnosed before age 50, the impact of age on efficacy and toxicity of recent innovations in standard-of-care curative intent therapy are not well-defined. For example, subgroup analyses from KEYNOTE-522, wherein 11% of the population was aged ≥65y, demonstrated an overall benefit with pembrolizumab (pembro) in addition to neoadjuvant chemotherapy (NAC) in patients aged ≥65y and age <65y; however, the older cohort had an inferior pathologic complete response (pCR) rate of 54.3% vs. 66.2% in the younger cohort, with corresponding event-free survival (EFS) hazard ratios of 0.79 and 0.61, respectively. The aim of this analysis is to examine whether differences in management of older patients with early breast cancer exist and to identify appropriate patients who would benefit from neoadjuvant chemo-immunotherapy (IO).
Methods: Patients with early-stage TNBC diagnosed between 7/1/2021 and 12/31/2023 for whom treatment with the KEYNOTE-522 regimen was planned standard-of-care therapy were identified from 2 institutional databases. Patients were excluded if they did not receive at least 1 cycle of NAC, at least 1 cycle of neoadjuvant pembro, or did not undergo definitive surgery. Univariate and multivariate analysis was performed using logistic

regression to identify factors associated with pCR.
Results: Of the 264 patients (263 female/1 male) included in the analysis, the median age was 52.9 (range 20.8-87.7). 51 (19.4%) patients were age ≥65y, and 213 (80.6%) patients were <65y. Hypertension was more common in patients age ≥65y compared to those <65y (66.7% vs. 21.6%), as was hyperlipidemia (64.7% vs. 23.0%) and type 2 diabetes (33.3% vs. 13.6%) [p<0.001]. Although tumor size, nodal status, and grade were similar between the two age groups, patients age ≥65y were more likely to be diagnosed with non-ductal histologies (17.5%) compared to age <65y (5.1%) [p=0.005]. Patients age ≥65y received fewer cycles of NAC compared to age <65y, with a median of 7 cycles (2-8) and 8 cycles (2-8), respectively (p<0.0001). Similarly, patients age ≥65y received less cycles of neoadjuvant pembro compared to age <65y, with a median of 7 cycles (1-11) and 8 cycles (1-11), respectively (p=0.003). The number of carboplatin and taxane cycles were similar between the two age groups (p=0.34 and p=0.60, respectively). Although the median number of anthracycline-cyclophosphamide (AC) cycles was the same in both age groups [4 (0-4)], the distribution of AC cycles varied: 52% of patients age ≥65y vs. 78% of those age <65y completed 4 cycles of AC, while 36% of patients age ≥65y vs. 12% of those age <65y received 0 AC cycles (p=0.0003). Patients age ≥65y had inferior pCR rates compared to patients age <65y: 49.0% vs. 63.4%, respectively (p=0.059). Although the 65y cutoff was not predictive of pCR, we observed a considerable advantage in patients age <50y vs. those age ≥50y, both in the univariate and multivariate setting. Multivariate analysis showed that younger age (<50y vs. ≥50y) (odds ratio (OR) 1.94; 95% confidence interval (CI), 1.11-3.38; p=0.02), the absence of type 2 diabetes (OR 2.08; 95% CI 1.03-4.18; p=0.04), and higher grade (3 vs. ≤2) (OR 2.18; 95% CI 1.07-4.42; p=0.03) were independent predictors of pCR.
Conclusion: This study suggests that older patients with early TNBC are less likely to receive the planned course of neoadjuvant chemo-IO which may, in turn, impact pCR rates. Overall, older patients received fewer cycles of both neoadjuvant pembrolizumab and NAC, with AC most frequently discontinued. Further studies to identify the optimal treatment regimen for patients aged ≥65y with TNBC are needed.

P2-11-24: Racial and ethnic disparities in treatment outcomes of patients with early-stage triple-negative breast cancer following neoadjuvant chemoimmunotherapy

Megan Wong, Alexis LeVee, Bethania Santos, Nora Ruel, Heather McArthur, Joanne E. Mortimer

Introduction: Triple-negative breast cancer (TNBC) disproportionately impacts patients (pts) of select racial/ethnic groups, particularly evidenced by the higher incidence and poorer outcomes among African American pts with triple-negative disease. Since the KEYNOTE-522 trial did not report differences in pathologic complete response (pCR) by racial/ethnic groups following neoadjuvant chemotherapy (NAC) with or without pembrolizumab, it is critical to confirm the benefit of adding pembrolizumab to NAC in all pt populations. Our study sought to determine the impact of race/ethnicity on the clinical

management and treatment outcomes of NAC with pembrolizumab to confirm the generalizability of KEYNOTE-522 and to identify differences in outcomes according to race/ethnicity. Methods We performed a retrospective chart review of pts with early-stage TNBC receiving NAC with pembrolizumab from 2 institutional databases (City of Hope and University of Texas Southwestern). Pts included in the study were diagnosed with early-stage TNBC from 7/1/2021 to 12/31/2023, received at least 1 cycle of NAC with pembrolizumab, and underwent definitive surgery. Descriptive statistics were used to help understand the differences in clinicopathologic, treatment, and response variables across race/ethnicity groups. The Chi-squared test was used to compare categorical data and the Kruskal-Wallis test compared continuous data across the race/ethnicity categories.
Results: 264 pts (263 females and 1 male) were included in this study, with 116 (43.9%) pts identifying as non-Hispanic White (NHW), 61 (23.1%) as Hispanic/Latino (HISP), 43 (16.3%) as Black/African American (AA), and 34 (12.9%) as Asian. The remaining 10 (3.8%) pts were of other/unknown ancestry. At diagnosis, there were no differences in age, prevalence of hyperlipidemia, prevalence of type 2 diabetes, clinical tumor size, clinical nodal stage, histology, and tumor grade between the racial/ethnic groups ($p>0.2$). However, BMI ($p=<0.0001$) and obesity ($p=0.004$) varied significantly across racial/ethnic groups of pts receiving neoadjuvant chemoimmunotherapy. Asian pts had the lowest median BMI at 24.9 (interquartile range [IQR] 22.2-29.1) and the lowest prevalence of obesity (23.5%), whereas AA pts had the highest median BMI at 33.9 (IQR 26.4-37.5) and highest rate of obesity (62.8%). Similarly, the prevalence of hypertension varied by race/ethnicity ($p=0.0008$). Hypertension was more common AA pts (53.5%) than Asian (23.5%), HISP (24.6%), and NHW pts (24.1%).
During NAC, the receipt of ≥75% of carboplatin and taxane doses were similar across racial/ethnic groups ($p=0.06$). There was also no difference in the number of doses received of pembrolizumab ($p=0.6$) and doxorubicin with cyclophosphamide ($p=0.4$).
While pCR rates were not significantly different by race or ethnicity ($p=0.3$), AA (51.1%, 95% confidence limit [CL] 35.6-66.7) and Asian (50.0%, 95% CL 35.3-70.6) pts had the lowest rates of pCR, whereas HISP (67.2%, 95% CL 55.0-79.3) and NHW (63.8%, 95% CL 54.9-72.7) had the highest rates of pCR.
Conclusion: Although not statistically different, our study found that AA and Asian pts had the lowest rates of pCR following NAC plus pembrolizumab, whereas HISP and NHW had the highest rates of pCR. Since the limited number of Asian and AA pts may contribute to this lack of significance, further research with larger sample sizes of these groups is needed. In addition, the presence of obesity and hypertension was different among racial/ethnic groups, highlighting that underlying health disparities exist in the NAC setting. Understanding these disparities underscores the importance of including diverse pt populations in clinical trials to ultimately optimize treatment outcomes for all pts with TNBC.

P2-11-25: Comparison of Outcomes Among Women with Triple-Negative Breast Cancer Treated with Neoadjuvant Chemotherapy According to Age

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Background: Triple-negative breast cancer (TNBC) subtype and women diagnosed with breast cancer at a young age (<40) have worse outcomes despite a higher rate of achieving pathologic complete response (pCR) following neoadjuvant chemotherapy (NAC). We sought to identify whether young age contributed to worse outcomes in a contemporary group of TNBC patients treated with NAC.
Methods: A retrospective review of an institutional database identified women with TNBC who underwent NAC followed by surgery from 2010-2019. Clinicopathologic, treatment, and outcomes variables were compared between women <40 and ≥40 years of age at diagnosis. Multivariable models were used to identify factors independently associated with locoregional recurrence (LRR) and distant recurrence (DR), and survival.
Results: We identified 515 women with median age of 51 (range 21-82); 104 (20%) were <40 and 411 (80%) were ≥40 years of age. Compared to women ≥40, younger women presented at a similar clinical stage (of all patients, 6.4% were stage 1, 65% stage 2, and 29% stage 3) and had similar rates of lymphovascular invasion (25%) and extracapsular extension (9.4%). Younger women were more likely to have a high-penetrance gene mutation (35% vs 14%), breast pCR defined as ypT0/is (55% vs 34%), negative pathologic nodal status (81% vs 64%), higher overall pCR rate (47% vs 25%), undergo a mastectomy (75% vs 47%) and less likely to receive adjuvant radiotherapy (70% vs 84%)(all p<0.01). A smaller proportion of women <40 underwent an axillary lymph node dissection (16% vs 25%), but this difference was only marginally significant (p=0.053). There was no difference in type of chemotherapy regimens between groups, with nearly 87% of women receiving an ACT-based regimen. At a median follow-up of 2.8 years (interquartile range 1.6-4.7), there were 36 ipsilateral LRR events. The 5-year LRR rate was 7.9% in women <40 (95% confidence interval [CI] 1.4-14%) versus 8.7% in women ≥40 (95% CI: 5.5-12%) and did not differ between groups (p=0.6). There were 79 total DR events; the 5-year DR rate was 15.2% in women <40 (95% CI 7.4-22.4%) versus 18.4% in women ≥40 years (95% CI 14-22.5%) and did not differ between groups (p=0.6). Fifteen (14%) women in the <40 group died of disease versus 77 (19%) in the ≥40 group (p=0.2). On multivariable analysis, lymphovascular invasion, nodal positivity, and having residual disease were significantly associated with increased risk of breast-cancer mortality. Age, race, and type of surgery were not significantly associated with any of the outcomes.
Conclusion: Young women with TNBC were more likely to be gene carriers and to undergo mastectomy, although they presented with similar clinical stage and were more likely to achieve a pCR from NAC. Young age at diagnosis was not an independent predictor of recurrence and survival outcomes in women with TNBC treated with NAC. Further studies assessing the impact of neoadjuvant chemo-immunotherapy on outcomes in young women are needed.

P2-11-26: Adjuvant chemotherapy in lymph node-negative, T1 triple-negative breast cancer

Jesus Anampa, Alvaro Alvarez Soto, Samilia Obeng-Gyasi, Rachel B. Jimenez, Xiaonan Xue

Introduction: Adjuvant chemotherapy improves overall (OS) and disease-free survival in early-stage triple-negative breast cancer (TNBC) after upfront surgery. However, the role of adjuvant chemotherapy in small, node-negative TNBC has not been formally assessed in clinical trials, and high-quality data is scarce. To better understand this issue, we designed this study to evaluate the benefit of adjuvant chemotherapy in a nationwide dataset.
Methods: This is a retrospective study using the National Cancer Database (NCDB). We collected data on T1N0M0 TNBC patients treated with upfront surgery diagnosed between 2010 and 2019. Kaplan-Meier methods and cox proportional models were used to compare OS between chemotherapy and no chemotherapy cohorts. Logistic regression analysis was used to identify variables associated with chemotherapy use.
Results: In patients with T1N0M0 TNBC, adjuvant chemotherapy improved OS (HR, 0.51; 95% CI, 0.47 – 0.55; p<0.001). The benefit of adjuvant chemotherapy was evidenced across all tumor sizes but with different absolute improvements in OS; the 5- year OS rates for chemotherapy vs. no chemotherapy were 98% vs. 93%, 96% vs. 89%, and 93% vs. 80% for patients with T1a, T1b, and T1c tumors, respectively. Compared to NH white, NH Black women had the same odds of chemotherapy use (OR, 1.01; 95% CI, 0.94 – 1.10; p=0.75) but derived less benefit from chemotherapy (HR, 0.64; 95% CI, 0.54 – 0.76; p<0.001). Despite no difference in chemotherapy benefit in OS across age subgroups, old patients had lower odds of chemotherapy use (OR, 0.19; 95% CI, 0.18 – 0.21; p<0.001).
Conclusions: We report that adjuvant chemotherapy improves OS in patients with T1N0M0 TNBC. However, the magnitude of the benefit is larger for T1c tumors than for T1b and T1a tumors. Patients with T1aN0 had good prognosis with or without chemotherapy; thus, informed decision-making weighing risks, benefits, and comfort level is especially critical in this group.

P2-11-27: A Phase 3, Randomized Study of Adjuvant Sacituzumab Tirumotecan (sac-TMT) Plus Pembrolizumab vs. Treatment of Physician's Choice in Patients With TNBC Who Received Neoadjuvant Therapy & Did Not Achieve a Pathological Complete Response at Surgery

Heather McArthur, Jing Wei, Jaime Mejia, Wilbur Pan, Javier Cortes

Background: Trophoblast cell surface antigen 2 (TROP2) expression is higher in triple-negative breast cancer (TNBC) than in other breast cancer subtypes, and the high expression is associated with poor prognosis. Sacituzumab tirumotecan (also known as sac-TMT/MK-2870/SKB264) is a novel antibody-drug conjugate composed of anti-TROP2 antibody coupled to a cytotoxic belotecan derivative via a novel linker with an average drug to antibody ratio of 7.4. In a previous phase 3 study (OptiTROP-Breast01), sac-TMT

monotherapy provided statistically significant and clinically meaningful improvement in PFS (hazard ratio [HR], 0.31; 95% CI, 0.22-0.45; P < 0.00001) and OS (HR, 0.53; 95% CI, 0.36-0.78; P = 0.0005) compared with physician's choice of chemotherapy in patients with heavily pretreated advanced TNBC. The current standard of care for patients with newly diagnosed high-risk early-stage TNBC is neoadjuvant pembrolizumab plus chemotherapy followed by adjuvant pembrolizumab monotherapy after surgery. However, there is a high unmet need for patients who do not achieve pathological complete response (pCR) after receiving the current standard of care regimen as these patients experience higher rates of disease recurrence and mortality when compared with those who achieve a pCR. The current study (NCT06393374) evaluates adjuvant sac-TMT plus pembrolizumab versus treatment of physician's choice (TPC; comprised of pembrolizumab with or without capecitabine) in patients with TNBC who received neoadjuvant therapy and did not achieve a pCR at surgery.
Methods: This phase 3, randomized, multicenter, open-label study is enrolling patients aged ≥18 years with centrally confirmed TNBC per the most recent American Society of Clinical Oncology/College of American Pathologists guidelines. Eligible patients have non-pCR after ≥5 cycles of neoadjuvant pembrolizumab plus chemotherapy, including ≥1 cycle of anthracycline-based neoadjuvant therapy. All patients must provide tissue from the surgical specimen for TROP2 assessment (central testing) and be able to continue on adjuvant pembrolizumab. Randomization must be conducted within 12 weeks from surgical resection; the window for randomization may be extended on a case-by-case basis after consultation with the sponsor. Patients are randomized (1:1) to receive pembrolizumab 400 mg Q6W for 5 doses plus sac-TMT 4 mg/kg Q2W for 12 doses (arm 1) or TPC with pembrolizumab 400 mg Q6W for 5 doses alone or with capecitabine 1000-1250 mg/m2 BID on days 1-14 and days 22-35 every 42 days for 4 cycles (2 weeks on, 1 week off; arm 2) until completion of therapy or disease recurrence, another malignancy requiring active treatment, unacceptable toxicity, or patient withdrawal. Randomization is stratified by residual tumor and lymph node status, TROP2 expression, and intention to use capecitabine. The primary endpoint is invasive disease-free survival (iDFS). Secondary endpoints are overall survival (OS), distant recurrence-free survival (DRFS), patient-reported outcomes (PROs), and safety. Enrollment began in Q2 2024.

P2-11-28: Intra-arterial chemotherapy for triple-negative breast cancer: a systematic review

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Introduction: Triple-negative breast cancer (TNBC) is defined by the absence of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2). This subtype is known for its aggressive behavior and poor prognosis, marked by rapid progression and a heightened risk of distant metastasis. The lack of effective targeted endocrine therapies and anti-HER2 treatments contributes to high mortality rates and

limited options for patients. One promising strategy is intra-arterial neoadjuvant chemotherapy, which aims to deliver treatment directly to the tumor site. This technique involves catheterizing the main artery supplying the breast tumor, allowing localized delivery of chemotherapeutics. This method enables a substantial concentration of the drug at the tumor site, leading to greater anticancer efficacy compared to traditional intravenous infusion. Currently, this technique is being evaluated in clinical trials, especially for its efficacy in locally advanced cases via the superior epigastric artery. Preliminary results indicate that this neoadjuvant approach exhibits mild toxicity profiles and significant therapeutic potential for treating locally advanced TNBC.
Methods: This review evaluates the efficacy of intra-arterial chemotherapy in treating TNBC by analyzing recent studies and outcomes. A comprehensive literature search was conducted using the Boolean strategy: ("triple-negative breast cancer" OR "TNBC") AND ("intra-arterial chemotherapy" OR "superselective intra-arterial chemotherapy" OR "intra-arterial"). The search was performed in PubMed and Embase, including studies published from 2000 to 2023.
Results: In reviewing three studies on breast cancer treatment, distinct outcomes were observed across therapeutic approaches. In the Jinsong He et al. study, the intra-arterial group exhibited a clinical complete remission (cCR) rate of 19.15% and a partial remission (PR) rate of 27.66%, resulting in an overall response rate (ORR) of 46.0%. The control group had a cCR of 6.38% and a PR of 23.40%, leading to an ORR of 29.0%. The H. Y. Jin et al. 2016 study on intra-arterial chemoinfusion in locally advanced TNBC reported a cCR of 33.3%, PR of 51.0%, and a notably high ORR of 84.3%. This study also found a pathological complete remission (pCR) rate of 31.3% and a disease control rate (DCR) of 98.0%. The He J-S et al. 2009 study on targeted chemotherapy reported an ORR of 91.6%, compared to 85.0% in the control group. Notably, skin ulceration was the most common toxicity, affecting 21.6% of patients, with two patients (3.9%) experiencing grade 3/4 ulcerations, effectively managed with topical care.
Conclusion: TNBC remains a challenging subtype characterized by its aggressive nature and limited treatment options due to the absence of effective targeted therapies. The reviewed studies highlight the potential of intra-arterial neoadjuvant chemotherapy as a promising strategy for delivering high concentrations of chemotherapeutics to tumor sites, resulting in improved response rates compared to conventional treatments.

P2-11-29: IMPACT OF GERMLINE MUTATIONS ON pCR IN TRIPLE NEGATIVE BREAST CANCER PATIENTS TREATED WITH PEMBROLIZUMAB PLUS CHEMOTHERAPY: THE TIGER STUDY

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Background: In high risk early triple negative breast cancer (TNBC), the integration of pembrolizumab into chemotherapy regimens has enhanced clinical outcomes in neadjuvant setting. However, there is a paucity of data regarding its efficacy in routine clinical settings, and the therapeutic response in germline mutation carriers remains inconclusive. This retrospective study aims to describe outcomes and safety in patients (pts) with early TNBC who received neoadjuvant pembrolizumab in a real-world context.

Methods: Clinical, pathological and germaline evaluation data of patients with early TNBC treated with neoadjuvant chemio/immunotherapy were retrospective collected from 10 italian referral hospitals. CTCAE v5 guidelines were used for toxicity grading. Logistic regression assessed the effect of clinical variables on AEs and response, adjusting for covariates.

Results: One hundred twenty one (121) patients (pts) were included, with a median age of 50 years (26-79). Body mass index (BMI) was <18.5 in 3 pts, from 18.5 to 24.9 in 75, 25-29.9 in 21, >30 in 17 and not available (NA)in 4, respectively. 74 pts had stage II TNBC and 47 stage III. G3 disease was reported in 93 pts, G2 in 18 and not known in 10, respectively. Nodal status was N0 in 59 pts, N1-N2 in 59, and NA in 3 pts. Overall, 19% of pts fell into clinical T2 or higher TNBC (n=24). G3 or greater AEs in the combined phases were experienced by 28% of pts (n=35) and AEs led to discontinuation in 12% of cases (n=15). 74/121 pts received up to 8 Pembrolizumab cycles. Germline alterations were detected in 26 pts (21%). Among them, 19 patients presented BRCA1/2 germline mutations, while 7 pts showed other than-BRCA germline alterations (1 PALB2, 1 MRE11, 1 RAD51, 1 TINF2, 1 FANCD2, 1 ATM and 1 BRIP1, respectively). Globally 94/121 patients received surgery and, of them, 56 achieved pCR (60%). In germline mutated subgroup, pCR rate was 80% (n=20/25). In the pts population that received surgery, the presence of germline mutations was significantly associated with higher probability of pCR (OR 3.6, p=0.01). At multivariate analysis, correlation between pCR and germline alterations was confirmed (p=0.02). No significant association was found between pCR and toxicity and other variables [stage, nodal status, grading, BMI class, treatment discontinuation and pembrolizumab cycles number (p>0.05)].

Conclusions: Efficacy of neoadjuvant pembrolizumab plus chemotherapy was confirmed in our Italian real-world population. Toxicity and discontinuation rate were comparable with that previously reported. Carrier pts with germline alterations, both BRCA1/2 and other than-BRCA1/2, showed a significant higher pCR rate when compared to wild type population.

P2-11-30: RAD51AP1 Inactivation Induces Synthetic Lethality of PARP Inhibitors in Breast Cancer Patients Irrespective of BRCA1/2 mutation

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Introduction: Triple-negative breast cancer (TNBC), an aggressive variant of breast cancer (BC), is characterized by a lack of expression of estrogen receptor (ER-), progesterone receptor (PR-), and HER2/Neu amplification. TNBC shares striking similarities with basal-like as well as BRCA1-mutation-associated BC. Approximately 70% of BCs arising in BRCA1-mutation carriers and 23% of BCs arising in BRCA2-mutation carriers are associated with the TNBC phenotype. TNBC accounts for 15-20% of all BC subtypes and is considered the most lethal subtype due to the lack of targeted therapy and the development of resistance to existing chemotherapies. Recently, poly (ADP-ribose) polymerase inhibitors (PARPi), Olaparib (AZD2281), and Talazoparib (BMN673) have been shown to offer an attractive new therapeutic avenue for the treatment of TNBC patients who carry BRCA mutations. BRCA1/2 is involved in homologous recombination (HR)-mediated DNA damage repair and PARPi induces synthetic lethality in patients who have HR deficiency (HRD). The concept of synthetic lethality is based on the observation that when single-strand break (SSB) occurs the cells can repair it efficiently by DNA recombinases and cancer cells will survive. However, if a double-strand break (DSB) occurs the cells are a lot less efficient in repairing the cells and will go to cell death. The repair of DSBs mainly relies on homologous recombination (HR), and BRCA1 and BRCA2 are crucial proteins involved in the HR repair pathway. Therefore, FDA-approved PARPi for the treatment of TNBC patients who carry BRCA mutations. Although PARPis induce synthetic lethality in BRCA-mutant MBC, PARPis had no response in patients who do not have BRCA-mutations, which accounts for ~80% of the total TNBC. Therefore, targeting the DDR pathway is one of the most promising approaches to enhance the synthetic lethality of PARPi in TNBC patients. We have recently identified that RAD51AP1 (RAD51-Associated Protein 1), a DNA damage repair protein that plays an important role in HR. RAD51AP1 enhances RAD51 recombinase activity by binding to damaged DNA strands and increases the catalytic activity of RAD51. RAD51 is a recombinase that catalyzes HR which is essential for maintaining DNA integrity in both normal and tumor cells. Like BRCA1, RAD51AP1 is also a DNA repair protein, and it plays a specific role in HR that is dispensable in cells with a lower burden of DNA damage such as normal cells. However, it is crucial in DNA damage repair and cell survival when the burden is high as seen in cancer cells and cells treated with DNA-damaging agents.
Objective: To test the hypothesis that RAD51AP1 inactivation will induce synthetic lethality of PARPi in both BRCA1/2 mutant wild-type and TNBC as well as in ER-positive breast (ER+BC) cells.
Methods: We have used two ER+BC cell lines (T47D, express wild type BRCA1, and MCF7, loss of single BRCA1 allele) and two TNBC cell lines (SKBR7, express wild type BRCA1, and HCC1937, express mutant BRCA1, 5382insC). We obtained PARPi Olaparib and Talazaparib (Selleckchem). We also used genetically engineered mouse models (GEMM) of ER+BC (MMTV-Wnt1-Tg), ER-negative BC (ER-BC, MMTV-Neu-Tg), and TNBC (C3(1)-TAg) in wild-type and Rad51ap1 knockout (Rad51ap1KO) background. All animals were housed and handled according to approved protocols established by the Augusta University (AU) Animal Care and Use Committee and NIH guidelines.
Results: Analysis of RAD51AP1 expression in different BC subtypes shows significantly higher RAD51AP1 expression in basal TNBC when compared with other BC subtypes such

as luminal A, luminal B, and HER2+. This high expression of RAD51AP1 is associated with a poor prognosis. RAD51AP1 expression promotes tumor growth in both ER+BC and TNBC cell lines. RAD51AP1 knockdown limits tumor growth and induces synthetic lethality of PARPi in both ER+BC and TNBC cell lines irrespective of BRCA1/2 mutation. We recapitulated these findings in GEMM of ER+BC, ER-BC, and TNBC.
Conclusion: Overall, our results provide a novel therapeutic strategy to induce the synthetic lethality of PARPi for the treatment of TNBC patients irrespective of BRCA1/2 mutations.

P2-12-01: Survival Outcomes after Pathologic Complete Response with Neoadjuvant Endocrine Therapy vs. Neoadjuvant Chemotherapy

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Background: Neoadjuvant systemic therapy has emerged as the standard of care for many patients with breast cancer potentially leading to surgical downstaging and providing insight into treatment response and prognosis. Neoadjuvant therapies can result in pathologic complete response (pCR), which is predictive of long-term outcomes; patients who attain pCR are consistently found to have better survival outcomes than those with residual disease. Select patients with estrogen receptor positive (ER+) tumors may receive either neoadjuvant chemotherapy (NAC) or neoadjuvant endocrine therapy (NET). Although the role of NAC has been well established for various breast cancer subtypes, NET has become a more appealing option for patients with ER+ disease. As such, we sought to assess survival outcomes in those with early-stage ER+ breast cancer who received either NET or NAC, and achieved pCR.
Methods: All patients diagnosed with ER+/HER2- stage I-III breast cancer in 2010-2021, who received neoadjuvant therapy followed by surgery, and achieved pCR, were selected from the National Cancer Database. Patients were stratified by type of neoadjuvant systemic therapy: NAC vs NET. The Kaplan-Meier method was used to estimate overall survival (OS) and log-rank tests were used to test for differences in OS between groups. Cox Proportional Hazards models were used to estimate the association of NAC vs NET with OS, after adjustment for demographics, disease characteristics, surgery, radiation and immunotherapy; hazard ratios (HRs) and 95% confidence intervals (CIs) are reported.
Results: We identified 3313 patients meeting eligibility criteria: 3148 received NAC and 165 NET. The median follow-up for the entire cohort was 82 months. Patients who received NAC were significantly younger [median age (IQR): NAC 49y (41-58) vs NET 64y (57-69); $p<0.001$], more likely to have a comorbidity score of 0 (NAC 89.3% vs NET 81.2%, $p=0.004$), and more likely to have private insurance (NAC 68.9% vs NET 44.2%, $p<0.001$). There were no significant differences between the NAC and NET patients based on race/ethnicity, income level, education level, or community type (all $p>0.05$). Expectedly, NAC patients were more likely to have larger tumors [median tumor size (IQR): NAC 3 cm (2-4.3) vs NET 1.3cm (0.7-2.8); $p<0.001$), ductal histology (NAC 92.6% vs 81.2%, $p<0.001$), and grade 3 tumors (NAC 70.2% vs 10.3%, $p<0.001$), while NET patients were more likely to have T1

(NAC 22.1% vs NET 67.9%, p<0.001) and N0 disease (NAC 46% vs NET 95.8%, p<0.001). The median time from start of treatment to surgery was 159 days for the NAC cohort, and 147 days for the NET cohort (p=0.23). In the unadjusted Kaplan-Meier analysis, there was no significant difference in OS between NAC vs NET [5-year OS: NAC 0.935 (95% CI 0.926-0.944) vs NET 0.916 (95% CI 0.856-0.951); log-rank p=0.08]. After adjustment for demographics, disease characteristics, and other treatments, there was no association between OS and the study groups (NAC vs NET; p=0.63).
Conclusions: Patients with ER+/HER2- early-stage breast cancer who achieved pCR had similar overall survival, regardless of whether they were treated with NAC or NET. As such, pCR appears to have similar prognostic value regardless of the type of systemic therapy used to obtain this favorable outcome.

P2-12-02: Risk of recurrence in real-world (RW) NATALEE- and monarchE-eligible populations of patients with HR+/HER2- early breast cancer (EBC) in an electronic health record (EHR)-derived database

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Background: The phase 3 NATALEE and monarchE trials showed statistically significant invasive disease–free survival benefits with the addition of a CDK4/6 inhibitor to endocrine therapy (ET) in patients with HR+/HER2− EBC. This analysis reports patient characteristics and efficacy outcomes in the NATALEE- and monarchE-eligible populations in a US RW EHR-derived database.
Methods: De-identified data from the Flatiron Health US EHR database (January 2011-November 2023) were analyzed. Patients with stage I to III HR+/HER2− EBC who were aged ≥18 y when diagnosed, had undergone breast cancer resection, had initiated adjuvant ET, and met disease eligibility characteristics for NATALEE or monarchE were included in the analysis. NATALEE eligibility was defined as stage II/III disease (AJCC 8th edition) irrespective of nodal status, with additional criteria for stage IIA T2N0 disease (grade [G] 2 with Ki-67 ≥20% or high genomic risk; G3). monarchE eligibility was defined as ≥4 positive lymph nodes (LNs) or 1 to 3 positive LNs and G3 and/or tumor size of ≥5 cm (cohort 1) and 1 to 3 positive LNs and Ki-67 of ≥20% (G<3 and tumor size <5 cm; cohort 2). N1mi disease was also allowed in monarchE. Invasive breast cancer–free survival (iBCFS) and distant recurrence–free survival (DRFS) were assessed based on STEEP v2.0 criteria.
Results: Overall, 2534 patients were NATALEE eligible (NATALEE population [NAT]), while 1157 were monarchE eligible (monarchE population [monE]). Median age was 61 y in NAT and 60 y in monE. Approximately half of patients in NAT had previously received chemotherapy (1312 [51.8%]), while nearly two-thirds of monE patients had prior chemotherapy (735 [63.5%]). Most patients in NAT had stage II disease (stage II, 1843 [72.7%]; stage III, 691 [27.3%]), while most patients in monE had stage III disease (stage I, 37 [3.2%, all T1N1mi]; stage II, 484 [41.8%]; stage III, 636 [55.0%]). In both populations,

most patients had N1 disease (NAT: N0, 604 [23.8%]; N1, 1462 [57.7%]; N2/3, 468 [18.5%]; monE: N0, 0; N1, 689 [59.6%]; N2/3, 468 [40.4%]).
The median follow-up from initial diagnosis was 55.1 months in NAT and 53.4 months in monE. Risk of invasive breast cancer recurrence or death was clinically meaningful in both NAT and monE, with 3- and 5-y iBCFS rates of 89.2% and 81.4% in NAT and 83.4% and 72.1% in monE, respectively. 5-y iBCFS rates decreased with increasing stage (NAT: stage II, 87.1%; stage III, 66.5%; monE: stage I, 97.3%; stage II, 80.1%; stage III, 64.9%) and nodal status (NAT: N0, 86.4%; N1, 85.3%; N2/3, 63.8%; monE: N1, 78.2%; N2/3, 63.8%). Risk of distant recurrence or death was considerable in both NAT and monE, with 3- and 5-y DRFS rates of 90.4% and 83.1% in NAT and 85.2% and 74.6% in monE. 5-y DRFS rates by stage and nodal status had trends similar to those of iBCFS rates; however, the rates were comparable for N0 and N1 in NAT (NAT: stage II, 88.7%; stage III, 68.6%; N0, 87.4%; N1, 87.4%; N2/3, 65.0%; monE: stage I, 97.3%; stage II, 83.1%; stage III, 67.2%; N1, 81.6%; N2/3, 65.0%). Patients who did not receive chemotherapy had slightly better 5-y iBCFS rates (NAT: 83.3% vs 79.7%; monE: 75.1% vs 70.6%) and DRFS rates (NAT: 85.3% vs 81.1%; monE: 78.0% vs 72.9%) than those who received chemotherapy.
Conclusions: This RW analysis reflective of the current treatment landscape shows that approximately twice as many patients with HR+/HER2− EBC met NATALEE vs monarchE eligibility criteria. The relatively high incidences of distant recurrences within 5 y in the NATALEE- and monarchE-eligible populations receiving the current standard of care highlight the notable risk of early recurrence in these patients and the need for risk reduction strategies.

P2-12-03: The effect of Abemaciclib plus fulvestrant on overall survival in hormone-receptor positive, HER2-negative breast cancer that progressed on endocrine therapy – Comparing Real-World outcomes in England to MONARCH 2

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Introduction: Abemaciclib plus fulvestrant was approved for use in Europe following the publication of the Monarch-2 trial. The National Institute for Health and Care Excellence recommended reimbursement through the Cancer Drugs Fund (CDF) for all patients with hormone-receptor positive, HER2-negative breast cancer that had progressed on endocrine therapy. During this time, the National Disease Registration Service (NDRS) collected real-world evidence on patient characteristics and outcomes. We compared the results from the Monarch-2 trial to the Systemic Anti-Cancer Therapy (SACT) dataset within NDRS to evaluate the generalisability of the Monarch-2 trial to real-world treatment settings in the NHS.
Methods: The SACT dataset linked to BlueTeq identified all patients with a CDF application for abemaciclib with fulvestrant between April 2019 and December 2019 in England, with follow-up until 25 March 2024. Baseline characteristics reported include gender, age group,

Eastern Cooperative Oncology Group performance status (ECOG PS) and previous endocrine therapy status as reported by the treating clinician, and summary measures were calculated for these characteristics. The primary endpoint was overall survival (OS). Monarch-2 was a global, randomised, placebo-controlled, double-blind phase 3 trial which enrolled patients between August 2014 and December 20151. The patient inclusion criteria were more restrictive in the Monarch-2 trial. This study compared results from the real-world SACT cohort to those from the Monarch-2 trial.
Results: 1,113 applications for abemaciclib with fulvestrant were received through the CDF; after deduplication and exclusions (to restrict to patients who received treatment funded through the CDF and whose treatment ascertainment was complete in the SACT dataset) this related to 876 patients, compared to 669 patients in the Monarch-2 trial. The median age of the real-world cohort was 65 years, compared to 59 years in the Monarch-2 trial. All patients in the Monarch-2 trial were female, while 1% (N=11) of the real-world cohort was male. The median OS in the real-world prescribing setting was 25.9 months [95% CI: 23.7, 28.4], compared with the Monarch-2 trial median OS of 46.7 months. While the Monarch-2 trial consisted exclusively of patients with ECOG PS of 0 or 1, 8% (N=71) of the real-world cohort had an ECOG PS greater than 1, however sensitivity analysis showed that OS in the real-world cohort restricted to patients with ECOG PS 0-1 was consistent with the OS for the overall real-world cohort.
Conclusion: The trial data is not generalisable to the real-world setting for this cohort of patients as the median OS in this real-world cohort was 20 months less than the median OS reported in the Monarch-2 trial.

P2-12-04: Impact of Weight and Weight Variation in Adolescent and Young Adult (AYA) Women with Invasive Breast Cancer

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Background: Breast cancer (BC) is the most common malignancy in adolescent and young adult (AYA) women age 15-39, and the incidence continues to rise each year (1, 2). The reason for this remains unknown, but one potential etiology includes the increasing incidence of obesity (1). Studies in premenopausal women have demonstrated that a high body mass index (BMI) prior to BC diagnosis is associated with worse clinical outcomes (3). The prognostic value of weight gain after BC diagnosis remains unclear (4) as its impact on recurrence risk and overall survival has been inconsistently reported, with limited data among the AYA population (5).
Our aim was to describe the clinical-pathological characteristics of AYA women diagnosed with BC, its association with weight change, and the impact of weight variation during the initial treatment period on patient outcomes.
Methods: This retrospective study included patients with invasive stage I-III BC, age 15-39 at the time of diagnosis, that received treatment at the Cleveland Clinic between 1996 and

2023. Data was collected via computerized data extraction and manual chart review. The analysis was limited to patients who had both a pre-diagnosis (within 1.5 years (y) before diagnosis) and post-diagnosis (up to 1.5y after diagnosis) BMI measurement so that change in BMI could be calculated. A BMI reduction of ≥2 units was considered clinically meaningful weight loss and a BMI increase of ≥2 units was considered clinically meaningful weight gain. A change in BMI < 2 units was considered no change or stable weight. Continuous and categorical variables were summarized using median IQR and N (%), respectively. The Kruskal-Wallis test was used to test for differences according to BMI change categories in continuous variables, and either Fisher's exact test or the Chi-squared test was used to test for differences according to BMI change in categorical variables, as appropriate. The Kaplan-Meier method was used to estimate 5y overall survival (OS) probability, the log-rank test was used to test for differences according to weight change and hazard ratios (HR) were estimated using univariable Cox regression.
Results: A total of 345 patients were identified for analysis. 339 patients (98%) were ≥25 years, 5 were age 19-24, and one was ≤18 years. The median pre-diagnosis BMI was 25 kg/m2 (22,31). Stable weight was observed in 242 patients (70%), 52 (15%) experienced weight loss, and 52 (15%) had weight gain during the first 1.5y of treatment from the time of diagnosis.
While the measurement and analysis of weight changes focused on the initial BC treatment period, median follow-up time among survivors was 6.8y. During follow-up, 51 patients died from any cause. The 5y OS probability of patients with weight gain, weight loss, and stable weight were 66%, 85%, and 90%, respectively (log-rank P=0.006). Patients with weight gain had a statistically significant increased hazard of mortality as compared to those with no change and with weight loss combined, HR 2.71 (95% CI: 1.44-5.10), P=0.005
Discussion: Overall survival probability was reduced in AYA women with early stage BC that experienced weight gain during the initial treatment period. Approximately 70% of patients had no significant change in BMI during their initial BC treatment course, despite undergoing loco-regional and systemic therapies. These results support counselling AYA women regarding the risk of reduced survival associated with weight gain during the initial BC treatment period. Future research is necessary to examine the impact of longer-term weight changes on the risk of BC recurrence and mortality.

P2-12-05: Retrospective Study of Drug-Induced Interstitial Lung Disease in Breast Cancer Patients Treated at Mayo Clinic between the Years of 2018-2023 (Real World Experience)

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Background: Breast cancer treatment has seen significant advancements in recent years, particularly with the introduction of immune checkpoint inhibitors, antibody-drug conjugates, and CDK 4/6 inhibitors. Despite these improvements, side effects remain a major concern. Drug-induced interstitial lung disease (DIILD), a subtype of diffuse

parenchymal lung disease, occurs at different rates with different drugs posing a significant challenge for the treating medical oncologist. Understanding the real-world incidence of DIILD with newer breast cancer drugs is essential for enhancing patient outcomes.
Methods: In this retrospective study, we reviewed patients diagnosed with DIILD at Mayo Clinic Comprehensive Cancer Center from January 2018 to December 2023. Using Mayo Clinic's informatics tool, Mayo Data Explorer, with keywords "drug-induced," "pneumonitis," "lung injury," and "interstitial lung disease," and ICD codes for breast cancer (n=304), 101 patients were identified. All cases were reviewed by a pulmonologist to confirm the diagnosis. Patients with alternative diagnoses were excluded. Pneumonitis grading was based on NCI CTCAE v6.0 guidelines. Statistical analyses included Kruskal-Wallis tests and Fisher's exact tests.
Results: Among the 101 breast cancer patients with DIILD, 89% were white, the median age was 62.7 years (IQR: 54-69.9), and 94.1% had good performance status (ECOG 0-2). Median BMI was 27.9 (IQR: 23.3-32.9), with 33.7% having a smoking history. Comorbidities included prior radiation (51.5%), lung disease (COPD 6.9% & asthma 15.8%), prior COVID-19 infection (24.8%), and CKD (16.8%, with 11.8% stage IV/V). Tumor characteristics: 61.4% were estrogen positive 25.7% HER2 positive and 12.9% triple negative. Disease stages: 47.5% of patients were in early-stage (stages I-III), while 56.4% were metastatic (stage IV).
Forty-five patients required hospitalization, five required intubation (11.4%), and there were three deaths (all associated with fam-trastuzumab deruxtecan). Common culprit drugs included fam-trastuzumab deruxtecan (20%), everolimus (15.8%), abemaciclib (12.9%), and pembrolizumab (11.9%). Median time to DIILD diagnosis: 111 days (IQR: 69-210) with specific median times of 173.5 days for fam-trastuzumab deruxtecan, 95.5 days for everolimus, 205 days for abemaciclib and 85 days for pembrolizumab.
Regarding treatment, 78.2% received high-dose corticosteroids, 1% mycophenolate, and 7.9% other treatments. Seventeen patients were rechallenged with the culprit medication and 47% experienced recurrence. More than half (57.4%) resolved within six months, with a median resolution time of 62.5 days (IQR: 41-110).
We analyzed for predictors of grade severity, with DIILD severity defined as low-grade (grades 1-2, 56.4%) and high-grade (grades 3-5, 43.6%). COPD was the only significant predictor, with COPD being more common in patients with higher ILD grade (13.6% vs. 1.8%, p=0.041). Of note, a history of prior COVID-19 infection was not predictive of DIILD severity.
Conclusion: This study provides real-world experience on DIILD in breast cancer patients, identifying common culprit drugs and highlighting an association of higher ILD grading with COPD. The variability in time to DIILD diagnosis underscores the need for vigilant monitoring. High-dose corticosteroids were effective, but rechallenge posed a high recurrence risk, indicating the complexity of managing DIILD. Future research is needed to identify individualized genomic predictors of lung toxicity.

P2-12-06: Key factors influencing patient preferences for BRCA testing and adjuvant therapy in HER2-negative early breast cancer

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Background: PARP inhibitors (PARPis) have survival benefits for women with high-risk HER2-negative early breast cancer (eBC) with germline BRCA mutations (BRCAm). Identifying patients eligible to receive PARPis can be challenging, as many individuals who are BRCAm carriers are unaware of their status, despite increasing availability of genetic testing. This study sought to identify factors that influence patient preferences for BRCA testing and adjuvant therapy.
Methods: Women (≥18 years) in the United States with HER2-negative eBC were recruited between October 2023 - March 2024 via an online research panel to complete a cross-sectional survey. The survey included a Best-Worst-Scaling (BWS) exercise to identify barriers/facilitators to BRCAm testing among 16 factors. Two Discrete Choice Experiments (DCE) were included: one to evaluate preferences for BRCA testing by prompting patients to select between 2 hypothetical scenarios varying on 5 testing-related attributes (i.e., eligibility for targeted treatment, delays due to testing, information sharing with family, preventive surgery options, and out-of-pocket costs [OOPC]) and another to assess the influence of 7 treatment attributes on patients' preferences for adjuvant therapy (i.e., invasive disease-free survival, whether the treatment is targeted, risk of nausea, risk of serious side effect [SSE], regimen, treatment duration, and monthly treatment OOPC) versus no treatment. Attributes included in the BWS and DCE exercises were informed by qualitative interviews with 20 healthcare providers and 12 patients. BWS and DCE attribute preference weights were calculated using hierarchical Bayesian modeling.
Results: Women (N=360) on average were 61 years old and 77% were White; most resided in the South (37%) and Midwest (23%). Most women were first diagnosed with breast cancer after 2017 (72%), 61% were never tested for BRCAm or unsure of their status/testing history. Roughly one-third received adjuvant chemotherapy and another third received neoadjuvant chemotherapy. Relative to the other factors in the BWS, the top facilitators for BRCA testing were the possibility of test results showing eligibility for targeted therapy that may prevent or delay metastasis, having no OOPC for testing, and physician recommendation. OOPC of $250, potentially delaying treatment by 3-4 weeks and concerns that test results could lead to denial of life insurance/higher premiums were the top three barriers. The DCE evaluating preferences for BRCA testing showed that, on average, decreasing OOPC from $250 to no cost was most important, followed by learning if preventive surgery should be considered to prevent new cancer, and learning about eligibility for targeted treatment. The DCE on preferences for adjuvant therapy showed that 77% of the time, patients chose treatment over opting out, regardless of the combination of adjuvant treatment attributes shown. On average, reducing treatment OOPC from $900 to $0 influenced treatment choice most, followed by reducing risk of SSE from 77% to 24%, and having a BRCA-targeted treatment.
Conclusions: Patients reported that a key benefit of BRCA testing was using the results to

inform treatment decisions. Yet, OOPC may act as a barrier to testing. Patients' decision to receive adjuvant therapy was influenced by the perceived effectiveness and tolerability of the therapy, and like BRCA testing, financial burden was a concern. Comprehensive patient-provider discussions regarding the benefits of testing including eligibility for innovative treatment may be an effective strategy for increasing BRCA testing and enhancing shared treatment decision-making. Selection of effective adjuvant treatment likely requires balanced consideration of medication tolerability and financial burden to the patient.

P2-12-07: Effectiveness of Predictive Calculators for Breast Cancer Recurrence: A Comparative Study with the 21-Gene Score

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Background: In patients with early-stage breast cancer who have hormone receptor-positive and human epidermal growth factor receptor 2-negative (HER2-) tumors, the utility of adjuvant chemotherapy is determined by a recurrence score based on the expression of 21 genes. The limitations of using this recurrence score include the high cost and the long waiting time for results. Nomograms/calculators have been developed to predict the results of the 21-gene recurrence score, taking into account clinicopathological variables established in clinical practice as prognostic and/or predictive. The aim of this study was to evaluate the correlation between the recurrence score based on the expression of 21 genes and the calculators from the University of Tennessee (TUC) and Johns Hopkins University (JHUC).
Methods:We retrospectively reviewed clinical records from 408 patients diagnosed with early-stage breast cancer at Instituto Nacional de Enfermedades Neoplásicas (INEN, Peru) and Oncosalud with available ODX recurrence scores. ODX-RS were compared with scores from the Tennessee University calculator (TUC) and Johns Hopkins University (JHUC) using a confusion matrix to validate those nomograms with our cohort. We performed a comparative analysis of clinical characteristics between low-risk (RS ≤ 25, n=342) and high-risk (RS > 25, n=66) groups, followed by univariate and multivariate logistic regression to identify predictors of low-risk ODX scores. Statistical significance was set at $p<0.05$.
Results: In our study, the median age was 58 years, with a higher proportion of >50 years in the low risk group (p=0.027). High-risk patients had higher median tumor size and Ki67 expression (p=0.018 and p<0.001), with significant differences also noted in surgery type, histologic type, Elston grade, and PR status. Meanwhile, negative recurrence status was similar in both groups. Significant predictors for low recurrence risk included histologic type, Elston grade, PR status, and Ki67%. Lobular carcinoma (OR 5.15, 95%CI 1.43-33.2, p=0.032) and PR positive (OR 4.98 95%CI 2.42-10.3, p<0.001) were associated to a lower risk of recurrence compared to ductal carcinoma and PR negative. Patients with higher Elston grades and Ki67% were less likely to have low recurrence risk. Patients demonstrate a high specificity but extremely low sensitivity with the TUC. This indicates that it misses a

significant number of true positives (low-risk patients correctly identified as low-risk), the low AUC values also indicate poor performance regardless of age group (≤ 50: 0.297, > 50: 0.344). Patients showed significantly improved performance with the JHUC, where we excluded 262 patients due to indeterminate outcomes. The rate of true positives for both age groups is higher (>83%) with greater sensitivity and specificity. AUC values (≤ 50: 0.802, > 50: 0.75) also reflect robust performance across different age cohorts.
Conclusions: Only the established Johns Hopkins University calculator demonstrated good performance in correctly stratifying our patients. This highlights the need for further refinement and validation in predictive models across diverse patient populations to optimize medical decisions.

P2-12-08: Real world progression-free survival after CDK4/6 inhibitors plus endocrine therapy: results from the multicenter SISTER study

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Background: Endocrine therapy (ET) combined with CDK4/6 inhibitors (CDK4/6i) is the standard first-line treatment for ER+/HER2- metastatic breast cancer (mBC). However, the optimal treatment after disease progression on ET + CDK4/6i remains unclear.
Aim: This study aims to evaluate real-word progression-free survival (rwPFS) with various treatments in a post-CDK4/6i setting, with real-word overall survival (rwOS) as a key secondary endpoint.
Design and Setting: The multicenter SISTER study included patients with ER+/HER2- mBC who received ET-based or chemotherapy (CT)-based therapy after progressing on ET + CDK4/6i between July 2015 and May 2023. rwPFS and rwOS were analyzed based on treatment type, clinicopathological characteristics and duration of prior CDK4/6i therapy. Descriptive analyses included patient demographics, disease and treatment characteristics.
Results: Following progression on CDK4/6i, 221 patients (43.7%) received ET-based therapy and 56.3% received CT-based therapy. The ET-based group included 114 patients (22.9%) who received everolimus + exemestane (Eve+Exe) and 100 (20%) who received ET alone. The CT-based group included 106 patients (21.2%) who received intravenous (iv) CT and 179 (35.9%) who received oral CT. At a median follow-up of 4.75 months (IQR, 2.82-9.31) from the post-CDK4/6i treatment start, the median rwPFS in the overall population was 5.44 months (95% CI 4.59-5.97). Median rwPFS was 5.28 months (95% CI 4.36-5.74) and 6.43 months (95% CI 4.36-5.74) in patients who received CDK4/6i as first- or second-line therapy, respectively. No statistically significant difference in median rwPFS was found between CT-based (5.83 months, 95% CI 5.27-6.69) and ET-based (4.29 months, 95% CI

3.90-5.67, p=.54) groups post-CDK4/6i progression. Patients with visceral involvement had a worse median rwPFS (5.31 months, 95% CI 4.22-5.80) compared to those without (6.43 months, 95% CI 4.69-7.64, p=.0009). Patients on oral CT had better median rwPFS (6.89 months, 95% CI 5.28-8.72) than those on iv-CT (5.44 months, 95% CI 3.70-5.97, p=.005). Patients treated with CDK4/6i for at least 12 months had similar median rwPFS (5.57 months, 95% CI 4.23-6.46) compared to those treated for less than 12 months (5.31 months, 95% CI 4.36-6.16, p=.24). The overall population's median rwOS was 24.26 months (95% CI 21.70-27.46). Patients on ET had a better median OS compared to those on CT (31.74 months, 95% CI 24.79-38.66 vs 20.79 months, 95% CI 18.23-23.93, p=.00089). In a multivariate model for PFS, age was inversely correlated with progression risk (HR 0.99, 95% CI 0.98-0.99, p=.028), while de novo metastatic disease (HR 1.24, 95% CI 1.00-1.53, p=.047) and visceral involvement (HR 1.45, 95% CI 1.17-1.80, p=.008) were independently associated with higher risk. Longer prior CDK4/6i treatment duration was associated with a lower progression risk (HR 0.99, 95% CI 0.98-1.00, p=.049). Compared with oral CT, the use of iv-CT, Eve+Exe or ET only was associated with a higher progression risk (HR 1.43, 95% CI 1.10-1.87, p=0.008; HR 1.40, 95% CI 1.08-1.82, p=.011; HR 1.39, 95% CI 1.06-1.81, p=.016, respectively). Patients with a known BRCA1/2 mutation (16/103, 15%) had a significantly shorter median rwPFS (3.95 months, 95% CI 3.25-5.31) compared to those with wild type BRCA1/2 (5.97 months, 95% CI 4.10-6.92, p=0.033) and median rwOS (26.9 months, 95% CI 23.1-NA) compared to non-mutants (mOS not reached, p=.34).
Conclusions: This study identified visceral involvement and a shorter CDK4/6i therapy duration as independent prognostic factors for poorer survival outcomes. CT-based therapy demonstrated superior rwPFS for patients with brief prior CDK4/6i therapy and those with visceral metastases. Patients receiving oral CT showed improved rwPFS compared to iv-CT. These findings suggest that treatment strategies should consider the type of progression and prior treatment duration to optimize patient outcomes in ER+/HER2- mBC.

P2-12-09: Clinical, genetic and immunological features, HER2-low expression and treatment patterns in a large population of male breast cancer patient with long-term follow up

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Background: Male breast cancer (BC) is a rare entity, accounting for 1% of all BC diagnoses. It is characterized by distinct biological and clinicopathologic features, often driving more unfavorable clinical outcomes as compared to female BC, thus posing impelling clinical challenges.
Methods: We collected clinical and biological data from a large retrospective cohort of male patients (pts) with BC diagnosis. Relapse-free survival (RFS, pts with stage I-III at diagnosis) and overall survival (OS, all pts) were adopted as survival endpoints according to STEEP v2.0 definitions. Tumor-infiltrating lymphocytes (TILs) were assessed on primary tumors,

relapses or both, complying with the recommendations by the International TILs Working Group.
Results: We included 157 pts. Median age at BC diagnosis was 65.1 years.
The population exhibited high rates of cardiometabolic comorbidities (65.6%) associated with major medication burden (24.3% pts with ≥5 drugs/day).
We reported relevant rates of second malignancies (28.6%) and family history of cancer (69.1%). Among pts undergoing genetic testing, rates of germline mutations in BRCA1, BRCA2 and PALB2 genes were 1.3%, 17.3% and 2.7%, respectively.
Our male BC population was enriched for HR+/HER2- subtype (91.3% vs HER2+ 8% vs triple-negative 0.7%). Of HR+/HER2- BC pts with advanced BC (n=35), 82.9% had HER2-low phenotype on primary and/or recurrent tumor.
Among HR+/HER2- BC pts, 15.9% received CDK4/6i+ET (adjuvant, n=1; advanced BC, n=19). Among BRCA+ pts (n=14), 21.4% received PARP-inhibitors (adjuvant, n=1; advanced BC, n=2).
Of pts receiving CDK4/6i for advanced BC, 94.7% received palbociclib; the ET backbone was aromatase inhibitor (AI) in 78.9% and fulvestrant in 21.1% of cases. Median PFS with CDK 4/6i+AI and CDK 4/6i+fulvestrant was 22.8 and 11.9 months, respectively. Grade 3 adverse events (AEs) occurred in 41.2% of pts (grade 3 neutropenia 41.2%, grade 3 thrombocytopenia 5.8%). No grade 4-5 AEs nor treatment discontinuations due to AEs were recorded.
After a median follow up of 9.1 years, 5-yr RFS and 5-yr OS rates in HR+/HER2 subgroup were 70.9% and 81.5%, respectively. Patients with BRCA1/2-PALB2 germline mutations experienced a trend for poorer OS than those tested negative (5-yr OS in mutated versus non-mutated: 81.7% vs 93.0%, p=0.068). Among all pts experiencing OS event (known cause of deaths, n=37), 40.5% died from other causes than BC (including: other malignancies, 27.0%; cardiovascular event, 8.1%). Among pts with OS event and previous BC distant relapse (n=25), 16% died from non-BC causes.
Finally, TILs were available for 77 pts; median (Q1-Q3) TIL levels were 2.0% (1-5%) overall, 2.0% (1-5%) in primary tumor and 1.0% (0-5%) in metastatic samples. No pts exhibited a lymphocytic predominant phenotype. TILs did not retain any prognostic role.
Conclusions: We confirmed, in one of the largest available series, that male BC represents a distinct clinical entity as compared to female BC, with unique challenges and clinical needs. The high burden of comorbidities and second malignancies drove a substantial risk of non-BC-related mortality, even in pts with advanced BC. In addition, a signal for a negative prognostic impact of gBRCA1/2-PALB2 mutations was captured. Data regarding CDK4/6i-based treatment were reassuring both in terms of efficacy and safety. A substantial proportion of men with advanced HR+/HER2- BC showed HER2-low phenotype, thus arising the need to generate solid data regarding trastuzumab deruxtecan efficacy/safety specifically in male BC. Finally, as expected also given the enrichment for HR+/HER2- phenotype, male BC was associated with weak immune infiltration.
Our results advocate for more inclusive criteria in clinical trials and for the generation of high-quality real-world data.

P2-12-10: Dynamic Changes in Breast Cancer Immunochemistry Biomarkers at Recurrence and Their Impact on Survival

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Introduction: Variations in the expression of estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER-2), Ki-67, and histological grade (HG) between primary and recurrent/metastatic lesions have been reported as prognostic factors in breast cancer (BC). This study aims to evaluate the impact of discordance in these markers on overall survival (OS) and to describe the behavior of tumor-infiltrating lymphocytes (TiL) and T-cell infiltration.
Methods: This study is a retrospective analysis of 132 non-advanced breast cancer patients who experienced confirmed recurrence or metastasis at the Instituto Nacional de Enfermedades Neoplásicas between 2011 and 2022. Descriptive analysis assessed the expression levels of ER, PgR, HER-2, Ki-67, and histological grade in both primary and metastatic tumors. In a subset of 21 patients, CD8 (SP16) and CD163 (EP324) staining was performed on diagnostic and recurrence samples, and TiLs were evaluated by an independent pathologist. Fisher's exact and Pearson's Chi-squared tests were used to identify significant differences between primary and recurrent/metastatic tumors. For survival analysis, the Kaplan-Meier curve and the Cox regression model were employed to estimate the relative risk (HR – Hazard Ratio). A p-value of less than 0.05 was considered statistically significant.
Results: Median age at diagnosis was 49.6 yo, and most patients were diagnosed at stage III (62%). 15.7% of patients experienced a loss of ER at recurrence, while 14.6% acquired ER expression ($p<0.001$). Additionally, 48.6% of patients experienced a loss of PgR at recurrence, while 13.6% acquired PgR expression ($p<0.001$). 41.3% of patients changed from HER2-zero to HER2-low ($p<0.001$). Regarding histological grade, 73% of patients increased from HG1 to HG3, and 81.3% increased from HG2 to HG3 ($p=0.009$). There were no significant differences between the median Ki-67 at diagnosis and the median at recurrence (42.05% vs 42.14%, $p=0.98$).
Overall, 55.5% (n=73) of patients changed the subtype of breast cancer at recurrence ($p<0.001$). For the luminal subtype, 11% changed to the HER2 subtype and 1.4% to the Triple Negative (TN) subtype. For the HER2 subtype, 15.8% changed to the luminal and 15.8% to TN subtypes. Only 3.8% of patients changed from TNBC to the HER2 subtype and 7.7% to the luminal subtype.
Regarding the subgroup analysis for TiL, CD8 and CD163, the median levels at diagnosis were 42.94%, 28.89%, and 44.71%, respectively. At recurrence, the median levels were 47.78% for TiL, 30.50% for CD8, and 41.00% for CD163. No significant differences were found between primary and recurrent/metastatic lesions (TiL $p=0.55$, CD8 $p=0.79$, and CD163 $p=0.57$).
Median overall survival (OS) was 99.3 months (CI 82-118). Finally, the survival analysis

demonstrated that patients who did not lose ER (HR 0.49; CI 0.29-0.81, p=0.005) or PR (HR 0.5; CI 0.28-0.88, p=0.016) expression had better OS. No significant differences were found for the other variables.
Conclusions: Patients who maintained expression of ER and PR showed significantly improved overall survival, underscoring the critical prognostic value of hormone receptor status. It is noteworthy that no significant differences were found in the levels of Ki-67 between primary and recurrent/metastatic lesions. In our series, the levels of TiL, CD8, and CD163 in recurrent tumors remained relatively stable compared to the primary tumors.

P2-12-11: Lobular breast cancer: diagnostic complexity and delays to surgery.

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Introduction: Lobular breast cancers account for up to 15% of breast cancers but can be diagnostically complex and often require increased investigation burden. We investigated characteristics and outcomes of lobular breast cancer.
Methods: We performed a retrospective cohort study of consecutive diagnoses of lobular breast cancer at two high-volume surgical centres in Liverpool, UK between March 2023-March 2024. Clinical, histological, imaging and treatment pathway data were collected. Outcomes in terms of time to surgery were computed and compared using Kaplan-Meier plots. Mean (SD) or median (IQR) are presented throughout and p-value <0.05 was considered statistically significant.
Results: For the 12-month period, a new primary diagnosis of lobular breast cancer was diagnosed in 87 women with a median age of 65 years (36-96) and 25/87 (28.7%) were detected via the NHS screening programme. 3/87 (3.5%) first presented as metastatic disease (all 3 as gastrointestinal metastases). 71/87 (81.6%) were classical lobular cancer subtype, 11/87 (12.6%) were pleomorphic and 5/87 (5.7%) were mixed lobular/ductal. 59/87 (67.8%) underwent MRI, the mean tumour size on MRI was 43.7 mm (SD 28, range 6-140mm). Tumour size on MRI was similar to the tumour size on final histology (43.7mm vs 39.3mm, p=0.31), however was consistently larger than USS (43.7mm vs 25.1mm, p<0.001) and mammogram (43.7mm vs. 22.6mm, p<0.001).
25/60 (41.7%) undergoing MRI required second look imaging with 17 (68%) requiring further image-guided biopsy, of which 5 were positive for malignancy (PPV=20%).
Time from diagnosis to surgery for patients going direct to surgery was significantly longer for the lobular breast cancer patients compared with total new cancers going direct to surgery (66 days vs. 55 days, p=0.004).
In total, 32 (36.7%) patients had neoadjuvant endocrine therapy, 21 patients received neoadjuvant endocrine treatment as a bridging therapy to surgery (median 44 days, range 13-179 days) and 11 patients received neoadjuvant endocrine therapy for downstaging, for a duration of 9-12months. 10 frail and/or co-morbid patients were treated with primary

endocrine therapy only, age range 77-96 years.
Oncotype testing was undertaken in 30 patients, recurrence score range 3-25.
38/51(74.5%) patients underwent breast conserving surgery of which 8/38 (21.1%) required further surgery 4/8 (50%) underwent completion mastectomy. Pre-operative imaging consistently underestimated pathological tumour size in these patients.

Conclusion: In lobular breast cancer, MRI demonstrates strong correlation with final pathological tumour size with greater accuracy than mammography and/or USS but generates a high volume of incidental findings requiring further investigations. We observed a clinically relevant and statistically significant delay to surgery in patients with lobular breast cancer. Further work is needed to improve efficiency in care pathways in lobular breast cancer, as this may improve patient outcomes.

P2-12-12: Impact of the addition of pembrolizumab to neoadjuvant chemotherapy in triple negative breast cancer in Ireland: a retrospective, multi-centre, real-world study

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Background: The 60-month follow up data from the phase III KEYNOTE 522 (KN522) trial reported that the addition of pembrolizumab (pembro) to chemotherapy improved event free survival (EFS) and increased the rate of pathological complete response (pCR).1 As a result, the KN522 regimen is standard of care for stage II/III TNBC. The KN522 trial reported that immune-mediated adverse events (IRAEs) ≥ grade 3 occurred in 12.9% of pts in the pembro arm and 1.0% of pts in the chemo only arm.2

Methods: We conducted a retrospective observational cohort study of patients (pts) with TNBC, stage II-III, who received paclitaxel and carboplatin followed by doxorubicin plus cyclophosphamide, associated with pembro, as per the KN522 trial, between January 2023 and May 2024 at five Irish institutions. We examined the real-world pCR rate and IRAEs.

Results: 67 patients (pts) started the KN522 regimen. The median age was 49 years (range 32-77). 47 pts (70%) had T1-2 tumours, 19 pts (28%) had T3-4 tumours, data was unavailable for 1 pt. 38 pts (57%) had node positive disease.
16 pts (23%) experienced an IRAE of ≥ grade 3 during treatment. 13 pts (19%) experienced an IRAE ≥ grade 3 in the neoadjuvant phase and 3 pts (4%) experienced an IRAE ≥ grade 3 in the adjuvant phase. The most common ≥ grade 3 IRAEs observed were hepatitis (n=6, 9%); nephritis (n=4, 6%) and colitis (n=3, 4%). 34 pts (51%) were hospitalized at least once during treatment. 13 pts were hospitalized on more than once occasion, and a total of 54 hospitalisations were recorded for the group during treatment. To date, 3 pts have been hospitalised during the adjuvant phase.

Of the 40 pts who had undergone surgery at the time of data analysis, 26 pts (65%) had a pCR. Among those pts who experienced a pCR, a higher proportion had stage II disease (n=20, 77%) compared with the pts who did not experience a pCR (n=9, 64%), although this was not statistically significant (p=0.469). There was a lower instance of ≥ grade 3 IRAEs observed among pts who experienced a pCR (n=4, 15%) compared with those who did not experience a pCR (n=5, 36%), however this was not statistically significant (p=.234). A lower number of patients who experienced a pCR were hospitalised during the neoadjuvant phase of treatment (n=12, 46%) compared with pts who did not experience a pCR (n=8, 57%). The median number of cycles of pembro received among patients who experienced a pCR was 8 (range 1-10), compared with a median of 7 cycles among patients who did not experience a pCR (range 2-10).

Conclusion: We found a high pCR rate among pts who completed the neoadjuvant phase of treatment. The IRAEs observed were in line with the known side effects of the agents. IRAEs ≥ grade 3 were higher in our real-world cohort compared with KN522 published data.2 Higher rates of IRAEs ≥ grade 3 have been reported among other real-world cohorts.3 We found a high rate of hospitalization in our cohort, which is also in line with published real-world data.4 The hospitalization rates observed with this regimen are higher than hospitalisation rates with neoadjuvant chemotherapy alone, which range from 6.2%-10% for pts aged <65 years and 12.7%-24% for pts ≥65 years.5 In this cohort, pts who experienced a pCR had a higher frequency of stage II disease and lower rates of ≥grade 3 IRAEs compared with those who did not experience a pCR, although this did not reach statistical significance. This real-world study supports trends observed in other similar studies and highlights the complexity of managing the toxicities arising from the addition of immunotherapy to standard neoadjuvant chemotherapy.

P2-12-13: Clinico-Pathological Characteristics and Incidence Trends of Breast Cancer in Young Women: A 10-Year Single Institution Retrospective Study

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Introduction: Women diagnosed with breast cancer (BC) before age 40 (YWBC) experience a substantially higher risk of recurrence and mortality than their older counterparts. The global trends in BC incidence and mortality among YWBC are scarcely described, although recent studies suggest a rise among certain high-income countries.

Material and methods: We conducted a retrospective analysis including all consecutive newly diagnosed BC patients (NBC) between January 2014 and December 2023 at Hospital Clinic of Barcelona, Spain. Clinical and immunohistochemistry characteristics (estrogen

receptor [ER], progesterone receptor [PR], HER2 status and Ki67 expression) determined BC subrogated subtypes: hormone receptor-positive (HR+)/HER-2 negative (HER2-), HER2-positive (HER2+) and triple negative (TN). YWBC incidence (YI) was defined as the yearly ratio between YWBC and NBC. Our primary objective was to report the YI over the years and determine if there was any variation. We also assessed incidence differences among immunohistochemical subtypes, as well as the YI in very young BC pts (≤ 35 years, VYWBC). Incidence difference across years was evaluated using the Kruskal–Wallis test, with a significance p-value <0.05.

Results: A total of 262 YWBC pts were included, accounting for 9.6% of NBC over the 10-year period, ranging from a minimum of 7.38% in 2020 to a maximum of 14.02% in 2018. When analyzing YI over the years, we observed an initial increase until 2018, followed by a decline and stabilization at a steady percentage, with no statistically significant differences (p=0.44). With a median age of 37 years (range: 18 to 40 years), the majority (76.33%) were diagnosed at early stages (i.e., stage II or less). Stages II and III represented 38.50%, whereas 6.11% were metastatic at diagnosis, without significant variations throughout the period (p=0.44). Most YWBC were ER-positive (66.41%), PR-positive (53.05%), HER2-negative (62.6%), and had a Ki67 >20% (52.29%). Most tumors were HR+ (54.23%) throughout the entire period, with minimal variation across the years. The proportion of HR+ vs HR-negative (HR-) tumors remained stable over the 10 years studied (p=0.44). HER2+ tumors accounted for 23.66% of YWBC, ranging from 10.34% (2023) to 43.48% (2018). On the other hand, TN tumors represented 21.76% of the cases, with a range from 5.56% (2014) to 41.38% (2023) during the period, again with no significant change. VYWBC represented a steady 4% of NBC, with a non-significant tendency to decrease in recent years (reaching 1.84% in 2023). Stages II and III represented 38.51%, without significant changes throughout the period (p=0.437). Across subtypes, HR+ tumors were 47.19%, HER2+ 24.57% and TN, 27.03%. Interestingly, among YWBC, VYWBC represented a third of the new cases, peaking in 2020, followed by a numerical non-significant reduction in incidence (p=0.44).

Conclusions: YWBC incidence has remained stable over the years. In our population, the YI is consistent with previous studies, ranging from 5-10% depending on the country, and slightly higher than the YI reported for Spain (6.6% in 2022). Both in YWBC and VYWBC, no variation was observed regarding stage and subtypes over time. Overall, these results indicate that over the past ten years there have not been significant changes in the YI, nor a trend towards more aggressive phenotypes among YWBC at our institution.

P2-12-14: Adverse events in patients treated with neoadjuvant chemo/immunotherapy for triple negative breast cancer

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Background: Triple negative breast cancer (TNBC) accounts for 15% of newly diagnosed breast cancer cases and has higher risks of distant recurrence and death compared to other subtypes. The current standard-of-care regimen for locally advanced disease from the KEYNOTE-522 (KN-522) trial combines neoadjuvant chemotherapy (carboplatin, paclitaxel, doxorubicin, and cyclophosphamide) with neoadjuvant and adjuvant pembrolizumab. This regimen increased rates of pathologic complete response (pCR) and event-free and overall survival but had higher rates of adverse events (AEs). Of note, patient race was not reported in KN-522. Our goals were to examine rates of AEs, hospitalizations, and suspected immune-related adverse events (irAEs) in TNBC patients undergoing neoadjuvant chemo/immunotherapy in a diverse academic center population more representative of a real-world patient population.

Methods: TNBC patients who received neoadjuvant treatment with pembrolizumab were retrospectively identified through pharmacy treatment records at 7 academic medical centers across the U.S. Electronic medical record review was conducted, and de-identified data were managed using electronic data capture tools. Descriptive statistics were used to report all variables, and total rates of AEs, grade 3+ AEs, and hospitalization rates for patient groups were estimated with 95% confidence intervals (CIs) calculated by 5000 Monte Carlo simulations. Fisher's exact test was used to compare event rates between groups, and two sample proportions test was used to evaluate the difference in proportions between any two groups.

Results: At the time of analysis, 137 patients had data collected from 4 sites. Median age was 48 years old. All identified as female. Racially, 59% identified as white, 34% as black, and 7% as other. Ethnically, 7% identified as Hispanic/Latino. 75% were overweight/obese based on initial BMI. 61% achieved a pCR following neoadjuvant treatment, and 4 (3%) died from progressive/metastatic disease at the time of data collection. 89% had a reported AE, and 36% had a grade 3+ AE. The most common grade 3+ AE was myelosuppression, and 70% required growth factor support at some point during neoadjuvant therapy. The most common suspected irAEs were dermatitis (20%), hypothyroidism (18%), arthralgias/myalgias (18%), diarrhea (15%), hepatitis (11%), and adrenal insufficiency (5%). 12% also had cardiac or thrombotic AEs. 24% were treated with systemic steroids for a suspected irAE, and only 58% received a full course (8 doses) of neoadjuvant pembrolizumab due to toxicity concerns. Pembrolizumab was permanently discontinued in 58% of patients with very few (6%) re-challenged once stopped. 31% were hospitalized during treatment, with the majority hospitalized during neoadjuvant therapy. Neutropenic fever was the most common admitting diagnosis, and 29% of hospitalizations had an irAE reported. There were no statistically significant differences in AE or hospitalization rates between white versus black patients or between patients with normal BMIs compared to overweight/obese patients, although there was a trend towards significance in overweight but not obese patients having lower rates of grade 3+ AE ($p=0.07$) compared to patients

with normal BMIs.

Conclusions: This study presents an analysis of AEs in a more diverse and representative of the real-world patient population receiving neoadjuvant chemo/immunotherapy for TNBC. Rates of pCR and AEs were similar to those reported in KN-522. However, a large proportion of patients stopped pembrolizumab early due to a suspected irAE and did not complete adjuvant treatment. Additionally, high rates of hospitalization and myelosuppression suggest that incorporating growth factor support may benefit TNBC patients receiving neoadjuvant treatment. Our study is ongoing with final data collection pending; this will be reported in the future.

P2-12-15: Trends in Antiemetic Use in Patients Receiving Fam-trastuzumab Deruxtecan-nxki for Metastatic Breast Cancer: A Retrospective Analysis of Data from the IntegraConnect PrecisionQ De-Identified Database

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Background: Clinical practice guidelines recommend treatment with 3-4 antiemetic drugs for regimens with high emetic risk, such as fam-trastuzumab deruxtecan-nxki (T-DXd). This study uses real-world data to characterize the real-world use of antiemetics in patients with metastatic breast cancer (mBC) and explores the impact of antiemetic treatment on T-DXd outcomes. Methods: This was a retrospective, observational analysis of data from patients in the IntegraConnect PrecisionQ real-world de-identified database, enriched with information obtained by curation. The study included adults (age ≥18 years) with a confirmed diagnosis of mBC who initiated treatment with T-DXd between December 20, 2019 and December 31, 2023. The index date was the date of T-DXd initiation and follow-up was through May 20, 2024. Antiemetic classes were defined using data obtained within the first 7 days of T-DXd initiation using National Comprehensive Cancer Network (NCCN) criteria. Outcomes included time to treatment discontinuation (TTD), the rate of dose reductions for T-DXd, and the rate of antiemetic use over time in patients receiving T-DXd. Also included were demographics and clinical characteristics. Data are presented using descriptive statistics. Results: Overall, 2,132 patients were included in the analyses, most were female (98.4%) and white (62.2%). The median (interquartile range [IQR]) age at diagnosis was 59 (50, 67) years and the median (IQR) age at treatment was 63 (54, 71) years. Most patients (82.4%) had an Eastern Cooperative Oncology Group (ECOG) score of 0-1; 13.9% were ECOG 2 and 3.7% were ECOG 3+. A total of 113 patients received no antiemetics, 136 received 1 antiemetic class, 1090 received 2 antiemetic classes, and 793 received 3-4 antiemetic classes. Over a median follow-up duration (IQR) of 16.3 (11.0, 28.0) months, 11.0 (5.7, 21.0) months, 13.8 (7.0, 19.7) months, and 9.1 (5.9, 13.8) months for each group, respectively, the number of patients who discontinued/died was 96 (85.0%), 94

(69.1%), 745 (68.3%), and 433 (54.6), respectively. The median (95% confidence interval [CI]) TTD was 7.0 (5.6, 8.3) months, 7.6 (5.5, 12.4) months, 8.3 (7.6, 9.2) months, and 9.0 (8.0, 9.6) months for patients treated with 0, 1, 2, and 3-4 antiemetic classes, respectively. At 6 months, treatment probability (95% CI) was 58.6% (48.8, 67.1), 55.2% (46, 63.4), 59.6% (56.5, 62.5), and 61.5% (57.9, 64.8), respectively; and at 18 months it was 15.4% (9.1, 23.3), 22% (13.8, 31.6), 26.1% (23.2, 29.2), and 30.2% (25.4, 35.2), respectively. The number of patients receiving 3-4 antiemetics has been increasing since the end of 2021, and in 2023, more patients were receiving 3-4 antiemetic classes (n=574) than 2 antiemetic classes (n=294). In an analysis of patients who were on T-DXd for ≥6 months (n=1027), the rate of dose reduction decreased with the number of antiemetic classes (32.7% for 0-1 classes, 27.5% for 2 classes, 24.9% for 3+ classes). Conclusion: In this analysis of real-world data, persistence on T-DXd treatment increased with the number of antiemetic classes administered. Following the NCCN guideline update, patients receiving T-DXd are now treated with 3 or more antiemetic classes. The requirement for dose reduction appears to be reduced when more antiemetic classes are used.

P2-12-16: A PROSPECTIVE RANDOMIZED PLACEBO-CONTROLLED TRIAL EVALUATING EFFECT OF A WIDE SPECTRUM MICRONUTRIENTS SUPPLEMENT ON CANCER RELATED FATIGUE IN BREAST CANCER PATIENTS TREATED WITH ADJUVANT/NEO-ADJUVANT THERAPY

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Background: Fatigue is the most common symptom experienced by patients during the cancer trajectory from diagnosis to the end of life. Several studies in breast cancer (BC) patients confirm that fatigue is the most reported symptom (88-91%). Women with BC are the highest users of complementary therapies, including use of micronutrients, to prevent or reduce the Cancer-related fatigue (CRF) severity. This highlights how there is still today the need for non-pharmacological treatments that are able to relieve CRF in breast cancer (BC) patients. There is no unanimous consensus on the use of micronutrients in CRF and randomized controlled studies are needed to define the effect of these substances. Recently a nutritional supplement containing 19 nutrients including amino acids such as arginine and carnitine, highly absorbed minerals such as Sucrosomial® iron, magnesium, zinc and selenium, group B vitamins, and plant extracts as Panax ginseng and Eleutherococcus senticosus (Apportal®) demonstrated a significantly improvement of post-Covid related fatigue and to ameliorate muscle strength and physical performance in subject suffering from long-Covid syndrome. Besides, a recent cohort study including 458 individuals who reported fatigue from different causes (not Covid related) showed that the intake of APPORTAL® significantly improves both mental and physical fatigue (under submission).
Study Objectives: The primary objective of this study is to investigate if BC patients

undergoing adjuvant or neo-adjuvant therapy can have a beneficial effect from Apportal® supplementation. Secondary objectives are evaluating if the supplementation with APPORTAL® can improve patients' nutritional status, muscular strength, and quality of life (QoL).
Trial design: This is a randomized, placebo controlled, multicenter study with two parallel groups of patients. Patients with BC, suffering from fatigue after at least one cycle of adjuvant or neo-adjuvant chemotherapy will receive APPORTAL®, (Pharmanutra SpA, Italy), 1 sachet per day or placebo for 8 weeks. Visits were scheduled at baseline and after 4-8-12 weeks, assessing fatigue (BFI questionnaire), physical status, blood nutritional markers, quality of life (QoL), muscular strengths, compliance and adverse events.
Major Inclusion Criteria: - Females aged 18 or higher - Patients diagnosed with histologically confirmed BC - Patients having done at least one cycle of adjuvant or neo-adjuvant chemotherapy - ECOG performance status ≤1 at screening - CRF of moderate-severe intensity (NRS > 4) - Patients able to follow the recommendations on the physical exercise to do - Patients who accept to use adequate contraceptive methods, if they are of child-bearing potential.
Sample Size
92 patients randomized 1:1 ratio into Treatment group (APPORTAL® plus physical exercise) and Placebo group (Placebo plus physical exercise).
Estimated Dates for Completing Accrual
Patient recruitment will be completed by mid-2025. First patient was enrolled in December 2023.
Trial Registration Number
NCT 06137833

P2-12-17: A Phase 1, First-in-Human study of autologous monocytes engineered to express an anti-HER2 chimeric antigen receptor (CAR) in participants with HER2 overexpressing solid tumors

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Background: Myeloid cells are actively recruited to the solid tumor microenvironment (TME) and have the potential to mediate tumor control via phagocytosis, TME remodeling, and T cell activation. We previously developed human chimeric antigen receptor macrophages (CAR-Macrophage) and have shown potent anti-tumor activity in pre-clinical solid tumor models1. The anti-HER2 CAR-Macrophage cell therapy product, CT-0508, was evaluated in a Phase 1 trial as a monotherapy and in combination with pembrolizumab. Clinical data have demonstrated preliminary safety, feasibility, and validated the mechanism of action. We have developed a next-generation CAR monocyte (CAR-Monocyte) platform to increase the available dose, improve tumor trafficking and engraftment, and

shorten the manufacturing and vein-to-vein time as compared to CAR-Macrophage therapy. CT-0525 is an autologous anti-HER2 CAR-Monocyte cell therapy based on CD14+ monocytes engineered with an Ad5f35 adenoviral vector to express an anti-HER2 CAR. Pre-clinical studies have demonstrated the feasibility, phenotype, pharmacokinetics, durable CAR expression, cellular fate, antigen specificity, and anti-tumor activity of CT-0525. Pre-clinical studies have shown that CT-0525 differentiated into pro-inflammatory CAR-Macrophages in vivo and controlled tumor growth. The CT-0525 manufacturing process takes one day and enables the production of up to 10 billion cells from a single apheresis. CT-0525 is being investigated in a first-in-human, open-label, multi-center, Phase 1 study in participants (pts) with HER2 overexpressing solid tumors.
Methods: This Phase 1, first-in-human study evaluates the preliminary safety, feasibility, tolerability, trafficking, TME activation, and initial evidence of efficacy of the investigational CAR-Monocyte product, CT-0525, in pts with locally advanced unresectable/metastatic solid tumors overexpressing HER2. Pts previously treated with anti-HER2 therapies are eligible for this dose escalation study. Filgrastim mobilized autologous CD14+ monocytes are collected by apheresis, followed by manufacturing and cryopreservation. CT-0525 will be administered without conditioning chemotherapy. The 1st cohort of pts will receive 3 x 109 CT-0525 CAR positive monocytes administered in one infusion intravenously. If tolerated as per the modified toxicity probability interval algorithm (mTPI), the 2nd cohort of pts will receive up to 10 x 109 CT-0525 CAR positive monocytes in one infusion. A minimum of 3 evaluable pts is required at each dose level. Primary endpoints include assessment of safety and tolerability, as well as manufacturing feasibility. Secondary endpoints include initial evidence of efficacy. Correlative assessments include pre- and post-treatment biopsies and blood samples for safety, immunogenicity, pharmacokinetics, tumor trafficking, TME modulation, epitope spreading, and other translational biomarkers. This clinical trial (NCT06254807) is currently enrolling at multiple sites across the United States.
1. Klichinsky M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nature Biotechnology. 2020; 38: 947-953

P2-12-18: A Randomized Phase 2 Non-inferiority Trial of (Z)-endoxifen and Exemestane + Goserelin as Neoadjuvant Treatment for Premenopausal Women with ER+/HER2-Breast Cancer (EVANGELINE)

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Background: Ovarian function suppression (OFS) when combined with tamoxifen or an aromatase inhibitor is the standard of care for premenopausal women (preMW) with estrogen receptor positive (ER+) human epidermal growth factor receptor 2 negative (HER2-) breast cancer (BC) based on SOFT (Francis 2022) and TEXT (Pagani 2022) clinical

trials and confirmed in a metanalysis (EBCTCG 2022). However, estrogen deprivation in preMW is associated with significant morbidity and up to 40% of premenopausal women with ER+/HER2- BC are intolerant of OFS. For these women, tamoxifen monotherapy is the only FDA approved option where long-term patient outcomes are known to be sub-optimal. Therefore, there is substantial room to improve endocrine therapy for preMW with ER+/HER2- BC. (Z)-endoxifen is one of the most active anti-estrogen tamoxifen metabolites, with preclinical data demonstrating superiority to tamoxifen and letrozole in aromatase expressing xenograft models and evidence for antitumor activity in early phase clinical trials in patients with prior progression on endocrine therapy including tamoxifen. In addition to its ability to potently block ERα, (Z)-endoxifen at clinically achievable concentrations inhibits protein kinase C beta 1 (PKCβ1), resulting in downstream AKT inhibition and apoptosis. It is hypothesized that (Z)-endoxifen, when delivered as monotherapy at doses that dually target both ERα and PKCβ1, will potently inhibit BC proliferation while obviating the need for OFS in premenopausal women. If successful, this innovative approach could spare women with endocrine sensitive BC from the side effects of OFS.
Methods: To further study (Z)-endoxifen in preMW, a multicenter study in the United States, with possible expansion to other countries was designed (EVANGELINE; NCT05607004). This is a Phase 2, open-label, randomized, neoadjuvant study with a pharmacokinetic (PK) run-in phase in preMW with Stage IIA or IIB ER+/HER2- incident BC. Subjects are randomized 1:1 to either exemestane plus goserelin or (Z)-endoxifen monotherapy. The primary objective of the PK-run is to determine the dose that inhibits PKCβ1 and in the randomized Phase II is to determine whether the endocrine sensitive disease (ESD) rate (defined as Ki-67 ≤ 10% after 4 weeks of neoadjuvant therapy) with (Z)-endoxifen is non-inferior to exemestane plus goserelin. Subjects will be enrolled with the intent of surgical treatment in the involved breast after completing 6 months of neoadjuvant treatment. Safety, tolerability, response rates and surgical outcomes will be assessed. Disease progression will be monitored throughout study participation. Paired tumor biopsies and blood sampling will allow for a robust biomarker evaluation. Enrollment to the 40mg PK Run-was completed in June 2023 (n=7), and accrual to the 80mg PK Run-in will be complete by August 2024. The randomized portion of the trial is expected to commence in Fall 2024.

P2-12-19: A Randomized Phase II Window-of-opportunity Trial of Ruxolitinib in Patients with High Risk and Premalignant Breast Conditions

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Background: Current medications to prevent breast cancer (BC) are the antiestrogens tamoxifen, raloxifene, anastrozole and exemestane which can prevent 50% of ER+ BC. However, because they can have side effects and require 5 years of continuous use, women

who are yet cancer-free are often reluctant to take them. Furthermore, antiestrogens do not prevent ER- BC. Therefore, it is urgent that we develop alternative prevention drugs that require only a short-term treatment and are suitable for preventing both ER+ and ER- BC. No prevention strategies have been developed for ER- or HER2+ subtypes, the most virulent forms of the disease.

Ruxolitinib is a small molecule which selectively inhibits the activities of Janus Associated Kinases (JAK); activated JAK phosphorylate STATs leading to modulation of gene expression. In animal models, ruxolitinib prevents progression of premalignant breast lesions to invasive BC. Studies in several human tissue types suggest that there are anticancer "barriers," namely apoptosis and senescence, which are erected in early lesions to prevent the progression to cancer. We hypothesize that the JAK2-STAT5 pathway in human premalignant lesions promotes the progression of these lesions to malignancy by lowering the apoptosis anticancer barrier; therefore, inhibition of this pro-survival pathway could reduce the load of premalignant lesions in the breast and thus lower BC risk. We are conducting a window-of-opportunity trial through the Translational Breast Cancer Research Consortium (TBCRC) to test whether short-term ruxolitinib treatment of subjects with premalignant lesions will result in increased apoptosis.

Trial Design: This is a phase II, double-blind, placebo-controlled clinical trial. Our target population consists of patients with premalignant breast lesions requiring surgical excision. Subjects will be randomized 1:1 to receive either ruxolitinib 20 mg twice daily or placebo for ~15 days after which they will proceed with standard of care surgical excision within 12 hours of the last dose.

Key Eligibility Criteria: 1) Breast biopsy showing atypical ductal hyperplasia, atypical lobular hyperplasia, lobular carcinoma in situ, or ductal carcinoma in situ requiring surgical excision

2) Adequate hematologic and organ function

3) Willing to not use concomitant strong CYP3A4 inhibitors

Statistical Methods: The primary endpoint is change in apoptosis, as measured by cleaved caspase-3 staining, in surgical samples relative to baseline biopsy. Lesions of different types will be characterized separately, and the participant's most advanced lesion present at baseline will be used for the primary analysis. The difference in changes between groups will be evaluated using the Wilcoxon rank sum test.

The secondary objectives primarily focused on the effects of ruxolitinib treatment on biomarkers. The secondary endpoints include pre- and post-treatment pSTAT5, TUNEL, Ki67, BCLxL, MCL1, BCL2, PRLR, pSTAT3, and serum prolactin levels. Changes in these markers will be compared between groups using the Wilcoxon rank sum test.

Accrual:

We expect to enroll 100 subjects from 7 sites in the US in order to obtain 70 evaluable subjects. Currently there are 80 subjects enrolled.

Contact Information:

For more information on this trial, please visit clinicaltrials.gov NCT02928978

P2-12-20: A Real-World Study of the Effectiveness and Safety of Trastuzumab Deruxtecan (T-DXd) in HER2-low Metastatic Breast Cancer (mBC) among Racial and Ethnic Minorities and Older Populations in the United States

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Background: Approximately 45-55% of all primary breast cancer (BC) cases are classified as human epidermal growth factor receptor 2 low (HER2-low). Currently, trastuzumab deruxtecan (T-DXd) is the only approved HER2-targeted therapy for HER2-low metastatic BC (mBC). In the US, Black/African-American and Hispanic women have more advanced BC stage at diagnosis, less favorable tumor characteristics, and a lower chance of receiving guideline concordant care compared to non-Hispanic white women. Similarly, women ≥65 years of age have higher BC incidence and mortality, are more likely to be under-treated, and are less likely to comply with treatment compared to younger women. Given these health inequities, we sought to further evaluate the safety and effectiveness of T-DXd among racial/ethnic minorities and older patients in the HER2-low mBC setting.

Specific Aims: The primary objective of this study is to assess the effectiveness of T-DXd measured by real-world progression-free survival among HER2-low mBC patients treated with T-DXd, stratified by race, ethnicity, and age group. Secondary objectives are twofold: (1) describe demographic/clinical characteristics and prior anticancer therapy among HER2-low mBC patients and (2) assess the real-world targeted safety profile of T-DXd among HER2-low mBC patients, both stratified by race, ethnicity, and age group. Targeted adverse events (TAEs) include interstitial lung disease/pneumonitis, left ventricular dysfunction, neutropenia/febrile neutropenia, and nausea/vomiting. As an exploratory objective, we will assess the effectiveness of T-DXd using additional endpoints including real-world overall survival, real-world time to next treatment, and real-world time to treatment discontinuation.

Eligibility criteria: To be included in the Source Population, patients must be ≥18 years old with HER2-low mBC. Major exclusion criteria include history of a non-BC primary cancer, history of HER2 positive disease, and having neither race nor ethnicity data. The T-DXd Treated Cohort will include the subgroup of patients from the Source Population who receive T-DXd during the study period.

Study Design: This is a non-interventional, retrospective cohort study conducted in the real-world setting. The data source is the Guardian Research Network (GRN), a de-identified electronic medical record database covering US oncology and non-oncology care. The planned study period is from August 2022 to December 2026. All necessary variables will be obtained from structured data elements or curation of unstructured data elements, as needed (e.g., physician notes, radiology reports). Present and target accrual. A sample size

projection for the T-DXd Treated Cohort was completed based on current patient accrual in GRN with assumptions for T-DXd uptake. At the time of this analysis, 60 patients fulfilled all eligibility criteria for inclusion in the T-DXd Treated Cohort, and approximately 450 patients are projected to be eligible for inclusion in this cohort through the end of the study period.

Statistical Methods: All analyses will be descriptive; no hypothesis testing is planned. The Source Population will be used to summarize demographics, clinical characteristics, and treatment patterns. Effectiveness endpoints will be analyzed using a time-to-event approach (Kaplan-Meier estimator). The proportion of patients with each TAE will be summarized and select TAE characteristics will be described. All analyses will be conducted overall and stratified by race, ethnicity, and age group (≤65, >65, and >75 years).

P2-12-21: A Video Intervention to Improve Patient Understanding of Tumor Genomic Testing in Patients with Metastatic Breast Cancer: Primary Results of a Prospective Intervention Trial

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Background: Tumor genomic testing (TGT) has become standard-of-care for all patients with metastatic breast cancer (MBC). Guidelines for patient education prior to TGT from the American Society of Clinical Oncology (ASCO) and the American College of Medical Genetics (ACMG) are not widely followed. We have previously demonstrated disparities in general genomic knowledge across race and income. The purpose of this study was to evaluate the impact of a concise (3-4 minute) video for patient education prior to TGT in a prospective interventional trial. We report the results of the MBC cohort (ClinicalTrials.gov NCT05215769).
Methods: We created a base animated video incorporating culturally diverse images, available in English and Spanish, applicable to any cancer type, with MBC-specific content included for patients with MBC. From March 2022 to June 2024, a total of 54 patients with MBC were enrolled at a single tertiary academic institution, and three community cancer centers. Participants completed validated survey instruments immediately prior to video viewing (T1), immediately post-viewing (T2) and after receipt of their own TGT results (T3). Instruments included: 1) 10-question objective genomic knowledge/understanding (GKU); 2) 10-question video message-specific knowledge/recall (VMSK); 3) 11-question Trust in Physician/Provider (TIPP); 4) attitudes around TGT. The primary objective was to assess change in VMSK between T1 and T2. A cohort of fifty patients provided 90% power to detect an effect size of 0.47 in change of recall accuracy from pre- to post-video using two-sided Wilcoxon signed-rank test with alpha of 0.05. The associations of VMSK, GKU, and TIPP with categorical demographic variables were explored with Kruskal-Wallis test.
Results: To date 50 of the 54 patients with MBC enrolled have completed surveys at all

timepoints. The MBC cohort had a median age of 57; all were female; most were Caucasian (49/54, 91%); most were married/in domestic partnership (41/54, 76%). For the primary endpoint, there was a significant increase in VMSK ($p<0.001$) but there was no significant change in GKU ($p=0.89$) or TIPP ($p=0.59$). Improvement of VMSK was consistent across demographic groups, including race/ethnicity, age, income, and education. Of the 10-questions in the VMSK survey, results for four questions significantly improved after viewing the video, including questions informing the likelihood of TGT impact on treatment decision, incidental germline findings, and cost of testing. However, the improvement seen was not consistently sustained at T3 in seven of ten questions. Higher baseline genomic knowledge was significantly associated with higher participant income.
Conclusions: A single viewing of our concise, 3-4 minute, broadly applicable video incorporating culturally diverse images administered prior to TGT significantly improved VMSK across all demographic groups. While video content recall regressed over time, patient's perception of the merits of TGT and their TIPP remained unchanged. This approach can be a valuable tool for empowering patients and educating them on the risks, benefits, and limitations of TGT in alignment with ASCO and ACMG guidelines.

P2-12-22: A multicenter, single-arm, phase II clinical trial of oral CDK4/6 inhibitor darciclib in combination with endocrine therapy in adjuvant treatment for hormone receptor-positive, HER2-negative female breast cancer.

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Background: Although the role of endocrine therapy in the adjuvant treatment of HR+/HER2- breast cancer has been established, Early HR positive and HER2 negative breast cancer show a long-term risk of recurrence. Studies have shown that about 20% of patients experience recurrence and metastasis after receiving adjuvant endocrine therapy for 5 years. CDK4/6 inhibitor combined with endocrine therapy for HR+/HER2- breast cancer has been proven to have synergistic effects. Previous studies have shown that Abemaciclib and ribociclib can reduce the risk of recurrence and increase iDFS in early high-risk HR positive breast cancer, but conventional doses lead to excessive adverse events and patients interrupt treatment. Compared with different CDK4/6 inhibitors combined with AI as first-line treatment of advanced HR+ breast cancer, dalpiciclib achieved the highest ORR. It was found that dose reduction had no significant identifiable negative impact on the progression-free survival evaluated in the study. This study will be evaluated the effectiveness and safety of a small-dose dalpiciclib combined with endocrine therapy in hormone receptor-positive, HER2-negative breast cancer in women.
Methods: This multicenter, single arm, phase II clinical trial(ChiCTR2400080962) will enroll 317 women with hormone receptor-positive early breast cancer from Jan 2024 to Dec 2025.

Eligible patients are aged ≥18 years and ≤75 years, either postmenopausal or pre/perimenopausal. Histologically confirmed stage II-III (a) IIA-N0: Grade 2 with high risk [Ki67≥20% or high-risk gene characteristics], Grade 3, N1 ; (b) Anatomical stage IIB: N0 or N1;(c) Anatomical stage III: N0, N1, N2, or N3. HR positive defined as ER positive: Positive staining tumor cells accounting for ≥10% of all tumor cells (confirmed by the pathology department of the research center), regardless of PR expression status.
Patients will receive dalpiciclib 100mg daily for 21 days followed by a 7-day break(every 28 days as a cycle) for 3 years. All patients receive standard endocrine therapy for 5-10 years after standard postoperative adjuvant chemotherapy and/or adjuvant radiotherapy. The primary endpoint is Intermediate Disease-Free Survival(IDFS), while the secondary endpoints including Disease-Free Survival(DFS), Distant Disease-Free Survival(DDFS),Overall Survival(OS).The safety of patients will be assessed by the quality of life rating scale (EORTIC QLQ-C30).
Conclusions: This multicenter, single-arm, phase II clinical trial will provide valuable insight into the efficacy and safety of a small-dose dalpiciclib in combination with endocrine therapy in adjuvant treatment for hormone receptor-positive, HER2-negative female breast cancer. If proven effective and well-tolerated, this regimen may offer a more convenient treatment option for this patient population, potentially improving quality of life and treatment adherence.

P2-12-23: A phase 2 trial combining tiragolumab and atezolizumab with neoadjuvant or first line chemotherapy for triple negative breast cancer (SKYLINE)

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Introduction: TIGIT is an immune inhibitory receptor that is a member of the Ig superfamily and that acts to limit anti-tumor immune responses in the context of the tumor microenvironment. TIGIT expression has been shown to be elevated in the tumor microenvironment in many human tumors and is coordinately expressed with other immune checkpoints such as PD-1/PD-L1. Prior works identified that expression of both PD-1 and TIGIT at the surface of CD4+ T lymphocytes is increased in presence of an immunosuppressive subset of cancer-associated fibroblasts (CAF) which express FAP (Fibroblast Activated Protein). Interestingly, new advanced metabolic imaging tracers, such as FAPI-46, could allow whole body imaging and quantitative mapping of FAP+ CAF and might represent a predictive marker of immunotherapy efficacy.
SKYLINE is a phase 2 trial exploring the use of an anti-TIGIT therapy, tiragolumab, in combination with anti PD-L1 atezolizumab and chemotherapy in patient with triple negative breast cancer (TNBC), as neoadjuvant (cohort A) or first line (cohort B) therapy.
Methods: SKYLINE (NCT06175390) is an ongoing trial in at Institut Curie Hospitals (France), prospectively enrolling 160 patients with TNBC (ER & PR<10%, HER2-). In cohort

A, patients with stage II-III TNBC undergo neoadjuvant therapy that consists in a sequential use of nab-paclitaxel, carboplatin, atezolizumab and tiragolumab at standard doses for four 3-weeks cycles, followed by AC, atezolizumab and tiragolumab for four 3-weeks cycles, before curative surgery. Patients in cohort A could also receive additional atezolizumab and tiragolumab in the adjuvant setting. In cohort B, patients with de novo or relapsed stage IV TNBC receive nab-paclitaxel, atezolizumab and tiragolumab (4-weeks cycles) until disease progression or unacceptable toxicity. The study primary objective focuses on anti-tumor efficacy: pCR rate and 6-months PFS rate in cohorts A and B, respectively. Multiomics characterization of repeated tissue samples, blood samples and imaging (including next generation imaging with 68Ga-FAPI-46 PET/CT) is planned to better understand and predict the efficacy of immuno-chemotherapy in patients with TNBC.

Acknowledgments
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Disclosures
FC Bidard received personal fees (advisory board) from Roche.

P2-12-24: A phase I/Ib trial of the CDK4/6 antagonist ribociclib and the HDAC inhibitor belinostat in patients with metastatic triple negative breast cancer and recurrent ovarian cancer with response prediction by genomics (CHARGE).

Talicia Savage, John G Lamb, Elyse D'Astous, Shashank Sama, Kristen Kelley, Christos Vaklavas, Namita Chittoria, Lauren Mauro, Kathleen Harnden, Takada Harris, Adam L Cohen, Theresa L Werner

Rationale: Metastatic triple negative breast cancer (TNBC) and recurrent ovarian cancer have poor prognoses with survival rates of approximately twenty-three months and thirty-two months, respectively. New treatment paradigms for these malignancies are needed. In Vivo studies have demonstrated promising results in single agent activity of histone deacetylase inhibitors (HDACi) against TNBC. In vitro studies have demonstrated cell cycle inhibition with the use of CDK4/6 inhibitors (CDKi) and synergistic inhibition with the combination of both HDACi and CDKi. We hypothesized that the CDKi ribociclib (RIB) plus the HDACi belinostat (BEL) would be well-tolerated and effective in people with metastatic TNBC or recurrent ovarian cancers.
Research objectives: This open-label, multi-center, phase I study follows a modified 3+3 dose escalation design allowing independent escalation of the dose of each of the agents with a ten-person expansion cohort at the maximum tolerated dose (MTD) level. Dose escalation was open to patients with TNBC or ovarian cancer, and only TNBC will be enrolled in the dose expansion. The first cohort at dose level 0 was RIB 200 mg daily and BEL 600 mg/m2 daily on days 1-5 of a 28-day cycle. The RIB dose could be escalated to 400mg on days 8-28, and the BEL could be escalated to 1000mg/m2 daily on days 1-5 of the 28 day cycle. The primary objective of this trial was to assess the maximum tolerated dose

of RIB in combination with BEL in patients with metastatic triple negative breast cancer or recurrent ovarian cancer. The secondary objectives were to assess the frequency, severity, and type of adverse events (AEs), and serious adverse events (SAEs). Here we report the final safety results from the 3+3 dose escalation cohort.
Results: We enrolled three patients (two ovarian, one TNBC) at dose level 0: RIB 200 mg daily + BEL 600mg/m2 days 1-7 with no dose-limiting toxicities (DLTs).
Three patients (one ovarian, two TNBC) were enrolled at dose level 1B: RIB 200 mg daily + BEL 1000 mg/m2 days 1-5 [increased dose of BEL compared to dose level 0]. Two patients experienced a DLT (one prolonged QTc, one allergic reaction).
Six patients (three ovarian, three TNBC) were enrolled at dose level 1A: RIB 400 mg days 8-28 + BEL 600 mg/m2 days 1-5. One patient experienced a DLT (prolonged QTc). Therefore, the MTD has been established at dose level 1A: RIB 400 mg days 8-28 + BEL 600 mg/m2 days 1-5.
The most common side effects experienced in at least 50% of patients were fatigue, dyspnea, constipation, headaches, and nausea with most AE being grades 1 or 2.
Conclusions: The maximum tolerated dose for this regimen has been determined to be 400 mg RIB on days 8-28 with 600mg/m2 BEL dosing on days 1-5. EKG monitoring is needed due to risk of QTc prolongation. We saw no unexpected or IRB reportable serious adverse events, and no indications of significant liver toxicity. The trial has now proceeded to dose expansion and efficacy assessment.

P2-12-25: A phase III trial evaluating De-escalation of Breast Radiation (DEBRA) following breast-conserving surgery of stage 1, HR+, HER2-, RS ≤18 breast cancer: NRG-BR007

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Background: Approximately 50% of newly diagnosed invasive breast cancers are stage 1, with the majority being ER/PR-positive, HER2-negative. Genomic assays such as the Oncotype DX® have identified patients (pts) with reduced risk of distant metastasis and without benefit from chemotherapy added to endocrine therapy (ET), freeing them from excess toxicity. Genomic assays are also recognized as prognostic for in-breast recurrence (IBR) after breast-conserving surgery (BCS) and could similarly allow de-escalation of adjuvant radiotherapy (RT). Reducing overtreatment is of interest to pts, providers, and payers.
Methods: We hypothesize that BCS alone is non-inferior to BCS plus RT for IBR and breast preservation in women intending ET for stage 1 invasive breast cancer (ER and/or PR-positive, HER2-negative with an Oncotype DX Recurrence Score [RS] of ≤18). Stratification is by age (<60; ≥60), tumor size (≤1 cm; >1-2cm), and RS (≤11, >11-18/MammaPrint Low). Pts are randomized post-BCS to Arm 1 with breast RT using standard methods

(moderate or ultra hypo- or conventional-fractionated whole breast RT with/without boost, or APBI) with ≥5 yrs of ET (tamoxifen or AI) or Arm 2 with ≥5 yrs of ET (tamoxifen or AI) alone. The specific regimen of ET in both arms is at the treating physician's discretion. Eligible pts are stage 1: pT1 (≤2 cm), pN0, age ≥50 to <70 yrs, s/p BCS with negative margins (no ink on tumor), s/p axillary nodal staging (SNB or ALND), ER and/or PR-positive (ASCO/CAP), HER2-negative (ASCO/CAP), and Oncotype DX RS ≤18 (diagnostic core biopsy or resected specimen). A "low risk" MammaPrint is permissible if completed as part of usual care prior to screening. Primary endpoint is IBR (invasive breast cancer or DCIS). Secondary endpoints are breast conservation rate, invasive in-breast recurrence, relapse-free interval, distant disease-free survival, overall survival, patient-reported breast pain, patient-reported worry about recurrence, and adherence to ET. We assume a clinically acceptable difference in IBR of 4% at 10 yrs to judge omission of RT as non-inferior (10-yr event-free survival for RT group is 95.6% v 91.6% for the omission-of-RT group). BR007 is powered to detect non-inferiority with 80% power and a one-sided α=0.025, assuming that there would be a ramp-up in accrual in the first two years (leveling off in Yrs 3-5); 1,670 pts (835 per arm) are required for randomization. Conservative loss to follow-up is 1%/yr. Some T1a pts screened may have Oncotype DX scores >18, making them ineligible for the study. In the accrual process, 1,714 pts will be required to register to ensure that our final randomized cohort is 1,670 pts.
As of July 3, 2024, 1,033 pts have been screened and 927 randomly assigned.
NCT #: NCT04852887.
Support: U10 CA180868, -180822, UG1 CA189867.

P2-12-26: A phase Ib study of novel combination (New) of low dose oral cyclophoSphamide (S) To potentiate axatilimab (A) + retifanlimab (R) in treating metastatic Triple negative breast cancer (NEW START)

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Background: A large proportion of triple negative breast cancer (TNBC) patients (pts) will have residual disease detected post-neoadjuvant systemic therapy (NAST), which is associated with a significant risk of recurrence within 2-3 years following surgery. Moreover, if resistance develops to first-line therapy, the response rate to second or higher lines of therapy is poor (5-10% historically), representing a vast unmet need. Evidence suggests that one mechanism causing resistance is associated with the tumor immune microenvironment (TIME), particularly low T cell infiltrates and high levels of tumor-associated macrophages (TAMs). This led to the hypothesis that eradicating TAMs could re-sensitize NAST-resistant TNBC to tumor growth inhibition. Preclinical models using the preclinical version of axatilimab + low dose cyclophosphamide (CTX) successfully depleted macrophages and treated several primary TNBC models. However, intratumoral

heterogeneity within lung metastasis was observed following treatment cessation, along with elevated PD-L1 levels. Suspecting elevated PD-L1 levels as the likely cause of T cell exhaustion, an immune checkpoint blockade drug was added, which showed higher efficacy and prolonged the overall survival of mice bearing established lung metastasis. Given the target relevance in inflammatory breast cancer (IBC), the TNBC and low ER/PR IBC will also be included due to the high infiltration of TAMs and resistance to chemotherapy. After successful pre-clinical models, we propose a phase 1b study to demonstrate safety, response, and to find the optimal biologic dose (OBD) of CTX and axatilimab, along with standard dose retifanlimab to facilitate studies of efficacy. Axatilimab is a monoclonal antibody (mAb) with a high affinity against CSF-1R. Targeting CSF-1R is expected to reduce the circulating levels of pathogenic macrophage precursors and inhibit their activation in tissues. Retifanlimab is a mAb that targets PD-1, currently FDA approved for use in merkel cell carcinoma. It is designed to block checkpoint inhibitory interactions and sustain the function of PD-1–expressing cells. Methods: Up to 24 pts will be enrolled in cohorts of 3 pts. Pts will receive a fixed dose of retifanlimab, one of two dosage levels of CTX, and one of three dosage levels of axatilimab. The utility-based Bayesian optimal interval (U-BOIN) design will be used to determine the OBD weighing both dose limiting toxicities and objective responses. We will study baseline and follow-up biopsy after pharmacodynamics window (4 weeks +/- 7 days) to test exploratory biomarkers such as TAMs, epithelial-mesenchymal transition features, and other TIME cells. Tumor staging will be done at baseline and after every 2 cycles, each cycle defined as 4 weeks. All tumor responses will be evaluated by iRECIST 1.1. The study was sponsored by Incyte and the UT MD Anderson Cancer Center Morgan Welch Inflammatory Breast Cancer Clinic and Research Program. Key inclusion criteria: - Metastatic or locally recurrent unresectable malignancy lacking standard curative treatments - Histologically or cytologically confirmed non-IBC TNBC, defined as ER<1%, PR<1% and HER2 negative per ASCO CAP 2018 guideline, OR - IBC confirmed according to international consensus criteria that is ER<20% and PR<20% - At least 1 week since prior chemotherapy or radiation therapy - Age ≥18 years - Has at least one measurable lesion per RECIST 1.1 - ECOG performance status ≤ 2 - Adequate organ and marrow function. Key exclusion criteria: - Unrecovered >Grade 1 adverse events due to prior anti-cancer therapy, with some exceptions - Evidence of progression in treated brain metastases. The primary endpoints are OBD, dose limiting toxicity, and objective response. The secondary endpoints include clinical benefit, duration of response, progression-free survival, 2-year overall survival, pharmacodynamic markers, and detailed safety and tolerability of the triple combination.

P2-12-27: A phase Ib study of the safety, tolerability, biological effect, and efficacy of allogenic natural killer cells in combination with trastuzumab and pertuzumab in patients with refractory HER2-positive metastatic breast cancer.

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Background: Survival of HER2-positive (HER2+) metastatic breast cancer (MBC) patients (pts) has improved with targeted therapies, but there is still a need of developing new therapeutic approaches. Double blockade alone with trastuzumab (T) and pertuzumab (P) showed significant clinical activity. T and P exert part of their activity based on antibody dependent cell mediated cytotoxicity (ADCC), mediated by natural killer cells (NK). Through the binding of the CD16 receptor of the NK cell to the Fc domain of T, NK cells can eliminate tumor cells covered by these antibodies. Furthermore, ADCC activation of an innate immune response induces cytokine secretion and antigen release, which may trigger an adaptive immune response against tumor antigens. Solid tumors usually present poor NK infiltration due to limited homing and immunosuppressive microenvironment. Our hypothesis is that the effect of T and P can be improved by regulating the efficiency of the ADCC activity through the infusion of ex-vivo activated allogenic NK cells. We propose a proof-of-concept phase I clinical trial for pts with HER2+ MBC refractory to antiHER2 therapies, to test the infusion of allogenic NK cells in order to enhance the ADCC of T and P to overcome this resistance and improve clinical outcome.
Methods: This is a single arm, open label, multi-center, proof of concept investigator-initiated phase Ib trial to assess the safety and the tolerability of NK adoptive cell therapy (NK-ACT) and T+P when used in combination in refractory HER+ MBC. A total of 6 pts will be included in the safety lead-in phase. If signs of both clinical and biological activity are seen, and no more than 1 treatment-limiting toxicity (TLT) is observed, the study will expand with 14 additional pts. The NK investigational cell product comprises a live cell suspension of allogenic NK cells obtained from peripheral blood from a healthy donor, that will be infused on Day 2 at a minimum dose of 5x107 NK and a maximum dose limit of 5x108 NK. IL-2 will be administered on Days 2 (within 24h after NK infusion), 4 and 6 as a subcutaneous dose of 5x105 UI/m2. A preparative IV single-dose cyclophosphamide (600mg/m2) is given between Days -3 and -5 before NK cell infusion. On day 1, T is given at a dose of 8mg/kg IV for the loading dose, and 6mg/kg IV for the maintenance dose and P at a dose of 840mg IV for the loading dose, and 420 mg IV for the maintenance, both every 3 weeks, until disease progression, unacceptable toxicity or study termination.
Major eligibility criteria: HER2+ pts as per ASCO/CAP guidelines; ECOG performance status ≤1; measurable disease; received at least two lines in the metastatic setting including

trastuzumab/pertuzumab and an anti-HER2 ADC; progressed to previous therapy; normal organ and marrow function; patient has potential NK allogenic donors. Key exclusion criteria: Prior treatment with adoptive cellular therapy; Symptomatic or untreated primary or metastatic CNS malignancy. The primary objectives are to assess the safety and the tolerability of NK-ACT and T/P when used in combination. The secondary objective is to evaluate the initial clinical activity of NK-ACT concomitant with T/P. Exploratory objectives include describing the mechanisms of action and assessing biomarkers of the immunomodulatory effect and anti-tumor activity of the combination of NK-ACT and T/P. Statistical methods: no formal hypothesis testing and no formal sample size calculation. The safety lead-in phase will include the first 6 pts. If no more than 1 TLT is observed amount those pts, the study will expand with 14 additional pts.

P2-12-28: A prospective randomized, controlled study to evaluate device efficacy for cutting and/or coagulation of tissue during mastectomy procedures.

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Background/Purpose: The ConMed HelixAR Electrosurgical Generator with Argon Beam Coagulation Technology (CHEST) trial focuses on patients undergoing unilateral or bilateral mastectomy with immediate breast reconstruction including breast implants, tissue expander placement, or autologous flap. The study design compares time to hemostasis between two devices: the HelixAR Electrosurgical Generator (HEG) and the Conventional Electrosurgical Coagulation (CEC) system. To evaluate the device effectiveness between the HelixAR Electrosurgical Generator and Conventional Electrosurgical Coagulation systems for cutting and/or coagulation of tissue during mastectomy and reconstruction surgery, we report their implementation in a randomized clinical trial. The study is powered to show superiority of the HEG to the CEC as it relates to the primary objective of time from post-mastectomy to hemostasis. The HEG is designed to deliver argon gas and high frequency electrical current compared to the CEC delivering high frequency electrical current only. The key efficacy measure is post-mastectomy time to hemostasis with the secondary endpoints of wound infection, drain duration, total drain output, blood loss and device related adverse events. The study hypothesizes that the time for post-mastectomy hemostasis is significantly less for the HEG than the CEC. Additional hypotheses include overall fewer device related adverse events, lower blood loss, and reduced operative time. Methods: This ongoing 1:1 randomized controlled trial plans to enroll 82 patients undergoing mastectomy with reconstruction. The population includes women with breast cancer and/or high risk of developing breast cancer from genetic mutations and family history. Exclusion factors include known history of bleeding diathesis or coagulopathy, advanced refusal of blood transfusion, active systemic or cutaneous infection or inflammation. Participants are randomly allocated to either the HEG or the CEC at the time

of surgery. The surgeons are instructed to use the randomized device throughout the procedure with primary endpoints focused on evaluating device efficacy for cutting and coagulation. Subjects are monitored for 2 months post-operatively to assess perioperative outcomes.
Results: At the time of abstract submission, 80 patients were enrolled, with 39 allocated to HEG and 40 to the CEC (1 pending randomization). 48 subjects experienced an adverse or serious adverse event including bruising, infections, and wound dehiscence. It has been determined that none of these reported events are related to device use. Preliminary findings indicate a marked reduction in hemostasis time with the HEG compared to the CEC.
Conclusion: The trial demonstrates the feasibility of conducting a randomized controlled trial at a single center in a complex operative setting comparing two FDA approved surgical devices. The final analysis will show whether the HEG improves hemostasis in women undergoing mastectomy with reconstruction.

P2-12-29: A randomized clinical trial comparing ctDNA-directed therapy change with standard of care in patients with metastatic triple negative breast cancer (mTNBC)

Jessica Sharpe, Ben H. Park, Vandana G. Abramson

Background: Despite treatment advances in mTNBC, most patients presenting for first-line therapy will require cytotoxic chemotherapy (CT). Sacituzumab govitecan (SG) is an antibody-drug conjugate to the Trop-2 antigen that is expressed in the majority of TNBCs. SG is approved as second- or third-line therapy for patients with mTNBC per the phase III ASCENT trial, but its utility as a first-line treatment is unknown. ctDNA correlates with cancer burden and response to local and systemic therapies, but there is not an approved use for quantitatively monitoring patients' ctDNA levels during treatment. Previous studies showed a direct correlation between a patient's ctDNA mean variant allele frequency (VAF) and tumor burden as well as response or resistance to treatment. We hypothesize that a lack of a significant decrease in ctDNA VAFs shortly after initiating a first-line CT for mTNBC could serve as a biomarker of de novo resistance and could warrant a change of therapy earlier than conventional imaging studies would normally dictate. This study therefore evaluates switching to SG in mTNBC patients without a 50% decrease in ctDNA VAFs less than one month after starting a first-line CT with the goal of improving progression-free survival (PFS) and overall survival (OS) and of decreasing toxicity by stopping an ineffective treatment earlier.

Methods: In this two-arm randomized clinical trial, 120 patients will receive standard of care (SOC) physician's choice CT. Patients will be randomized 1:1; one group will undergo SOC treatment with conventional staging imaging to guide treatment decisions, while the other group will undergo ctDNA evaluation on cycle (C) 1 days (D) 1 and 15 of treatment. If the mean ctDNA VAF on-treatment does not decrease by 50% on C1D15, patients will change SOC CT to SG. Both groups will undergo radiological assessment every 9 weeks.

ctDNA will be banked on C4D1, C7D1, and at the end of treatment. Patients with a biopsy-confirmed new diagnosis of mTNBC that is PD-L1 negative (CPS <10) or who are otherwise not eligible for immune checkpoint inhibitor therapy are eligible for this study. Patients will not have received prior treatment for mTNBC and must have an ECOG PS of 0-2 as well as measurable disease by RECIST. The primary endpoint is PFS. This study has 80% power to detect a 2-month difference in PFS between patients treated with a ctDNA-guided therapeutic approach compared to patients assessed by conventional imaging alone (one-sided type 1 error of 10%). Key secondary endpoints include evaluating the overall response rate (RECIST v1.1), PFS2, and OS. Correlative studies include assessing ctDNA-defined clonal mutation profiles and their predictive value and correlating changes in ctDNA with standard imaging. This study (NCT05770531) is open at Vanderbilt University Medical Center and will open at 7 other sites through the ACCRU network. Six patients have been enrolled as of 6/2024.

P2-12-30: A randomized phase III clinical trial of acupuncture for chemotherapy-induced peripheral neuropathy treatment (ACT)

Ting Bao, Mingxiao Yang, Ivana Lopez-Nieves, Anna M. Tanasijevic, Lauren Piulson, Mehul Shrivastava, Matthew Weitzman, Iris Zhi, Jun J. Mao

Background: Chemotherapy-induced peripheral neuropathy (CIPN) is a common and potentially dose-limiting side effect of neurotoxic chemotherapy among people who experience cancer. CIPN presents as pain, numbness, tingling, and motor weakness, which worsens quality of life and can disrupt active treatment regimens. Currently, duloxetine is the only ASCO-recommended painful CIPN treatment after chemotherapy completion. However, due to its undesirable side effects, such as fatigue, sleep disturbance, and digestive symptoms, there is a pressing need for more well-tolerated, evidence-based interventions. Based on our pilot study that demonstrated the feasibility and preliminary efficacy of acupuncture in relieving CIPN pain, we developed the ACT trial to evaluate the effect of acupuncture on improving pain and other related symptoms among cancer survivors with CIPN pain.
Methods: ACT is a prospective phase III multicenter, parallel two-arm randomized clinical trial at Dana-Farber Cancer Institute (DFCI) and Memorial Sloan Kettering Cancer Center (MSK) (ClinicalTrials.gov Identifier: NCT04917796). ACT aims to determine the efficacy of an eight-week electroacupuncture (EA) treatment vs. sham acupuncture (SA) on improving CIPN symptoms severity in cancer survivors. We plan to enroll 250 participants to detect an effect size of at least 0.45 for the primary pain outcome at 12 weeks post-randomization between EA vs. SA, with 80% power and a 1% Type I error rate, assuming a 10% loss to follow-up. Major eligibility criteria include adults who 1) have no evidence of disease or stable diseases, 2) have completed neurotoxic chemotherapy such as platinum agents, taxanes, vinca alkaloids, and bortezomib at least three months before enrollment, and 3) have a clinical diagnosis of CIPN with moderate to severe pain, defined as a score of at least four on the Brief Pain Inventory (BPI) average pain item. Participants will be randomly

allocated to receive ten real or sham acupuncture treatments over eight weeks at two centers and their regional clinics. Aside from patient-reported outcome measures (i.e., BPI, Functional Assessment of Cancer Therapy/Gynecologic Oncology Group-Neurotoxicity, EORTC Quality of Life Questionnaire-CIPN 20), we conduct quantitative sensory testing to assess changes in sensory function. The primary study endpoint is week 12, and the secondary study endpoint is week 24.
Progress: 1) We transitioned this trial to a multicenter trial in June 2024, with DFCI as the coordinating center and MSK as a subsite; 2) The DFCI site started patient enrollment in June 2024; 3) The DSMB last reviewed the trial in December 2023 and suggested that the trial continue as planned at MSK; 4) As of July 9, 2024, a total of 149 of the planned 250 participants have been enrolled. Accrual completion is expected by December 2024.

P3-01-01: Innovative Approaches to Breast Cancer Awareness: The Impact of Virtual Navigation and Future Digital Solutions

Manisha Salinas

Background: Breast cancer remains a significant public health challenge, with persistent disparities in screening and outcomes among Black women. Black women are more likely to be diagnosed at later stages and have higher mortality rates compared to White women. These disparities are often due to barriers such as limited access to healthcare, lack of awareness, and socioeconomic factors. Innovative digital solutions are critical for bridging these gaps and enhancing breast cancer awareness, screening behaviors, and survivorship in underserved communities.
Objective: This study aims to evaluate the impact of virtual health navigation on breast cancer awareness and to introduce a forthcoming mobile application designed to support breast cancer survivorship using AI and digital health tools.
Methods: We employed a mixed-methods approach, utilizing a virtual platform to deliver educational sessions on breast cancer risk factors, mammography, and preventive behaviors. Participants' knowledge, attitudes, and screening intentions were assessed through pre- and post-intervention surveys. Qualitative insights were gathered from focus group discussions to understand participants' experiences and perceptions.
Results: The virtual navigation program significantly increased participants' knowledge and awareness regarding breast cancer. Baseline surveys indicated that only 25% of participants had undergone mammography screening in the past year. Post-intervention surveys revealed that 75% of participants intended to undergo mammography screening within the next year, demonstrating a threefold increase in screening intentions. Additionally, 94% of participants found the virtual sessions beneficial, and 88% reported high satisfaction with the platform. Qualitative findings highlighted enhanced engagement and understanding of breast cancer risks, with 77% of participants expressing a desire to seek further information on breast cancer prevention and early detection post-intervention. These results emphasize the program's success in improving education and prevention behaviors, setting the stage for enhanced survivorship support.

Future Directions: Building on these successful outcomes, we are developing a mobile application designed to extend support into the survivorship phase for minority women. This app will leverage AI and digital health tools to provide personalized health information, follow-up screening reminders, and virtual support groups. By offering continuous support, the app aims to help survivors maintain their health post-treatment and address ongoing disparities in breast cancer care.
Conclusion: The integration of virtual navigation and digital health tools represents a transformative approach to enhancing breast cancer awareness, screening behaviors, and survivorship among Black women. This research demonstrates the significant potential of innovative digital solutions in addressing healthcare disparities. By developing a mobile application focused on breast cancer survivorship, we are positioned to provide continuous, personalized support that can significantly reduce disparities and improve health outcomes. The implications of this work highlight the critical role of targeted digital health interventions in fostering a more equitable healthcare landscape, ultimately leading to better outcomes for underserved populations.

P3-01-02: Impact of race, socioeconomic status and clinicopathological features on clinical outcomes in triple negative breast cancer in the ECOG-ACRIN EA1131 trial

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Background: Racial disparities in breast cancer outcomes persist despite a decline in overall breast cancer mortality. Black women have a higher incidence of triple negative breast cancer (TNBC), worse social determinants of health (SDOH), and quality of cancer care, which contributes to racial survival disparities. The EA1131 study evaluated patients with clinical stage II-III TNBC with residual disease after completion of neoadjuvant chemotherapy to determine whether invasive disease-free survival (IDFS) would be improved with adjuvant platinum compared with capecitabine. Herein we report a post hoc analysis of EA1131 to evaluate whether racial disparities were observed among patients with residual TNBC.
Methods: Our study population included Black and White patients in EA1131. Race was self-reported; participants who identified as part of a racial group with <15 patients or unknown race were excluded from this analysis (n=39). Our primary objective was to evaluate clinicopathological features, basal-subtype, SDOH, and survival outcomes by race in EA1131. The Agency for Healthcare Research Quality (AHRQ) neighborhood socioeconomic index (nSES) was calculated using residential zip codes linked to county level data on occupation, income, poverty, wealth, education, and crowding. Binary and

categorical data were analyzed with Fisher's exact test to evaluate differences in baseline characteristics by race. Cox regression analysis was used to estimate hazard ratios for DFS and overall survival (OS), adjusting for treatment arm, intrinsic subtype (basal vs non-basal), tumor grade, clinical stage prior to neoadjuvant treatment, pathologic stage after neoadjuvant treatment at the time of surgery, BMI, nSES index, and insurance type.
Results: Of 415 patients enrolled in EA1131, 376 were included in this analysis (308 White [82%] and 68 Black [18%]). Most patients had basal-subtype TNBC (77%), grade 3 disease (71%), pathologic stage II disease (49%), private insurance (70%), and a BMI ≥30 (49%). There were no racial differences in grade, clinical or pathological stage. Black patients were more likely to have basal-subtype TNBC (89% vs 75%; p=0.009) and a BMI ≥30 (62% vs 46%; p=0.026) compared to White patients. Black patients were more likely to be in the lowest nSES index quartile (39% vs 22%; p=0.008) and have Medicaid (32% vs 13%; p<0.001), while White patients were significantly more likely to have private insurance (73% vs 57%; p=0.013). The median DFS for White patients was 42.6 months compared to 25.1 months for Black patients, but this numerical difference was not statistically significant (p=0.24). There were also no significant differences in DFS or OS by race (HR 1.03, 95% CI: 0.65-1.61 and HR 0.67, 95% CI: 0.36-1.22, respectively), after adjusting for potential confounders.
Conclusion: Black patients with residual TNBC enrolled on EA1131 were more likely to have more aggressive basal-subtype tumors, to be obese, and to have lower socioeconomic status. Despite these factors, no significant differences in survival by race were observed. While this analysis was limited due to sample size, one possibility is that enrollment in a clinical trial, by minimizing differences in treatment received, has the potential to mitigate racial inequities in care. Larger studies are necessary to confirm these findings.

P3-01-03: The impact of race on outcomes of immune checkpoint inhibitor in triple-negative breast cancer patients: NCDB data analysis

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Background: Immune checkpoint inhibitor (ICI) in combination with chemotherapy has become the standard of care for early-stage and first-line metastatic triple-negative breast cancer (TNBC) patients. TNBC disproportionately affected African Americans and Hispanic patients. Previous studies prior to ICI approval in TNBC demonstrated racial disparity with worse outcomes in these groups of patients. However, the impact of race on the outcomes of TNBC patients treated with ICI remains unknown. In this study, we analyzed the National Cancer Database (NCDB) to evaluate the clinical characteristics and outcomes of TNBC patients from different racial groups.
Methods: The NCDB patient database from 2004-2017 was used. Continuous variables were summarized using sample mean and standard deviation, and categorical and discrete variables were summarized using count and percentage of patients. Independent samples t-test or ANOVA were used to compare continuous variables, and categorical and discrete variables were compared using a chi-square test. Patient survival by race was calculated

using the Kaplan-Meier method. Associations of race with death were evaluated using unadjusted Cox regression models with death as the outcome. Hazard ratios (HRs) were estimated. All statistical tests were two-sided. Statistical analyses were performed using R Statistical Software.
Results: A total of 2015 patients were included, consisting of 71.4% White, 19.7% African American, and 8.9% Hispanic. The African American and Hispanic cohorts had more patients < 60 years old than the White cohort, representing 63.9%, 66.1%, and 56.8%, respectively ($p = 0.005$). African Americans and Hispanics also had more patients with Charlson-Deyo scores above 0 compared to Whites, representing 17.2%, 16.1%, and 10.6% of their respective cohorts ($p = 0.003$). African Americans and Hispanics tended to receive treatment at academic centers compared to Whites, representing 47.0%, 55.2%, and 34.0%, respectively ($p < 0.001$). Additionally, a greater proportion of African Americans and Hispanics received treatment in metro areas compared to White patients, representing 93.3%, 96.0%, and 85.5% of their respective cohorts ($p < 0.001$). There was no significant difference in insurance coverage between races ($p = 0.333$). In contrast, the different race cohorts presented similar distributions of clinical features, with no significant differences in tumor grade ($p = 0.276$), tumor size ($p = 0.285$), lymph node status ($p = 0.422$), lymphovascular invasion status ($p = 0.088$), clinical and pathologic staging ($p = 0.096$), and whether they received chemotherapy ($p = 0.230$), radiation therapy ($p = 0.587$), and surgery ($p = 0.242$). For stage II-III TNBC, there were 538 White, 142 African American, and 62 Hispanic patients who received at least 1 dose of ICI. Using Kaplan-Meier analysis, African American patients ($n = 142$) had significantly worse overall survival (OS) compared to whites (HR = 1.362, $p = 0.012$). For patients with Stage IV TNBC receiving ICI, there were 108 Whites, 30 African American, and 12 Hispanic patients. There was a trend toward worse outcomes in African American patients when compared to whites (HR = 1.950, $p = 0.210$). There was no significant difference in patient survival when comparing Hispanic/other patients with whites in any stage of TNBC while receiving ICI (HR = 0.956, $p = 0.777$ for Stage II and III; HR = 2.089, $p = 0.200$ for Stage IV).
Conclusion: Despite similar clinical characteristics, African-American TNBC patients receiving ICI had significantly worse outcomes compared to Whites. Future studies are needed to further validate these results and elucidate the biological basis for the racial disparity observed with ICI treatment in TNBC patients.

P3-01-04: Racial and ethnic disparities in the development of second primary solid tumor malignancies in breast cancer survivors

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Background: Cancer is the second most common cause of death in the United States and breast cancer (BC) is projected to be the most common cancer diagnosis in 2024. Further, BC, lung/bronchus, colorectal and pancreatic cancers are projected to contribute to nearly 50% of all cancer deaths. Prior SEER analysis of early-stage BC survivors demonstrated a

statistically significant increase in the development of lung, tongue, salivary gland, gastric, colon and genitourinary cancers compared to the general population. However, very little is known about the racial differences in the development of second primary malignancies (SPM) in BC. Hence, we investigated the development of solid tumor SPM in BC survivors across different racial and ethnic groups.
Methods: We conducted a retrospective analysis of the Surveillance, Epidemiology and End Results (SEER) 17 registries database and identified the cases diagnosed with BC as their primary malignancy between the years 2000 to 2021. These cases were followed for several years, and the standardized incidence ratios (SIR) for the development of SPMs in BC were calculated. The analysis was stratified by the following races and ethnicities: Non-Hispanic Whites (NHW), Non-Hispanic Blacks (NHB), Non-Hispanic American Indian/Non-Hispanic Alaska Native (NHAI/NHAN), Non-Hispanic Asian or Pacific Islander (NHAPI), Hispanic (H).
Results: A total of 703991 NHW, 105014 NHB, 4712 NHAI/NHAN, 90295 NHAPI and 118500 H were included in the SPM analysis. The risk of developing solid tumor SPMs after BC was increased in all groups with SIR ranging from 1.14 in NHW to 1.83 in NHAI/NHAN ($p<0.05$). Within this, all groups had a statistically significant increased risk for developing second primary BC and endocrine malignancies, particularly thyroid cancer. The mean age for developing second primary BC ranges from 61.2 years (y) in Hispanics to 67.99 y in NHW. The total SIR for second primary BC was highest in NHBs (NHW – 1.31, NHB 2.07, NHAI/NHAN – 1.84, NHAPI 1.85, H – 1.58, $p<0.05$), which peaks (SIR: 2.61, $p<0.05$) at a latency of 10+ years after initial BC diagnosis. NHAPI were at the highest risk for development of thyroid cancer (SIR 2.22), followed by Hispanics (SIR 1.96), NHAI/NHAN (SIR 1.91), NHB (SIR 1.63) and NHW (1.4) ($p<0.05$). Further, there was a statistically significant increase in the risk for developing kidney and pancreatic cancers in various groups. The total SIR for kidney cancer was highest in NHAI/NHAN at 2.57, followed NHAPI at 1.55, NHB at 1.44, H at 1.4 and NHW at 1.1 ($p<0.05$)). Pancreatic cancer was relatively more common in BC survivors among NHAI/NHAN (SIR 1.76) and NHAPI (SIR 1.49) ($p<0.05$). NHAI/NHAN BC survivors seem to be particularly prone to developing SPMs with elevated SIRs such as – other oral cavity and pharynx (40.3), larynx (10.97), melanoma of skin (12.54), skin excluding basal and squamous (9.18), anus, anal canal and anorectum (5.48), cecum (2.86), colon, rectum and anus (1.79) and lung/bronchus (1.78) (all $p<0.05$). Interestingly, Hispanic BC survivors were more prone to stomach cancer with SIR 2.23 ($p<0.05$). There was also a higher incidence of salivary gland and soft tissues malignancies including the heart in all groups except NHAI/NHAN ($p<0.05$).
Conclusion: There is a statistically significant increase in the development of solid tumor SPMs across all races and ethnicities, with a disproportionate increase in the NHAI/NHAN (head and neck, skin and GI cancers), NHAPI (thyroid, BC, kidney and pancreas) and NHB (BC) emphasizing the importance of survivorship care that is accessible to all races and ethnicities. Further, there is a need to tailor the survivorship practices to each race and ethnicity depending on their specific risk for certain malignancies.

P3-01-05: The Effect of Individual and Neighborhood-level Socioeconomic Disparities in Advanced and Early-Onset Female Breast Cancer

Anumita Chakraborty, Lauren Correia, Jill Hasler, Gaetano Romano, Antonio Giordano, Shannon Lynch, Aruna Padmanabhan

Background: Race is a significant factor in the diagnosis and outcomes of breast cancer (BC); Black women are diagnosed with more aggressive disease, and have higher mortality from BC compared to White women. Race may be a surrogate for other social determinants of health (SDOH), which may explain observed disparities. Further study is needed to assess the effect of all 5 domains of SDOH (education access and quality, health care access and quality, neighborhood and built environment, social and community context, and economic stability) on BC. In the absence of individual-level information, neighborhood-level socioeconomic status (nSES) can be useful in determining patterns of disparity. This study aims to determine associations between early-onset BC and advanced-stage BC at diagnosis across all 5 domains of SDOH using both individual and nSES measures.

Methods: A retrospective analysis of female patients who presented to Fox Chase Cancer Center and Temple University Hospital between January 1st, 2018-December 31st, 2022, was conducted. Patient demographics (age, race, insurance, address, family history of cancer) and clinical characteristics (stage, receptor phenotype) were obtained. Patient addresses were used to geocode patients to a US Census Tract corresponding to known nSES variables obtained from the US Census Bureau, the Centers for Disease Control and Prevention, and the SEER Database. Primary outcomes included early-onset BC (defined as diagnosis <45 years) and advanced disease (AD; defined as stages 3 and 4 at diagnosis). Data was analyzed via univariate logistic regression models to compare patient, clinical, and neighborhood characteristics. Multivariate logistic regression models controlling for age at diagnosis, year of diagnosis, insurance, BC phenotype, and family history of cancer were used to assess associations between individual variables or nSES and the outcomes. Lasso regression controlling for race, insurance, and family history of BC was used to assess for significant associations between nSES variables and outcomes.

Results: 5172 female patients were diagnosed with BC, of which 1248 (24.1%) patients were Non-Hispanic Black, 3023 (58.4%) Non-Hispanic White, 151 (2.9%) Asian/Pacific Islander, 419 (8.1%) Hispanic and 331 (6.4%) Other/Unknown. Mean age at diagnosis was 60.1 years. 374 (7.2%) patients were diagnosed with ER-/PR-/HER2- breast cancer (TNBC). 641 (12.4%) patients were diagnosed with early-onset BC, and 744 (14.4%) patients had AD at diagnosis. On multivariate analysis, patients with Medicaid and Medicare had higher odds of being diagnosed with AD compared to those with private insurance (OR 2.116 [1.494-2.998], p<0.001; and OR 1.383 [1.013-1.888], p=0.041, respectively). Hispanic patients had lower odds of AD compared to non-Hispanic White patients (OR 0.544 [0.322-0.917], p=0.022). Patients with TNBC and ER-/PR-/HER2+ disease had increased odds of AD at diagnosis (OR 3.391 [2.543-4.521], p<0.001; and OR 1.382 [1.019-1.874], p=0.037, respectively). Patients with TNBC and ER-/PR-/HER2+ disease also had increased odds of

early-onset disease (OR 1.756 [1.256-2.455], p=0.001; and OR 1.427 [1.036-1.966], p=0.029, respectively). Lasso regression indicated the nSES variables of Technical/Professional/Managerial Employment, Household Income, and Index of Concentration at the Extremes (ICE; high-income White vs. low-income Black) was associated with advanced vs. localized disease at diagnosis.

Conclusion: Patients with Medicare and Medicaid have higher risk of AD at diagnosis compared to patients with Private insurance. Patients with TNBC and ER-/PR-/HER2+ BC have higher risk of both AD at diagnosis and early onset disease. Lasso regression indicates that rate of Technical/Managerial/Professional Employment, Household Income, and ICE (high-income White vs. low-income Black) within a patient's census tract is associated with advanced vs. localized disease.

P3-01-06: Differences in health-related quality of life among breast cancer survivors by Hispanic origins

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Background: Breast cancer is the most frequently diagnosed cancer and the second-leading cause of cancer-related death in American women. With prolonged survival, health-related quality of life (HRQOL) has become the primary concern of the survivors, and Hispanic women are more vulnerable than other race/ethnic groups. Despite many interventions available for the Hispanic population, they still report worse HRQOL than other groups, which warrants investigation of subpopulations that have their own culture, lifestyle, and health beliefs, which challenges healthcare providers in helping them improve their HRQOL. Therefore, this study assessed intra-disparities in HRQOL by their Hispanic origin.
Methods: The Florida Statewide Cancer Registry, a trusted source of cancer data, facilitated the patient recruitment process for this population-based epidemiologic study. Following state-mandated recruitment procedures, up to two invitation letters and four phone calls were made to gauge potential participants' interest. Surveys were then sent to interested participants according to their preferred mode (mail or online) and language (English or Spanish). The Functional Assessment of Cancer Therapy – Breast (FACT-B) was utilized to assess five domains of HRQOL: physical, social, emotional, and functional well-being, as well as breast cancer symptoms. Total and domain scores were calculated. Higher scores indicate better HRQOL. Scores were compared across Hispanic origins using ANOVA tests.
Results: A total of 365 women completed surveys; participants included 33 Colombians, 24 Cubans, 22 Dominicans, 20 Mexicans, 198 Puerto Ricans, and 68 in the other category. The mean FACT-B total score was 104.0 (SD 24.7), with a significant difference among Hispanic subgroups ($P < 0.001$), the highest score from Cubans (mean 117.6, SD 17.2) and the lowest score from Dominicans (mean 93.7, SD 24.2). As the total score was highly correlated with all five domain scores (r ranging between 0.59 – 0.85, all $P < 0.0001$), similar results were observed from ANOVA tests for five domains. In addition to Hispanic origin, factors such as

income, education level, marital status, smoking status, alcohol consumption, laterality, cancer stage, treatment type, and surgery type were all associated with the total and domain scores.
Conclusions: The study has shown intra-disparities in HRQOL among Hispanic breast cancer survivors. These disparities, which could be explained by differences in sociodemographic and clinical characteristics of Hispanic subgroups, present a significant challenge. However, the insights gained from this study can be used to develop culturally tailored intervention programs that have the potential to significantly improve the HRQOL of Hispanic breast cancer survivors.

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P3-01-07: Breast Cancer Survival According to Health Insurance Coverage in Brazil: Results from the Prospective AMAZONA III Study (GBECAM 0115)

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Background: Breast cancer (BC) is the most commonly cancer diagnosed in women in Brazil. Prior analysis of AMAZONA III study showed that Brazilian patients with public health coverage were diagnosed with symptomatic disease, later stages, and more aggressive BC subtypes when compared to privately insured patients. The aim of this study is to evaluate the impact of BC subtypes and type of health insurance in clinical outcomes of stage I-III BC from a large, prospective, cohort study in Brazil.
Methods: AMAZONA III study (GBECAM 0115) is a prospective cohort study that included patients newly diagnosed with invasive BC during the period of January 2016 to March 2018 within 22 sites in Brazil, including patients covered by public and private health systems. Here we present survival data from patients with stages I to III BC. Data were collected from medical visits and information from type of health insurance, public or private, were extracted from medical charts. BC subtypes were classified according to estrogen receptor (ER) status, progesterone receptor (PgR) status, and HER-2 status analyzed in local laboratory as follows: Luminal (ER and/or PgR positive and HER2 negative), HER2 positive (any ER/PgR and HER2 positive) and Triple negative (ER and PgR negative, HER2 negative). The 5-year disease-free survival (DFS) and overall survival (OS) rates were assessed by Kaplan-Meier analysis and hazard ratios with 95% CI from Cox regression. Trial Registration: NCT02663973.

Results: A total of 2,839 patients were included in this analysis, of whom 1771 (62.4%) had public insurance, 1569 (55.3%) were postmenopausal, 1413 (59.4%) had luminal tumors, 613 (25.8%) had HER2-positive (HER2+) and 352 (14.8%) triple-negative (TN) BC. After a median follow-up of 60.6 (60.4-60-8) months, there were 521 (15.1%) DFS events and 353 (12.4%) OS events. The 5-year DFS (5yDFS) was 91% in the private and 75% in the public system (HR 3.1, [95% CI, 2.4-3.99], p<0.0001). DFS was similar for stage I patients in the public and private systems (p=0.276). For stages II and III BC, 5yDFS was higher in the private when compared to the public system (89.4% vs. 79.6%, respectively, for stage II, p=0.0039; and 80.8% vs. 55.6%, respectively, for stage III, p<0.0001). The 5-year OS (5yOS) according to insurance coverage were 94.4% for private and 80.3% for public coverage (HR 3.91, [95% CI, 2.82-5.42], p<0.0001). Five-year OS (5yOS) was 98.2% for stage I, 88.2% for stage II, and 68% for stage III BC (p<0.0001). According to BC subtype, 5yOS was 90.2% for luminal, 83% for HER2+, and 72.7% for TN BC (p<0.0001). For stage I, 5yOS was 99% for both luminal and HER2+ BC and 89.8% for TN BC (p=0.005). There were no differences in OS according to insurance coverage in stage I, 99.1% in private vs. 97.2% in public system (p=0.27). For stage II, 5yOS was 92.1% for luminal, 86.7% for HER2+ BC, and 77.2% for TN BC (p<0.0001). There were no differences in OS according to insurance coverage in stage II BC. For stage III, 5yOS was 73.3%% for luminal, 68.4% for HER2+ BC, and 57.5% for TN BC (p=0.0028). 5yOS was worst for stage III BC treated in the public system when compared with the private system (63.2% vs. 88.9%, respectively; HR 4.21 [95% CI, 2.29-7.75], p<0.0001).
Conclusion: The AMAZONA III study demonstrated that patients from the public health system have worse clinical outcomes in Brazil. When stratified by BC stage similar DFS were noted in stage I disease but patients with stage II and III had better outcome in private health system. Patients with stage III BC from the public health system have worse OS when compared to privately insured patients. Overall, BC subtypes demonstrated significant impact on DFS and OS outcomes regardless of the insurance coverage. This study demonstrates inequities in early BC clinical outcomes in Brazil that may be influenced by timely diagnosis and treatment, different locoregional and systemic therapies, limited access to novel therapies, among others, which must be further investigated.

P3-01-08: The prevalence of biomarker alterations, treatment patterns, and survival in metastatic triple-negative breast cancer by race: A national cohort study

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Background: The prevalence of triple-negative breast cancer (TNBC) is higher in Black women than in White women. However, in the era of precision oncology, treatment decisions informed by diagnostics and outcomes by race in patients with metastatic TNBC (mTNBC) are not well understood. This study assessed the prevalence of biomarker alterations, treatment patterns, and clinical outcomes in Black and White patients with

mTNBC in the United States (US) who received next-generation sequencing (NGS)-based testing.
Methods: This observational cohort study utilized the nationwide, electronic health record (EHR)-derived, de-identified Flatiron Health–Foundation Medicine Clinico-Genomic Database (primarily community oncology settings). Adult patients with mTNBC diagnosed from January 2017 to March 2022 were included and followed until September 2022. Patients who lacked race data, participated in a clinical trial, or had <6 mo of follow-up were excluded. All patients had NGS-based tumor tissue testing and/or liquid biopsy. The current portion of the study included only patients with mTNBC and assessed BRCA, PIK3CA, AKT1, and PTEN alterations. Pathogenic variants, or those likely to be pathogenic, were used to identify alteration status. BRCA1/2 results were available with no distinction between somatic and germline mutations. Programmed cell death ligand 1 (PD-L1) expression was not included in the NGS panel test, but this information was extracted from linked EHR data if documented in the physician notes. PD-L1 expression was categorized as positive for a combined positive score (CPS) of ≥10. Descriptive statistics were used to summarize biomarker alterations and treatment patterns. The Kaplan–Meier method and a Cox regression analysis were used to assess overall survival (OS) from diagnosis of mTNBC.
Results: Of 670 patients with mTNBC, 185 (28%) were Black and 485 (72%) were White. Median time from mTNBC diagnosis to NGS testing was similar in both groups (2.1 vs 1.8 mo; p=0.09). Compared with White patients, Black patients had a higher prevalence of BRCA1/2 alterations (12% vs 7%; p<0.04), but there were no significant differences in the prevalence of PIK3CA (19% vs 22%; p=0.5), PTEN (15% vs 17%; p=0.4), or AKT1 (2% vs 5%; p=0.13) alterations; there were too few patients (~15%) with PD-L1 CPS test results available in the database to discern differences. A total of 530 patients received first-line (1L) therapy: 81% of Black patients and 79% of White patients. Among patients who received 1L treatment, there was no significant difference between Black and White patients for the use of programmed cell death protein 1/PD-L1 inhibitor–based regimens (19% vs 27%; p=0.08) or chemotherapy (70% vs 63%; p=0.09). Median OS from mTNBC diagnosis was numerically, but not significantly, shorter in Black than White patients, respectively (13.7 vs 18.9 mo; p<0.15). After adjusting for baseline characteristics, treatments, and biomarkers, a nonsignificant trend toward a shorter OS remained in Black patients compared with White patients (HR 1.19; 95%: CI: 0.94–1.51; p=0.14).
Conclusions: In this US study involving patients with mTNBC who received NGS testing, Black patients were almost twice as likely to have somatic/germline BRCA1/2 alterations and, therefore, may benefit from poly (ADP-ribose) polymerase inhibition. Overall, although there was a trend toward worse survival outcomes for Black patients compared with White patients, significant differences were not observed. Future studies, with larger sample sizes, more complete and comprehensive testing results, and assessment of potential testing disparities, are needed to better understand ongoing disparities in treatments and outcomes in mTNBC by race.

P3-01-09: Feasibility of providing rural patients with telehealth consultation coupled with in-person intervention at tertiary oncology center

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Background: Progress in precision oncology diagnostics and treatment is proceeding at a rapid pace, but patients in geographically remote areas are being left behind, owing to lack of resources and infrastructure. Work performed by our group has demonstrated that nearly half of patients with presumed metastatic breast cancer (MBC) in Washington do not undergo biopsy at cancer recurrence, contrary to standard guidelines. One barrier to patients being offered this procedure is a lack of availability of high-quality bone biopsy for patients with bone-dominant disease in rural areas, owing to the technical demands of the procedure and subsequent pathologic analysis. Fred Hutch/UW Comprehensive Cancer Center (FHCC) serves all of Washington as its formal catchment area, with nearly 800,000 patients living in non-metro areas, and also the only comprehensive cancer center in a five-state region known as WWAMI (Washington, Wyoming, Alaska, Montana and Idaho).
Methods: In 2021-2023, we conducted a pilot feasibility study to bring patients to FHCC for high quality bone biopsy with genomic sequencing, the results of which could make them eligible for life-extending targeted therapy. We reached out to community oncology providers across the five state WWAMI region, describing our study and offering bone biopsy plus liquid biopsy to eligible patients. The PI obtained licensing in the relevant states to allow telehealth visits across state lines. Community providers referred patients, who met via telehealth with the PI for a comprehensive second opinion including review for study eligibility. A research coordinator then helped the patient arrange travel and accommodations for each patient and a caregiver. The patient was sent a blood draw kit to present to their local phlebotomy site for liquid biopsy and shipping to FCC molecular pathology lab. The primary aim of this study was to demonstrate logistical feasibility along with patient willingness to come for the procedure and send a blood sample drawn to our molecular pathology lab.
Results: We provided telehealth second-opinion consultations for 10 metastatic breast cancer (MBC) patients in remote sites (Washington, Alaska, Idaho and Montana), and subsequently referred them for high-quality bone biopsy with molecular evaluation at FHCC. Results from these samples are currently under analysis, and will be reported in a separate publication, combined with data from local Seattle patients enrolled in a similar study comparing bone to liquid biopsy. Average distance traveled was 390 miles, range 61 to 890, median 315. Patients spent an average of two nights in Seattle, and every patient was accompanied by a caregiver. Average reimbursement for travel, meals and accommodation was $1500. Accrual was limited by COVID-19 travel restrictions, so that patients enrolled in study and able to travel were lower than anticipated when the study was designed.
Discussion: This pilot trial demonstrated that patients were interested in traveling to a tertiary urban center for state-of-the-art interventions, and that these could be performed

with manageable cost, and a potentially scalable infrastructure. The fact that COVID-19 licensing restrictions were waived for telehealth visits between the relevant states during the period of the study obviated the need for PI licensing, but also emphasized the benefit that could be offered to patients were those regulations changed permanently to allow telehealth opinions to occur. Given that FHCC is surrounded by four states without a tertiary referral center, it is worth considering how changes in licensing regulations could lower barriers to enrollment in clinical trials for these rural populations. Expanded studies are required to explore the potential for this model in screening for clinical trials in combination with offering interventions not available at rural sites.

P3-01-10: Racial and ethnic disparities in the development of hematological second primary malignancies in breast cancer survivors

Kriti Ahuja, Malak Alharbi, Jayasree Krishnan, Zunairah Shah, Shipra Gandhi, Arya Mariam Roy

Background: Breast cancer continues to be one of the most commonly diagnosed malignancies. With improved survival in early-stage breast cancer due to significant advancement over the decades, survivorship care and monitoring for second primary malignancies (SPMs) have become increasingly important. A recent study has demonstrated significant increase in risk for developing SPMs including leukemia in stage I and II breast cancer survivors at a latency of 2 years. It is unclear whether the development of these SPMs is also due to shared abnormalities in underlying molecular pathways or is solely related to treatment. Therefore, we are investigating the development of second primary hematological malignancies in breast cancer survivors, stratifying them based on the race and receipt of chemotherapy.
Methods: We conducted a retrospective analysis of the Surveillance, Epidemiology and End Results (SEER) 17 registries database and identified the cases diagnosed with breast cancer as their primary malignancy between the years 2000 to 2021. These cases were followed for several years, and the standardized incidence ratio (SIR) for development of SPMs in breast cancer were calculated. The cohort was divided into two groups based on the receipt of chemotherapy (CT vs no/unknown CT). The analysis was stratified by the following races and ethnicities: Non-Hispanic Whites (NHW), Non-Hispanic Blacks (NHB), Non-Hispanic American Indian/Non-Hispanic Alaska Native (NHAI/NHAN), Non-Hispanic Asian or Pacific Islander (NHAPI), Hispanic (H).
Results: A total of 703991 NHW, 105014 NHB, 4712 NHAI/NHAN, 90295 NHAPI and 118500 H were included in the SPM analysis. We observed a statistically significant increase in development of leukemia across all racial and ethnic groups that received CT, however, NHAI/NHAN and NHAPI were observed to have the highest risk at total SIR of 3.44 and 3.45 respectively ($p<0.05$) (NHW: 1.87, NHB: 2.27, H: 1.83 $p<0.05$). There was a statistically significant increase in development of acute myeloid leukemia (AML) in patients who received CT across all racial and ethnic groups except NHAI/NHAN. Although the history of CT is said to increase the risk of therapy related AML, the effect of CT was different across

different racial and ethnic groups, being the highest in NHAPI, followed by NHB with overall SIR of 5.16 and 4.27 respectively as compared to NHW at 3.61 and Hispanics at 3.56 ($p<0.05$). This was primarily due to elevated SIR ($p<0.05$) at a latency of 1-5 years as follows: NHAP: 8.85, NHB: 7.4, H: 7.37, NHW 6.36. Among AML, the risk was observed to be elevated for Acute monocytic leukemia and the SIR was observed to be highest in NHB at 18.86 (H: 13.04, NHAPI 11.79, NHW 8.13, $p<0.05$). Further, even among the patients with no/unknown CT status, there was a statistically significant increase in development of AML across all groups except NHAI/NHAN. This was highest in NHAPI at an overall SIR of 1.94 and NHB at 1.84. It was highest at a latency of 1-5 years with SIR in NHB at 2.57 and NHAPI 2.44, $p<0.05$ (H: 1.95, NHW: 1.39, $p<0.05$). NHAI/NHAN were found to have a statistically significant increase in Myeloma in the No/unknown CT group at overall SIR of 3.91, with 7.25 ($p<0.05$) at a latency of 10+ years.

Conclusion: Breast cancer survivors exhibit an increased susceptibility to certain hematological malignancies across diverse racial and ethnic backgrounds. While all patients receiving CT understandably face a heightened risk of developing AML, this risk is particularly pronounced among NHAPI and NHB populations, warranting further investigation. Furthermore, an elevated risk of AML persists in the no/unknown CT population, with NHAPI and NHB groups showing the highest susceptibility, indicating a potential genetic predisposition warranting further study.

P3-01-11: Racial Disparity in the Screening and Diagnosis of Breast Cancer in the Public Health System (SUS) in Brazil

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Background: The effectiveness of screening and early diagnosis of breast cancer (BC) can significantly vary among different population groups and racial disparity can be a critical factor that influences access and quality of health services, including BC screening and diagnosis.

Objectives: To evaluate the racial disparity in screening and diagnosis of breast cancer within the Unified Health System (SUS) between the years 2015 and 2022 in Brazil.

Methods: A cross-sectional ecological observational study was made utilizing public data from the Outpatient Information System, Hospital Information System, and Mortality Information System of the Department of Informatics of the Unified Health System, as well as data from the Hospital Cancer Registry (RHC) of the National Cancer Institute. For RHC data, the period from 2015 to 2021 was analyzed due to the lack of updated and consolidated data for 2022. Mammography coverage rate was evaluated in biennial periods: 2015/2016; 2017/2018; 2019/ 2020; and 2021/2022. The proportions of early diagnosis (stages 0, 1, and 2) and late diagnosis (stages 3 and 4) were calculated. The time between the specialist consultation and receiving the diagnosis and the time between receiving the

diagnosis and starting the treatment was evaluated. Records with incomplete, invalid or missing data were excluded from the analysis.
Results: Over 18 million screening mammograms were performed on women aged 50 to 69 in SUS and because of Covid-19 pandemic there was a significant reduction in the number of mammograms, with a 41.5% decrease in 2020 and an 18.9% decrease in 2021 compared to 2019. In 2022, there was a slight increase of just over 1% in the number of mammograms compared to 2019. Regarding race/ethnicity, 52.4% of screening mammograms were performed on white women, 28.5% on brown women, 5.8% on black women, 13.4% on yellow women, and only 0.1% on indigenous women. The impact of the Covid-19 pandemic on mammography coverage is evident: the country's coverage ranged from 26.3% in the 2015-2016 biennium, 25.1% in 2017-2018, to 18.4% between 2019-2020, and 20.5% in 2021-2022. Analyzing staging it was observed that approximately 38% of breast cancer cases were diagnosed in advanced stages of the disease and the proportion of stage 3 or 4 diagnoses for white women was 35.5%, brown and black women had proportions of 44.2% and 46.5%, respectively. From 2015 to 2021, early diagnosis in white women decreased from 65.2% (2015) to 59.7% (2021). In brown women, the percentage of early diagnosis was 54.8% in 2015, increasing to 58% in 2021. For black women, these percentages were 54.9% and 52.6% for 2015 and 2021, respectively. The average number of days between the specialist consultation and receiving the diagnosis was 36 days and white women had a shorter average time (37 days) compared to black and brown women (51 days). Additionally, 65.5% of white women were diagnosed within 30 days, while this proportion was lower for black women (58.9%) and brown women (61.1%). The proportion of black women who started treatment after 60 days (65.5%) was higher than that of white women (60.8%) and brown women (59.7%). Furthermore, the average number of days until treatment initiation for brown women was 167 days, while black and white women had averages of 169 and 201 days, respectively.
Conclusions: The incidence of breast cancer among the black population in Brazil reflects the complex interactions between biological, social, and economic factors. Addressing these disparities effectively requires a collective effort from public policies, health institutions, and civil society. Only through integrated and inclusive approaches will it be possible to ensure that all women, regardless of their race or ethnicity, have equal access to quality healthcare and thus improve the impact on breast cancer survival rates and quality of life.

P3-01-12: Enhancing Adherence to Hormone Therapy among Latina Breast Cancer Patients Impacted by Social Determinants of Health through a Bilingual, Culturally Tailored Mobile App and Patient Navigation

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Background: Hormone therapy (HT) is highly effective for nearly all breast cancer patients with hormone receptor-positive tumors, which account for approximately 80% of all breast cancer diagnoses. Long-term use of HT significantly reduces cancer recurrence rates and cuts the risk of mortality nearly in half during the second decade after diagnosis. Despite these proven benefits, approximately 33% of women prescribed HT do not adhere to their medication as recommended (<80%). Latina patients are disproportionately impacted by Social Determinants of Health (SDoH), which hinder their adherence to HT, placing them at a higher risk of breast cancer recurrence, lower quality of life, increased medical costs due to disease progression and treatment, and greater risk of cancer-related mortality.
Objective: The goal of this randomized controlled study is to assess the effectiveness of the existing bilingual, culturally tailored, and interactive HT Helper app, in combination with patient navigation (PN), on improving adherence to HT among Latina breast cancer patients experiencing SDoH barriers, i.e., income, health insurance, education, health literacy, and language, impacting their medication adherence.
Study Design: The proposed 4-year study involves a parallel 3-group randomized controlled trial with 5-time assessments (baseline, 3, 6, 12, and 18 months) and will enroll 159 Latina breast cancer patients who are prescribed HT and are attending the breast clinic at the Mays Cancer Center (MCC) at UT Health San Antonio. Group 1 will receive a) the HT Helper app; and b) patient navigation (PN). Group 2 will receive patient navigation only. Group 3 will receive the usual care and information provided by the MCC's breast clinic to patients undergoing oral HT. Intervention components are based on Social Cognitive Theory and elements of Motivational Interviewing.
Methods: We will compare the effectiveness of 3 groups on HT adherence: 1) the HT Helper app + PN group vs. the PN alone group vs. the usual care group, and will assess the effect of each study condition on patient self-efficacy to identify HT side effects, use self-care to manage side effects, and communicate with the medical team. We hypothesize that the HT Helper app + PN and the PN alone groups will have greater rates of HT adherence and higher patient self-efficacy than the usual care group, with the HT Helper app + PN achieving better results than both PN alone and the usual care groups.
Conclusion: This theory-based, multilevel intervention will increase patient education, enhance self-efficacy, facilitate communication with the medical team and coordination of resources to address SDoH barriers and help patients develop self-care skills and optimal adherence to HT, ensuring patients the most equitable treatment outcomes possible, including improvement in quality of life, survival, and life expectancy.

P3-01-13: Race and response to neoadjuvant chemotherapy for nonmetastatic breast cancer using the SEER database.

Alvaro Alvarez Soto, Wenqi Gan, Jesus Anampa, Susan Tannenbaum

Background: Pathological complete response (pCR) is a surrogate for improved overall survival (OS) and cancer-specific survival (CSS) in patients with nonmetastatic high-risk breast cancer (BC) who received neoadjuvant chemotherapy (NACT). Multiple cohort

studies have suggested racial disparities in the odds of achieving pCR between Non-Hispanic White (NHW) and Non-Hispanic Black (NHB) patients. Those studies were based on local databases or the National Cancer Database (NCDB), which is a hospital-based dataset. The goal of our study is to investigate racial disparities in the odds of achieving pCR along with disparities in CSS and OS between NHW and NHB women using SEER, a nationwide, population-based NCI database that represents around 48% of all the new cancer diagnosis in the US.

Methods: We identified female NHW and NHB patients aged ≥18 years old who were diagnosed with BC between 2010-2018 and who received NACT. We excluded patients with in-situ disease only, metastatic disease, neoadjuvant radiation use, patients who did not have surgery, and patients in which BC was not their first primary. SEER defined pCR as the absence of all primary tumor or residual in situ cancer only, and non-PCR was defined as patients who had no or partial response to neoadjuvant chemotherapy.

To estimate differences in the odds of achieving pCR between races, we used a logistic regression analysis adjusted for tumor biology, treatment-related, and demographic variables. Hazard ratios of breast cancer-specific mortality and all-cause mortality calculated using Cox regression models, stratified by race and pCR status. We created survival curves using Multivariate Cox models.

Results: We identified 8,355 NHW patients and 2,071 NHB patients diagnosed with stage I-III pathologically confirmed BC who received NACT between 2010 and 2018. There were 2,945 NHW and 668 NHB with hormone receptor positive (HR+)/HER2-, 2,085 NHW and 394 NHB with HR+/HER2-, 1,124 NHW and 245 NHB with HR-/HER2+, and 2,201 NHW and 764 NHB with TNBC.

Baseline characteristics were significantly different between groups. In terms of demographics, NHW women were older (age ≥55; 46.6% vs. 38.6%), more frequently married (63% vs. 37.7%), and had higher income (income ≥ 60,000 USD; 41.2% vs. 23.7%). In terms of tumor biology, NHW were less frequently TNBC (26.3% vs. 36.9%) and high-grade tumors (56.9% vs. 66.2%). A delay in diagnosis to chemotherapy ≥2 months was seen less frequently in NHW than in NHB (18.3% vs. 29.1%).

After adjusting for all covariates, we found no difference in the odds of achieving pCR between NHW and NHB women (OR=0.89, 95% CI 0.80 – 1.00). Subsequent stratified analysis for each clinical BC subtypes, showed no differences in the odds of achieving pCR between NHB and NHW for HR+/HER2-, HR-/HER2+, or TNBC, (OR=1.07, 95% CI 0.86 – 1.32), (OR=0.81, 95% CI 0.60 – 1.10), and (OR=0.85, 95% CI 0.71 – 1.01), respectively. For women with HR+/HER2+, NHB women had lower odds of achieving pCR as compared to NHW, (OR=0.78, 95% CI 0.63 – 0.98).

No differences in the risk of death from any cause was found in NHB patients who achieved pCR compared to NHW who achieved pCR (HR=1.10, 95%CI 0.85 – 1.42). However, NHB who did not achieve pCR had worse OS compared to NHW without achieved pCR (HR=1.32, 95%CI 1.17 – 1.49). Those results were more prominent in the HR+ groups. Similar results were seen for CSS.

Conclusions: We found no differences in the odds of achieving pCR between NHB and NHW patients with nonmetastatic BC. After stratifying by BC subtype, only NHB patients with

HR+/HER2+ tumors were less likely to achieve pCR. All-cause mortality was similar between NHW and NHB that achieved pCR. However, in patients with no pCR, NHB had a higher risk of death than NHW. Biologic or socio-economic factors might explain these disparities in patients with no pCR.

P3-01-14: DARC/ACKR1, Duffy-Null and African ancestry influence on immune response in triple negative breast cancer

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Breast cancer (BC) related mortality remains approximately 40% higher among African American women, where women of African ancestry are more frequently diagnosed with the more aggressive molecular subtype of BC, triple negative BC (TNBC). We have previously reported that increased west African ancestry is associated with TNBC diagnoses, and that the African ancestry-specific Duffy-null allele (Fy-) is a risk factor for TNBC diagnoses compared to other BC subtypes. Fy- is an ancestral allele that provides immunity from Plasmodium vivax malaria and is found at frequencies of >90% across Sub-Saharan African populations, and >60% among African Americans. Fy- removes erythrocytic expression of the Duffy Antigen Receptor for Chemokines gene, recently renamed Atypical Chemokine Receptor 1 (DARC/ACKR1). DARC/ACKR1 functions to modulate systemic chemokine levels, and via expression on endothelial cells influences immune cell diapedesis into tissues. In the context of BC, Fy- individuals retain DARC/ACKR1 expression on endothelial cells, and we have recently reported variable DARC/ACKR1 expression on breast tumor epithelial cells using bulk transcriptomics and immunohistochemistry-based methods. Our preliminary work conducted among women of African ancestry suggests that immune cell infiltration is correlated to DARC/ACKR1 epithelial expression. Currently, we aim to describe the combined impact of DARC/ACKR1 on immune response in both the tumor microenvironment and circulation dependent on DARC/ACKR1 expression status.
To determine Fy- impact on circulating inflammatory markers of BC patients, we have conducted Luminex multiplex assays to detect and quantify over 40 circulating chemokines and cytokines. Fy- status was determined by WGS of germline, or single-plex PCR genotyping methods. We previously reported univariate associations with CCL2, CXCL2 and CXCL11. In models adjusting for BMI and TNBC status, significant associations with CCL2 and CXCL11 are retained, where we also report significance in univariate and fully adjusted models with CCL1, CCL19, MIF and Resistin. Our ongoing work is evaluating levels associated with Fy- among African Americans only in univariate and adjusted models, and exploring how these profiles may change between molecular subtypes, including TNBC.
We have quantified the circulating and tumor immunological/inflammation profiles of (n=100+) TNBC patients using Luminex multiplex assays and tumor-specific transcriptomics. We have discovered associations with (1) Fy- status and chemokine levels in the tumors versus circulation, and (2) differential levels of chemokines in tumors or

circulation based on DARC/ACKR1 expression in the breast tumors. To better define these phenotypes spatially, we have utilized imaging mass cytometry to profile breast tumors with varying levels of DARC/ACKR1 expression on the tumor epithelial cells. DARC/ACKR1 positive tumors have increased levels of immune cells infiltrating, with increases in T cell, B cell and macrophage populations. This aligns with our previous reports using bulk transcriptomics data, specifically where we showed an African ancestry associated gene signature enriched in immune response pathways. We are currently working to quantify the spatial resolution of these phenotypes.
In conclusion, African ancestry women are more frequently diagnosed with TNBC with aggressive tumor phenotypes that are hard-to-treat. The mechanisms driving this aggressive tumor progression likely involve systemic inflammation. DARC/ACKR1 expression helps regulate systemic inflammatory profiles, which are altered among Fy- patients. We are working to define the impact of these DARC-regulated systemic profiles on the tumor microenvironment among TNBC patients, associated with Fy- DARC status. Specifically, DARC/ACKR1 driven immune profiles may provide new opportunities to for treatment to better serve this population of women.

P3-01-15: Exploring the Impact of Race, Ethnicity, and BMI on Neoadjuvant Hormonal Treatment Outcomes

Traci King, Priscila Barreto Coelho, Youley Tjendra, Judith Hurley, Sofia George

Introduction: Neoadjuvant hormonal therapy (NHT) is a potential treatment strategy for estrogen receptor-positive (ER+) breast cancer (BC) in postmenopausal women. Despite its clinical benefits, utilization of NHT varies significantly and anecdotal experience suggests that treatment responses may vary across different patient populations.

Methods: This is an IRB approved retrospective cohort study of 170 consecutive postmenopausal patients with ER+/HER-2 negative BC who received neoadjuvant hormonal therapy (NHT) at a single institution from 1/2005 to 6/2018. All subjects were treated with anastrozole 1 mg daily until maximal clinical response. In a subset of 96 patients with surgical pathology available for review by a single pathologist, quantitative ER and PR were measured by decile and Ki67 was categorized as low (0-15%), intermediate (16-30%) and high (>30%). BMI was classified as <18.5 (underweight), 18.5-24.9 (normal), 25-29.9 (overweight), and ≥30 (obese). Comparative analyses assessed the correlation between race, ethnicity, BMI, and clinical outcomes.

Results: The median age at diagnosis was 62 (range 46-91), and median size of the breast mass was 5.3 cm (range 1.5-19.0 cm). At start of treatment, 27% of patients were stage IIA, 29% IIB, 22% IIIA, and 18% IIIB. The majority of patients were White (77%) and Hispanic (71%). 23% of patients were Black. Regarding BMI, 37% were in the normal range, 38% were overweight, and 25% obese. Pathology review showed that the large majority of tumors had 91-100% ER positivity (66%) or 81-90% ER positivity (19%). PR positivity was

more variable with only 21% at 91-100% PR positivity and 35% of patients with <50% PR positivity on IHC. The largest amount of patients (48%) were Ki67 low, 17% were intermediate, and 34% were Ki67 high.
Higher BMI was associated with higher ER expression (p=0.007) and higher Ki67 (p=0.042). There was no association between ER/PR expression and race or ethnicity.
The median duration of NHT was 6 months, with treatment durations extending up to 28 months. Pathological complete response (PCR) was observed in 3 tumors. All patients with a PCR were stage IIB at diagnosis, White, Hispanic, and overweight. Progression of disease occurred in 10 patients of which 40% were Black, 60% were Hispanic, 30% were overweight, and 40% were obese. Although pCR can be used as a surrogate endpoint for response to neoadjuvant therapy, there is no standard method of determining a significant response to NHT. In our cohort, the decision to give adjuvant chemotherapy was individualized for each patient based on the perceived response to NHT. The patients given adjuvant chemotherapy were felt to be at higher risk of relapse than those who received adjuvant hormonal therapy alone. Overall, 61% of patients required adjuvant chemotherapy after NHT. White women were more likely to receive adjuvant chemotherapy than Black women 66% vs 45% (p=0.019). There was a significant correlation between higher Ki67% and the need for adjuvant chemotherapy (p=0.030), but not between degree of ER positivity, PR positivity, or BMI.

Conclusion: The use of NHT potentially prevented adjuvant chemotherapy use in 39% of postmenopausal women with ER+/HER-2 negative BC. There appear to be disparities in response to NHT based on race but not on BMI or ethnic group. Additional research is needed to investigate molecular underpinnings between race/ethnicity/BMI and response to NHT.

P3-01-16: Evaluation of the Combination of Vepdegestrant, a PROTAC Estrogen Receptor (ER) Degrader, Plus Palbociclib in CDK4/6 Inhibitor-Resistant WT ER and ER Y537S Mutant Patient-Derived Xenograft (PDX) Models

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Background: Vepdegestrant is an oral PROteolysis TArgeting Chimera (PROTAC) ER degrader that directly ubiquitinates and degrades wild-type ER and clinically relevant mutants. Vepdegestrant demonstrated superior ER degradation and antitumor activity compared with the selective ER degrader, fulvestrant, in endocrine-sensitive and resistant xenograft models and has shown substantial ER degradation and promising clinical benefit in heavily pretreated patients with ER+/HER2- breast cancer. Inhibitors of cyclin-dependent kinase 4 and 6 (CDK4/6) are now standard of care (SOC) in combination with endocrine therapy (ET) in patients with ER+/HER2- advanced breast cancer, demonstrating significantly increased progression-free (and with some combinations, prolonged overall

survival) compared with ET alone. Previously, our studies revealed evidence of synergistic interactions between vepdegestrant and CDK4/6 inhibitors (palbociclib, abemaciclib and ribociclib) in ER+ breast cancer cells.
Methods: Here, we evaluated the mechanism underlying the synergy of vepdegestrant and palbociclib in vitro. Since resistance to CDK4/6 inhibitors eventually occurs, we also evaluated the combination of vepdegestrant and palbociclib in 2 CDK4/6 inhibitor-resistant PDX models, ST941-PBR (ESR1 Y537S) and ST1799-PBR (ESR1 WT).
Results: In vitro studies revealed enhanced apoptosis in vepdegestrant plus palbociclib treated ER+ breast cancer cells, underpinning the synergistic interactions between vepdegestrant and palbociclib. In vivo, as a single agent, vepdegestrant is highly efficacious in both CDK4/6 inhibitor-resistant models (ST941-PBR 101% TGI, ST1799-PBR 76% TGI) unlike fulvestrant (ST941-PBR 2% TGI, ST1799 PBR-11% TGI) which showed minimal efficacy. In combination with palbociclib, vepdegestrant displayed greater anti-tumor activity (ST941-PBR 107% TGI, ST1799-PBR 84% TGI) than that observed with vepdegestrant alone (ST941-PBR 101% TGI, ST1799-PBR 76% TGI) or fulvestrant plus palbociclib (ST941-PBR 23% TGI, ST1799-PBR 12% TGI) in both CDK4/6 inhibitor-resistant models tested. No significant body weight loss in mice was observed with the combination treatment.
Conclusions: These data suggest that in vitro, the combination of vepdegestrant and palbociclib leads to enhanced apoptosis in ER+ breast cancer cells and in vivo, vepdegestrant alone or vepdegestrant plus palbociclib is superior to fulvestrant alone or fulvestrant plus palbociclib, respectively, in the CDK4/6 inhibitor-resistant setting.

P3-01-17: Transcriptomic and phenotypic profiling of circulating tumor cells from metastatic breast cancer patients

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Background: Metastatic breast cancer (MBC) is considered an incurable disease and understanding the molecular mechanisms at the basis of tumor progression has become mandatory. Circulating tumor cells (CTCs) constitute a population of rare cells that are shed in the bloodstream by primary and metastatic tumors and migrate towards distant organs. The molecular characterization of CTCs is a powerful tool to better investigate the mechanisms underlying metastasis. In this study, we combine phenotypic and transcriptional profiling of CTCs isolated from MBC patients to infer the mechanisms involved in their biology, also in relation to the metastatic site and their phenotypic features.
Methods: Blood samples for CTC analysis were collected from luminal MBC patients recruited in the KENDO study (Protocol code: IRST174.19); CTCs were enriched using the RosetteSep CTC Enrichment Cocktail and frozen until downstream analyses as viable cells. To validate the antibody staining for phenotypic analysis, tests were conducted using the

cancer cell line SKBR-3. In brief, SKBR-3 cells were spiked in peripheral blood of a healthy volunteer and enriched using RosetteSep CTC Enrichment Cocktail. Enriched SKBR-3 and patients' CTCs were incubated with antibodies targeting CD45 (leukocyte marker) and epithelial markers (E-tag; EpCAM, E-cadherin) for phenotypic analysis using the DEPArray NxT platform. For transcriptomic profiling, single CTCs were isolated as single, viable cells. Libraries were prepared using the QIAseq UPX 3' Transcriptome Kit and sequenced using MiSeq on V3-150 cycles cartridges. Bioinformatic and statistical analyses were carried out using the CLC Genomic Workbench.
Results: Through feasibility analysis conducted on SKBR-3 cells, we demonstrate that our workflow involving phenotypic analysis on DEPArray NxT is an efficient tool to identify cancer cells (CD45-/E-tag+) and white blood cells (WBCs; CD45+/E-tag-). In MBC samples, we detected different cell populations: WBCs (CD45+/E-tag-), CTCs (CD45-/E-tag+), and dual-positive CTCs (CD45+/E-tag+; dpCTCs). Transcriptomic profiling was successfully performed on 37 single CTCs isolated from 7 patients. The transcriptional profile of CTCs is consistent with breast malignancy for different databases including ClinVar2019, DisGeNet, and Human Phenotype Ontology. Moreover, Gene Set Enrichment analysis highlighted pathways associated with synapse organization and calcium channel activity, suggesting the implication of the interplay between cancer cells and neurons in metastasis. Compared to a commercial RNA, CTCs express at high levels transcripts associated with cancer dissemination and genome architecture (i.e., STAG2 and H2AFZ). In CTCs from patients with bone metastasis we detected the expression of genes associated with metastatic osteotropism (i.e., S100A4, VAPA, HMGB1), while CTCs from a single patient with gastric metastasis expressed genes associated with gastric cancer metastasis and cell redox homeostasis. Transcriptional profiling of dpCTCs revealed the expression of transcripts encoding for members of the CD47/SIRPα axis, supporting their macrophage-cancer cell fusion origin, and increased glycogen biosynthetic and metabolic activity.
Conclusions: Our data suggest the potential of CTC based transcriptomics in providing new insights on the clinical-biological evaluation of MBC and its mechanisms underlying metastatic cascade and organotropism. In addition, our data provide hints for the characterization of dpCTCs, a poorly studied population whose role in metastasis is still far to be fully elucidated.

P3-01-18: Clinical validation of image-based AI predictive biomarkers for precision neoadjuvant triple-negative breast cancer treatment (PEAR-TNBC): Interim results from Cohort A

Peter Hall, Matthew Williams, Eleonora Peerani, Elli Tham, Francesco Iori, George de Fraine, Kerrie Loughrey, Andreas Kaffa, Thomas Richardson, Carolina Liberal, Angeliki Velentza-Almpani, Demi Wiskerke, Farah Sangkolah, Aston Crawley, Jay Kearney, Edgar Molina, Nourdine Bah, Marios Tasoulis, Cliona Kirwan, Susan Cleator, Steve Chan, Duleek Ranatunga

Background: Pathological complete response (pCR) to neoadjuvant therapy strongly predicts the risk of recurrence and death in triple-negative breast cancer (TNBC). There are

multiple neoadjuvant regimens available for TNBC but no biomarkers to match patients to treatments. Pear Bio has developed a functional precision medicine test coupling an organ-on-a-chip with a computer vision (CV) pipeline through which an individual patient's response to different treatments can be monitored simultaneously ex vivo using time-lapse 3D microscopy. The results are available within 1 week of the biopsy being received.
PEAR-TNBC (NCT05435352) is an ongoing prospective, two-cohort, observational trial to assess the accuracy of the Pear Bio test in predicting pCR to neoadjuvant treatment in TNBC patients. Here we report the interim results from Cohort A of the trial.
Methods: To be eligible, patients need to have ≥10mm tumor, stage I-III, TNBC, planned for neoadjuvant treatment (chosen by the treating oncologist) followed by surgery and be willing to undergo a study-specific core needle biopsy. To be evaluable, the tumor sample needs to be successfully cultured in the 3D ex vivo assay and the patient has to complete at least 4 cycles of neoadjuvant chemotherapy. The primary endpoint is the accuracy of the Pear Bio test in predicting pCR (ypT0/Tis ypN0), defined as specificity, with secondary outcomes as sensitivity, and positive and negative predictive values.
The Pear Bio test is run in parallel with the patient's treatment and the treating oncologist is blinded to the results.
We used the change in dead cell count between Day 0 and Day 3 as the metric to predict pCR, optimised the cut-off by plotting a Receiver Operating Characteristic (ROC) curve, and calculated the area under curve (AUC) for the ROC curve, as well as other measures of classification performance. The treatment responses in the test to different regimens were ranked and compared to the response seen in the patient with the regimen chosen by the treating oncologist.
Results: Cohort A recruitment began on 27th May 2022 and completed on 4th July 2024, having recruited 34 patients from five UK sites. Four patients had to be excluded (one bacterial contamination, one declined NAC, two yielded too few cells) and 18 were still awaiting clinical outcomes. In the 12 evaluable patients, median age was 53 (IQR: 42 – 64) years and median tumor size was 27mm (IQR: 20 – 34). One patient was node positive; seven patients were Stage 1 – 2B, one stage 3A and four undetermined staging. Seven patients received the KEYNOTE-522 regimen, four received AC-CarboTaxol and one EC-CarboTaxol. The median number of cycles of neoadjuvant therapy received was 8.5. Nine patients achieved a pCR at surgery.
The AUC for predicting pCR was 0.81 (p=0.046), with the Pear Bio test correctly predicting outcomes in 10 of the 12 patients.
For the three patients who did not achieve a pCR, the assay suggested that other regimens may have been more effective.
Discussion: We have presented interim results of a novel functional precision medicine assay to predict pCR in patients with TNBC receiving neoadjuvant therapy. We have shown the successful culture of tumor cells and demonstrated intra- and inter-patient variation in response to different therapies. The assay shows promise in predicting pCR, although the number of patients analyzed is small currently. The trial data continues to mature, and Cohort B is now recruiting. Future trials will run the Pear Bio test prior to therapy selection,

guiding the choice of regimen to assess the test's ability to increase pCR rates or de-escalate treatment safely.

P3-01-19: Proteomics-based degradome analysis of mammary tumor adjacent adipocyte reveals adipocyte-derived peptide MASP6 promotes breast cancer cell invasion and migration

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Breast cancer is the most common female malignancy all over the word, at a risen rate of over 1.7 million cases per year globally, where metastasis is the leading cause of death. Accumulating epidemiological evidence indicates that abnormal fat status such as obesity is an important risk and poor prognostic factor for breast cancer. Adipose, a significant component of the stromal tissues, also a type of metabolically active endocrine organ, is the most abundant stromal constituent in the mammary gland surrounding breast cancer. In addition, a growing number of studies have confirmed that adipocytes adjacent to invasive cancer cells, known as cancer-associated adipocytes (CAAs), participate in the regulation of breast cancer progression. Breast tumor adjacent adipocytes have the potential to drive the malignancy of tumor cells by secreting hormones, proinflammatory cytokines, and accelerating tumor metabolic reprogramming. Despite of the diversity effects of adipokines on breast cancer, adipocytes secreted protein derived peptidome remains a black hole to our knowledge, since degradome has been expanding its roles in biology and pathology. In addition to glucose and lipid metabolic products, the fragments/peptides cleaved from extracellular matrix and membrane proteins degradation by proteinase or hydrolase at the level of cancer-tissue microenvironment, are supposed to carry cancer-specific information and further reveal the reciprocal interconnected network of tumor and surrounding stroma tissues, which could be a potential anti-tumor therapeutic target. In addition, little evidence proved that adipocyte-derived peptide could participate in cancer metastasis progression. In this study, we aim to assess the biological effects of breast tumor adjacent adipocyte secreted protein derived peptides on breast cancer cells from multiple angles, and systematically analyze the degradome characters, expecting to realize a basic understanding of its role in the development and progression of breast cancer. Here we found a novel adipocyte-derived peptide MASP6 that may affect the invasive and migrative ability of breast tumor, which is of great significance for exploring new targets for breast cancer.

In order to explore the underlying mechanism of communication between breast cancer and adipose tissue, we isolated adipocytes from adipose tissues adjacent to breast tumor (TAA) and breast benign lesions (BAA) and collected the supernatant. The results showed that peptides of adipocyte secreted degradome could increase breast cancer malignancies dominantly. LC-MS/MS and bioinformatic analysis data showed there are about 100 identified peptides contribute to aberrant cell adhesion of tumor cells through regulating focal adhesion. Sparklingly, 2 lipophilic peptides are identified stable, creating sustained

effect on tumor cells.What's more, wound healing test showed that MASP6, one of the adipocyte-derived peptide, promoted the invasive and migrative ability of breast tumor cells. Dual-luciferase assay, quantitative real-time polymerase chain reaction (qRT-PCR) and western blots verified that the expression of Matrix Metalloproteinase (MMP-2/9) was increased after MASP6 treated in breast cancer cells. Mouse xenograft model demonstrated that MASP6 enhanced tumor metastasis in vivo. The PI3K/Akt signal pathway was stimulated after MASP6 treatment. Nuclear and cellular protein extraction, indirect cellular immunofluorescence revealed that MASP6 could enter into cytoplasm and activate nuclear factor kappa B (NF-κB-p65) via promoting its nuclear translocation.
In conclusion, our results confirmed mammary gland surrounding adipocytes induce malignancies of breast tumor cells, and delineated the interaction network between secreted degradome of adipocytes and tumor cells, which are believed to afford novel insights into breast cancer therapy.

P3-01-20: A novel, semi-automated Pipeline for HER2 Quantification on CTCs in breast cancer patients. Is cytopathology of peripheral blood a new diagnostic option?

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Introduction: The Human Epidermal Growth Factor Receptor 2 (HER2) plays a central role in breast cancer (BC). Nowadays, the assessment of HER2 status and the selection of patients eligible for anti-HER2 therapy rely on immunohistochemistry (IHC) and in situ hybridization on tissue biopsy, an invasive approach, unable to capture intratumor heterogeneity and dynamic of HER2 expression. Circulating tumor cells (CTCs) offer an alternative material to evaluate HER2 expression in real-time, through a simple blood draw. Limitations to the use of CTCs for HER2 assessment derive from limited sensitivity in detection methods and lack of standardization.To overcome these limits, we developed a semiautomated pipeline combining label-independent CTC enrichment and HER2 expression quantification and we compared it to the gold-standard CellSearch®, in a cohort of patients (pts) with metastatic BC (mBC).
Methods: Blood samples (7.5 mL) were collected in EDTA tubes from mBC pts enrolled at Weill Cornell Medicine and processed within 1 hour for CTC analysis. CTCs were captured using Parsortix™ and stained for epithelial (EpCAM, cytokeratins [CK]), leukocyte (CD45) and HER2 markers as well as for nuclear staining. CTCs were identified as nucleated cells, EPCAM/CK+ and CD45-. For each CTC, 4 color digital images were processed with the automated post-processing developed tool: a combination of an open-source image analysis software CellProfiler and a custom MATLAB code allowing for CTC identification and HER2 expression categorization into high, low and no expression. A second aliquot for each sample was processed in parallel with CellSearch® for comparison.
Results:A total of 16 whole blood samples from mBC pts were collected: 5 at baseline

(31%), 4 at progression (25%) and 7 at restaging (44%). The study cohort included 5 pts (31%) with HER2+ BC, 5 pts (31%) with hormone receptor-positive (HR+)/HER2- BC, and 6 pts (3%) with HR-/ HER2- BC.By using our pipeline, CTCs were identified in all the samples, whereas CellSearch® analysis detected CTCs in 77% of samples (10/13 of evaluable samples).A total of 320 CTCs were detected in the 16 CTC-positive samples by our pipeline, versus 368 CTCs detected by CellSearch® in 10 CTC-positive samples. We were able to detect HER2+ CTCs in all the 16 processed samples (100%), while CellSearch® only did in 4/13 of the evaluable samples (31%). Among all detected CTCs, 142/320 were HER2+ (44.4%) by using our pipeline, compared to 40/368 (10.9%) identified by CellSearch®. Of the CTCs detected with our pipeline, 81.7% (116/142) had a high HER2 expression and 18.3% (26/142) a low expression. Our pipeline was therefore able to identify HER2+ CTCs in 11/11 (100%) of pts with a HER2 negative status on tissue as opposed to 2/9 (22.2%) of the samples processed by CellSearch®. Interestingly, in addition to EPCAM+/CK+ CTCs, with our pipeline we were also able to identify 83 cells with low/negative EPCAM/CK expression. These cells were CD45-negative and 12% of the cases presented a HER2 expression.
Conclusion: The current study showed the feasibility of a real-time HER2 assessment on CTCs enriched from mBC pts. The developed pipeline was able to count and identify HER2-positive CTCs with higher efficiency than the gold-standard CellSearch® overall and interestingly, also in the HER2-negative subgroup. This is a preliminary analysis that should be confirmed in larger cohorts. HER2 marker is a therapeutic target and an accurate and real-time assessment of the HER2 status could be used to better guide treatment in a larger cohort of patient with advanced breast cancer with any detectable HER2 expression that can be effectively treated with antibody-drug conjugates.

P3-01-21: Gene expression changes following pre-operative letrozole therapy among postmenopausal women with estrogen-receptor positive HER2/neu-negative invasive breast cancer

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Introduction: Pre-operative endocrine therapy is an evolving area with the advantage of gaining information on tumor response early the treatment course. There is limited data on how pre-operative endocrine therapy affects tumor gene expression. Our goal was to characterize changes in gene expression associated with pre-operative letrozole therapy among postmenopausal women with estrogen-receptor positive (ER+) HER2/neu-negative (HER2-) breast cancer. Approximately 75% of ER+ breast cancers are androgen receptor (AR)+, and there is emerging evidence that the balance between ER and AR signaling is a critical determinant of tumor growth. This study examines changes in overall gene expression, as well as gene expression related to ER and AR signaling.
Methods: Our study population included 33 post-menopausal females with mean age of 65 years and ER+/HER2- breast cancer who underwent surgical resection of the tumor after

receiving neoadjuvant letrozole (median days of treatment = 23 (IQR 21-30 d)). Patients had pT3 and pN2a disease or less, and grade 1 (66.6%) or grade 2 (33.3%) disease. Most patients had ER > 90% by IHC (91% of population). Gene expression was assessed on the initial diagnostic biopsy and the operative specimen. Samples were sequenced on an Illumina NextSeq P4 flowcell. Alignment was performed against the iGenomes hg38 reference genome using STAR v-2.7.10b, with quality checks using FastQC and RSeQC. We used STAR2's –quantMode flag with Gencode v38 gene definitions to perform gene expression quantification and generate counts, followed by edgeR to perform the differential expression analysis in R. A significance threshold of log2FC >= 1 or log2FC <= -1 at 5% FDR was used to define the genes of interest.
Results: 14971 genes were analyzed, of which 347 genes were differentially expressed following letrozole therapy. 203 genes were significantly downregulated, such as SERPINA6 (log2FC -4.2), CYP2A6 (log2FC -3.9), IGSF1 (log2FC -2.7), PDZK1 (log2FC -2.7), TMC3 (log2FC -2.6). The five most upregulated genes include FOSB (log2FC 5.2), FOS (log2FC 4.3), DUSP1 (log2FC 3.7), RGS1 (log2FC 3.6), and ATF3 (log2FC 3.1). 41 genes related to ER/AR signaling were significantly differentially expressed following letrozole treatment, of which the majority were related to AR signaling (37 genes). Most genes related to AR signaling were significantly downregulated (92%), such as PDZK1 (log2FC -2.7), TROAP (log2FC -2.1), NOY1R (log2FC -2.1), PGR (log2FC -1.9), and ESPL1 (log2FC -1.9).
Discussion: This study revealed modulation of gene expression after short-term pre-operative letrozole therapy. We are currently examining pathway level changes associated with pre-operative letrozole treatment to better understand how aromatase inhibitors impact AR signaling. Additionally, our team is investigating whether our observed changes in gene expression are associated with quantitative AR receptor expression by IHC and other outcome measures, such as changes in the proliferation marker Ki67. Further studies should aim to understand how aromatase inhibitors impact AR signaling and the potential impact on the efficacy of endocrine treatment and overall patient outcomes.

P3-01-22: Pilot Feasibility Study of ctDNA Testing in Breast Cancer and its Association with Pain, Stress and Anxiety

Mrinalini Ramesh, Yasmin Fakhari Tehrani, Arya Mariam Roy, Kristopher Attwood, Shipra Gandhi

Background: Molecular residual disease (MRD) assessment by circulating tumor DNA (ctDNA) has shown great promise in predicting therapeutic effects and recurrence risk. Prior studies have demonstrated that colorectal cancer patients with ctDNA negativity have superior disease-free survival compared to those with detectable ctDNA. We aimed to determine whether there was any association between ctDNA detection and clinical outcomes in breast cancer patients. Our study also investigated the association between ctDNA levels, stress, anxiety, and pain experienced by patients in this cohort. In particular, we wanted to investigate whether ctDNA positivity impacted stress and anxiety levels in these patients.

Methods: A prospective study was designed to obtain the ctDNA levels in patients treated in the breast clinic at Roswell Park Comprehensive Cancer Center from 2022 to 2024. This was done by obtaining peripheral blood with serial ctDNA analysis using a personalized, tumor-informed assay (Signatera™). Patient demographics, distress thermometer screening scores, pain scale scores, vital signs, absolute neutrophil count, and active benzodiazepine/antidepressant/antipsychotic use were compared to ctDNA status using the mean, median, and standard deviation for continuous variables, and frequencies, and percentages for categorical variables. Pain scores were obtained on a numerical scale of 1-10 with acceptable pain defined as a score less than their pain scale result. Comparisons were made using the Mann-Whitney U and Fisher's exact tests, as appropriate. The association between ctDNA, measures of pain, stress, and anxiety with recurrence were also assessed.

Results: A total of 49 patients with stage I-III breast cancer had ctDNA testing performed in the adjuvant and neoadjuvant setting after completion of locoregional and systemic treatment from 2022-2024, with 16% stage I, 45% stage II, and 39% stage III. The patient age at diagnosis ranged from 33 to 76 with a median age of 56. Of these 49 patients, 7 had detectable levels of ctDNA, including 3 patients with stage III cancer, 2 with stage II, and 2 with stage I. The median number of ctDNA tests collected was 4 with a range of 1 to 12. Testing was performed every 3 months. The study also included 7 stage IV patients who had detectable levels of ctDNA for the same period. The median age of stage IV patients was 53 years with a range of 34 to 74. ctDNA level was tested every 2 months. The median number of ctDNA collected was 6 with a range of 3 to 13. ctDNA was higher in patients with T3 versus T1 (226 MTM/mL vs 100.8 MTM/mL,0.034), and M1 versus M0 (271.5 MTM/mL vs 47 MTM/mL, <0.001). ctDNA was also positively correlated with the progression of disease on imaging ($p < 0.001$). Additionally, patients with well-controlled pain had significantly lower ctDNA expression compared to patients with uncontrolled pain (42.4 MTM/mL vs 183.3 MTM/mL, p=0.007). No association of ctDNA positivity with anxiety or distress was observed.

Conclusion: This pilot study shows the feasibility of testing ctDNA in clinical practice and explores its association with symptoms and outcomes. Further analysis with a patient population followed for a longer period will be required to understand the impact of ctDNA testing on the well-being of patients with breast cancer.

P3-01-23: MELK controls stromal components through fibronectin modulation in highly aggressive breast cancers.

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Background: Triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC) have limited therapeutic options due to advanced stage at diagnosis, high rate of metastasis, and lack of actionable targets. In a previous study, we showed that MELK is a potential target in highly aggressive breast cancers; a driver of cell stemness, and promotes epithelial-mesenchymal transition and metastasis in a TNBC xenograft model. Fibronectin (FN1) produced by mesenchymal cells has been implicated in breast cancer metastasis, and previous studies have shown that FN1 expression is higher in TNBCs than in HER2-positive or hormone-receptor-positive breast cancers. Studies have also shown that an increase in FN1 can lead to the formation of extracellular fibronectin fibrils, which promote directional cancer cell motility. We previously showed that inhibition of MELK using siRNA against MELK and using a MELK inhibitor reduced the expression of FN1 in vitro and in a xenograft mouse model, suggesting that MELK kinase activity may be important for maintaining FN1 levels in TNBC. We hypothesized that MELK promotes the expression of FN1, which is involved in reorganization of the extracellular matrix.
Methods and results: To determine if MELK promotes transition to mesenchymal-like stromal components, we used qPCR to evaluate the expression of FN1 in MDA-MB-231 clones with CRISPR-based MELK knockout. The clones C3 and C28 showed 0.54-fold ± 0.03-fold ($p \leq 0.01$) and 0.76-fold ± 0.01-fold ($p \leq 0.001$) reduction in expression of FN1 compared to Cas9 control cells. To determine if MELK promotes the motility and invasion of TNBC through FN1, we performed a migration assay by plating MELK-knockdown and MELK-inhibitor-treated TNBC/IBC cells in FN1-coated migration chambers. When MELK was knocked down using three siRNAs against MELK in MDA-MB-231 cells, the relative percentage of migration was reduced by 69.01% ± 0.03, 44.53% ± 0.05, and 11.80 ± 0.10, respectively, in the MELK-knockdown cells compared to the control cells transfected with non-targeting siRNA ($p \leq 0.001$). Similarly, treatment of MDA-MB-231 and SUM149 cells with the MELK inhibitor 30e reduced the relative percentage migration by 30.45% ± 0.10 and 20.00% ± 0.14 compared to the respective DMSO-treated controls ($p \leq 0.05$) for both comparisons. To evaluate the effect of MELK on stromal organization, we used the 3D Spheroid BME Invasion Assay to measure the invasion of MDA-MB-231 cells in the presence of FN1 and MELK-In-30e. The presence of FN1 enhanced the invasion of these cells compared to the control cells, and inhibition of MELK with MELK-In-30e reduced the invasion of these cells compared to the DMSO-treated control cells. To pave the path for clinical translation of MELK inhibitors, we evaluated three MELK inhibitors (MELK-In-17, MELK-In-30e, and OTS167) to find the inhibitor with the highest efficacy and lowest toxicity alone or in combination with paclitaxel in the murine TNBC 4T1 mouse model. All the treated groups exhibited a reduction in tumor growth compared to the vehicle-treated group. The MELK–In–30–treated group exhibited significant inhibition in tumor growth compared to the OTS167-treated group ($p=0.002$). The combinations of MELK inhibitors with paclitaxel did not show any synergism with respect to tumor growth inhibition.
Conclusions and Future Directions: Our results showed that MELK promotes FN1 expression, which may be involved in stromal reorganization and contribute to tumor progression in TNBC. Currently, we are evaluating MELK inhibitors with other rational combination therapies in a triple-negative IBC xenograft model.

P3-01-24: Triple negative and non-triple negative breast cancer have distinct metabolic characteristics associated with divergent survival outcomes

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Introduction: Triple negative breast cancer (TNBC) is an aggressive breast cancer subtype associated with high histologic grade and poor prognosis. TNBC is also known to be associated with modified metabolic activities of both tumor and stromal cells in the tumor microenvironment, potentially as a mechanism to enhance tumor development and survival in low-nutrient conditions. Here we aimed to investigate the differences in the metabolic activity of TNBC vs. non-TNBC and their impact on survival.
Methods: Comprehensive immune profiling, including the expression of 395 immune-associated genes measured by RNA-seq, and PD-L1 expression testing by IHC, was performed on 149 real-world breast cancer samples. 52 samples were, by definition, triple negative for ER, PR, and HER2 overexpression. Based on Reactome pathway database data, mRNA expression signatures of carbohydrate (7 genes), lipid (10 genes), protein (29 genes), vitamin/cofactor (6 genes), and overall (32 genes) metabolism were calculated by averaging the normalized gene expression of each gene set. Samples were grouped into high (greater than or equal to median expression) and low (less than median expression) groups for each metabolic signature. Statistical comparisons of biomarkers between groups were performed using the Wilcoxon Rank-Sum test for continuous variables and Fisher's Exact Test for categorical variables ($p \leq 0.05$ for significance). Survival differences were quantified by Kaplan-Meier analysis ($p \leq 0.05$ for significance).
Results: In general, TNBC demonstrated greater overall metabolic activity ($p=0.001$), including greater lipid ($p=5.8\times10\text{-}5$), protein ($p=0.0053$), and vitamin/cofactor ($p=0.04$) metabolism. However, there was no significant difference in carbohydrate metabolism between TNBC and non-TNBC. Among TNBC cases, only higher vitamin and cofactor metabolism was associated with better overall survival (OS) (61 months vs. 37 months, $p=0.029$), while carbohydrate, lipid, protein, and overall metabolic activity were not significantly associated with OS. For non-TNBC cases, high overall metabolic activity was associated with better OS (180 months vs. 78 months, $p=0.011$), as was high carbohydrate (147 months vs. 79 months, $p=0.0053$), high lipid (162 months vs. 73 months, $p=0.016$), and high protein metabolism (148 months vs. 73 months, $p=0.026$).
Conclusions: We found that, in general, TNBC is more metabolically active than non-TNBC, though the survival effects of this difference vary depending on the specific aspect of cellular metabolism being measured, with some signatures associated with OS in TNBC and others in non-TNBC. An improved understanding of the metabolic environment of breast cancer as it relates to both triple negative status and differences in patients may facilitate a more nuanced characterization of tumor subtypes, aiding in the development of treatment strategies taking this aspect of the tumor microenvironment into account.

P3-01-25: Molecular and immunological characterization of HER2-low, HER2 ultra-low, and HER2-null male breast cancer

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Background: Human epidermal growth factor receptor 2 (HER2) gene expression is an important predictive and prognostic biomarker in breast cancer (BC), which also guides treatment recommendations. The expression of HER2 is a continuum from null to positive and includes HER2-low and ultra-low as targets for anti-HER2 antibody–drug conjugates (ADC). However, HER2-low and ultra-low have not been studied in male BC. Here, we analyze whether there are any differences in molecular and immunological features between HER2-low, ultra-low and HER2-null/negative expression in males with BC.
Methods: 199 male breast tumor samples were included in this study. HER2-null expression was defined as infiltrating cancer cells completely free of HER2 immunohistochemistry (IHC) staining. HER2 ultra-low expression was defined as ≤10% cancer cell showing incomplete and faint/weak membrane staining. HER2-low expression was defined as HER2 (IHC) 1+ or 2+ with negative chromogenic in situ hybridization (CISH) assay. HER2-positive expression was defined as HER2 IHC 3+ staining or 2+ with positive CISH assay. Mutations and gene expression were detected by next-generation sequencing (NextSeq; WES, NovaSeq) and Whole Transcriptome Sequencing (WTS; NovaSeq) (Caris Life Sciences, Phoenix, AZ), respectively; tumor mutational burden (TMB) totaled somatic mutations per tumor (high>10 mt/MB). Immune cell fractions were calculated by deconvolution of WTS:Quantiseq. Statistical significance was determined using chi-square and Mann-Whitney U test and p-value <0.05 was considered significant.
Results: Of 199 samples, there were 70 (35.2%) HER2-null tumors, 49 (24.6%) HER2 ultra-low tumors, 64 (32.2%) HER2-low tumors, and 16 (8.0%) HER2-positive. The proportion of HR+ was 81.4% in HER2-null, 93.8% in HER2 ultra-low, 87.5% in HER2-low and 81.2% in HER2-positive tumors. HER2 ultra-low male BC had higher frequency of PIK3CA (44.19% vs 23.64%) compared to HER2-null and KMT2D (7.5% vs 0%) compared to HER2-low, all p<0.05. HER2-low male BC had numerically lower frequency of TP53 (8.7% vs 16.6%, p=0.2) and ESR1 (1.6% vs 5.4%, p=0.2) compared to HER2-null. No significant differences were noted in TMB-high frequency (7.1% vs 5.0% vs 6.9%) and PD-L1 positivity (22C3) (8.11% vs 9.0% vs 10.6%) between HER2 ultra-low, HER2-low and HER2-null (all p=1.0). Analysis of inferred immune cells revealed that HER2 ultra-low and HER2-low male BC had higher infiltration of B cells (6.6% vs 5.8% vs 4.9%) but lower infiltration of neutrophil (2.4% vs 2.0% vs 3.8%) (all p<0.05). HER2-low had lower expression of immune checkpoint gene LAG3 (fold change (FC): 1.6), stem cell-related genes (KLF4, POU5F1; FC: 1.3-1.7) and drug-efflux gene ABCB5 (FC: 2.2) compared to HER2-null tumors (all p<0.05). HER2 ultra-low had lower expression of drug-efflux gene ABCG2 (FC: 1.5) compared to HER2-null tumors (p<0.05). Data adjusted for HR-subtype will be presented at the meeting.
Conclusions: Our findings add valuable information to the current understanding of the HER2 spectrum in the male breast cancer, including frequency distribution and molecular

characterization. With some exceptions, HER2-low, ultra-low and null breast cancer in men shared genomic features, suggesting that the disease biology may not be different across the spectrum of what historically has been considered HER2-negative disease. Interestingly, HER2 ultra-low, HER2-low and HER2-null had differential tumor immune microenvironment that warrant further exploration.

P3-01-26: Amitriptyline potentiates Elacestrant in treating ER positive ESR1 mutant Breast cancer.

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Background: Breast cancer (BC) stands as the most prevalent cancer and the leading cause of cancer-related deaths among women globally, with an incidence rate of 1 in 8 women developing invasive breast cancer during their lifetime. A significant majority (80%) of these cases are Estrogen Receptor positive (ER+). The standard first-line treatment for locally advanced or metastatic ER-positive/human epidermal growth factor receptor 2 (HER2)-negative breast cancer includes endocrine therapy with aromatase inhibitors (AI) or Fulvestrant combined with a cyclin-dependent kinase 4/6 (CDK4/6) inhibitor. However, disease progression often leads to endocrine resistance, primarily due to acquired mutations in the estrogen receptor 1 (ESR1). These ESR1 mutations cause estrogen-independent ER activation, resulting in resistance to AIs but not to selective ER degraders (SERD) or selective ER modulators (SERM). Current treatment guidelines recommend continuing sequential endocrine therapy until a visceral crisis occurs or all options are exhausted, revealing a significant unmet clinical need. Repurposing existing or investigational drugs has emerged as a promising strategy for cancer treatment. Consequently, we explored the potential of repurposing the antidepressant Amitriptyline for treating ER+ breast cancer.
Methods: We employed both short-term and long-term cell viability assays, as well as apoptosis assay, to evaluate the anticancer activity of Amitriptyline across various BC cell lines. These included wild-type ERα cell lines (MCF7 and ZR75), cell lines with acquired resistance (MCF7-Tam and MCF7-LTLT), and mutant ERα cell lines (MCF7-ERα-D538G, MCF7-ERα-Y537S, ZR75-ERα-D538G, and ZR75-ERα-Y537S). To validate our in vitro findings, we employed patient-derived explants (PDEX), cell-derived xenograft, and patient-derived xenograft (PDX) models to test Amitriptyline's efficacy. Biotin/Streptavidin pull down assay followed by mass spec analysis and RNA sequencing (RNA-seq) analysis were conducted on vehicle-treated and Amitriptyline-treated breast BC cells to elucidate the target protein and mechanism of action respectively. Additionally, we investigated whether Amitriptyline could sensitize ER+ breast cancer cells to Elacestrant, an FDA approved SERD.
Results: Amitriptyline treatment significantly reduced both short-term and long-term cell viability of BC cells in a dose-dependent manner. Additionally, Amitriptyline markedly increased apoptosis in BC cells. These in vitro findings were corroborated in vivo, as Amitriptyline treatment inhibited the growth of ER+ BC in a preclinical orthotopic

syngeneic model. Furthermore, Amitriptyline significantly lowered Ki67 levels in ESR1 mutant patient-derived explants (PDEX). Notably, Amitriptyline enhanced the sensitivity of ER+ BC to Elacestrant, demonstrating highly synergistic effects. The combination of Amitriptyline and Elacestrant significantly improved cell viability, survival, and apoptosis outcomes compared to Elacestrant alone.
Conclusion: Our study highlights the potential of Amitriptyline as a safe and effective treatment option for patients with ER+ breast cancer.

P3-01-27: CYTOKINE LEVEL AND OUTCOMES OF NEOADJUVANT TREATMENT IN EARLY BREAST CANCER

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Background: Globally breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women. Neoadjuvant therapy (NAT) in locally advanced breast cancer resulted the achievement of pathological complete response (pCR) which is a valid predictor of good prognosis and influence in the future also on survival both in Her2 positive and TNBC.
Correlation between neutrophil to lymphocyte ration (NLR), levels of cytokines IL6,IL8,IL10 and pCR after neoadjuvant chemotherapy remains unclear.
Patients and methods:This prospective study was conducted at Sharett Institute of Oncology and included 37 patients with locally advanced breast cancer undergoing neoadjuvant treatment.The patients were sampled for the first time on the day they started chemotherapy, in the mid cycles and at the end of the treatment.IL-6,IL-8 and IL10 were measured by an automated solid-phase chemiluminescent immunometric assay.
Results:The rate of elevated NLR above 2.05 in non-pCR was significantly higher than in those with pCR (58.3% vs. 16.7%, P=0.032). High NRL (>2.06) at the start of treatment was predictor of non-pCR (odds ratio 7.00, 95% CI 1.3-39.1, P=0.027).The difference in the rate of elevated delta NLR among responders and non-responders was not significant (16.7% and 30.4%, P=0.450).
We found that the levels of IL-6 and IL-10 were consistently low in the subgroup of BRCA1/2 carriers, while IL-6 was higher in Her2-positive tumor. Analysis of changes in cytokines during NAT showed that elevated IL-6 above 4.8 pg/ml after first treatment was found to be a significant predictor of pCR.A significant correlation was found between IL-6 and IL-10. The changes of IL-10 showed similar to IL-6 kinetics during neoadjuvant treatment both in responders and non-responders, however difference between two subgroups did not reach statistical significance. For IL-8, no statistically significant findings were found.
We also collected saliva samples in order to check cytokine levels.First we found that cytokine level in Saliva were almost ten times higher than in serum.IL6 level was higher in patients with family history before the treatment (p= 0.010) and in mid treatment (p=0.042).IL6 and IL10 level were higher in the mid treatment in patients with advanced

disease, such as larger tumor size with significant p value (p=0.013 for IL6 and p=0.049 for IL10).IL8 and IL10 levels were lower during and at the end of NA treatment in patients with Her2 positive disease.There was no correlation in cytokines measured between serum and saliva during neoadjuvant treatment.
Conclusion: We found association between pCR and NLR. Also elevated IL-6 was a significant predictor of pCR.Furthermore the levels of IL-6 and IL-10 remained low in BRCA ½ carriers, while IL-6 was consistently higher in Her2-positive tumor.
A significant correlation was found between IL-6 and IL-10. The changes of IL-10 showed similar to IL-6 kinetics during neoadjuvant treatment both in responders and non-responders, however difference between two subgroups did not reach statistical significance. For IL-8, no statistically significant findings were found.
No correlation was found between salivary and blood cytokine levels,with salivary levels tending to be higher.This can be caused because of food,dry mouth as a side effect of the treatment, tooth and gum infections both as a side effect of the chemotherapy and so on. But we were able to find correlation between salivary cytokines,Her2 positive disease, family history and tumor size.
From the results of the study, it can be concluded that there is a relationship between an increase in IL-6 levels and response to treatment and there is room to deepen the research and examine the possibility of introducing IL-6 as a measure of response to treatment.

P3-01-28: Therapeutic Yoga Enhances Cognitive and Metabolic Markers in Breast Cancer Survivors

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Background: Cancer-related cognitive impairment (CRCI) frequently arises as a consequence of breast cancer treatments, manifesting in challenges such as impaired memory, attention, processing speed, and word finding. These cognitive deficits ranging from mild to moderate, can persist for months or even years. They can negatively impact a survivor's quality of life, mental health, and interpersonal relationships. Moreover, breast cancer and its therapies adversely affect various metabolic processes in the body influencing factors such as weight changes, fat metabolism, energy regulation, dyslipidemia, growth hormone regulation and cardiovascular health. This study aims to investigate whether a 16-week therapeutic yoga program (TYP) modulates the cognitive and metabolic biomarkers profile in plasma among heterogeneous breast cancer survivors.
Approach: Participants included in the study were adults aged 18 and older with a clinical cancer diagnosis. Informed consent was obtained from all participants. Nineteen participants completed three weekly 75-minute sessions of TYP combined with love and kindness meditation. Blood samples were collected from sixteen participants both before and after the TYP intervention. Eight neuro and metabolic biomarkers viz, Beta-NGF, BDNF, Ghrelin, IL12P70, Leptin, MCP-1, TNF-Beta, VEGF-A were measured by a U-Plex Custom Metabolic Group1 (hu) Multiplex Assay on the MESO Quickplex SQ 120MM, Model 1300.

The data was analyzed using Wilcoxen signed rank test.
Results: The participants had a mean age of 59.6 years (± 7.3 years). Over half of the cohort (56%) were classified as overweight or obese (BMI ≥ 25 kg/m^2). The majority were female (71%) and breast cancer survivors (65%), with 44% of these survivors being of Hispanic ethnicity. Statistically significant increases were observed in concentrations of BDNF (pre: 653.50 vs post: 1234.17 pg/ml; 88.85%, P=0.005) and ghrelin (pre: 576.10 vs post: 710.80 pg/ml; 23.38%, P=0.04). Notably there was a marked difference found [PDI1] in VEGF-A (45.51% increase post TYP) and MCP-1 (19.79% decrease post TYP).
Conclusion: TYP contributed to substantial improvements in cognitive biomarkers in plasma samples from breast cancer patients. Future research utilizing larger cohorts is needed to validate these results.

P3-01-29: Open, single-arm clinical trial with innovative inPROBE® technology for in vivo, real-time, quantitative HER2 expression assessment

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Background: The current standard of HER2 expression assessment on breast cancer (BC) cells is IHC/FISH; however the results are imprecise. This significantly implies prognosis, therapy and reimbursement, especially in relation to HER2-low and ultra-low BC subtype.
Methods: We developed a new, innovative tool for quantitative assessment of HER2 expression on BC cells. This is real-time, in vivo inPROBE®, merging molecular biology with photonics technology. We conducted an interventional, open-label, single-arm safety and efficacy clinical investigation in female BC patient aged 18 to 75 years with known HER2 status based on IHC/FISH. The primary endpoint was identification of HER2 concentration ranges detected with microprobe corresponding to HER2 receptor status identified by IHC/FISH. The key secondary endpoint was to assess the relation between inPROBE assessment in tumor mass and surrounding area in the direct tumor vicinity in HER2-positive pts.
Results: There were 22 women with confirmed BC and known HER2 status enrolled, with 18 pts. finally included in statistical analysis (6 pts. with HER2+ and 12 pts. with HER2- BC). All pts. were in good clinical stage (ECOG PS=0). Mean (SD) age was 56.6 (10.6) years, range 39-77 years. The study met its primary endpoint. The ranges of HER2 concentrations corresponding to HER2- or + status were identified, with significant difference for minimum values in Wilcoxon exact test (significantly lower for HER2- than for HER2+ BC, p=0.041). The secondary endpoint was not met, but comparison of HER2 receptor concentrations detected with the microprobe located within the tumor and in the near tumor area in overall population (HER2+ and HER2-) showed moderately significant difference (p=0.046). This group could be heterogenous, possibly including patients with truly HER2- (0), but also HER2-low expression. Safety profile of device was good and promising. No adverse events were observed.

Conclusions: The current standard IHC/FISH method for HER2 expression assessment could be questioned and advances in targeted therapy may require more sophisticated technologies. Further clinical development of inPROBE® can be continued with no risks for patients' safety. inPROBE® technology could become promising tool, providing the oncologist with new, modern, real-time, in vivo diagnostic method; thus, meeting a pressing clinical need.

P3-01-30: Precision and Repeatability of PD-L1 Detection using PD-L1 IHC 22C3 pharmDx in Breast Cancer

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PD-L1 IHC 22C3 pharmDx (SK006) is an FDA-approved qualitative immunohistochemical (IHC) assay validated for the detection of the programmed-death ligand 1 (PD-L1) protein in multiple tumor types, including triple-negative breast cancer (TNBC). TNBC is a breast cancer (BC) subtype that is negative for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2).
In addition to TNBC, BC also includes a collection of sub-types known as non-TNBC (ER+, PR+, and/or HER2+). While precision and repeatability of PD-L1 IHC 22C3 pharmDx have previously been demonstrated on TNBC, these performance characteristics are less known for non-TNBC. Although non-TNBC is not an FDA-approved tumor type for the device, the goal of the studies listed herein was to determine and report the precision and repeatability of PD-L1 IHC 22C3 pharmDx on TNBC and non-TNBC specimens, collectively.
To evaluate the analytical performance of PD-L1 IHC 22C3 pharmDx for BC, the following studies were executed on formalin-fixed, paraffin-embedded (FFPE) BC specimens: intra-run repeatability (n=42), day-to-day testing variability (combined precision; n=41), as well as inter- and intra-observer precision (n=61). The specimen pool included the dynamic range of PD-L1 expression (CPS = 0–100) for each study. To mimic real-world subtype prevalence, the specimen pool consisted of approximately one third TNBC and two thirds non-TNBC. All specimens were prepared and stained according to the PD-L1 IHC 22C3 pharmDx package insert. The stained slides were scored using the combined positive score (CPS) algorithm and assigned an expression status (positive/negative) at the CPS ≥ 1 cutoff. CPS was determined by dividing the number of viable PD-L1-staining cells (tumor cells, lymphocytes, and macrophages) by the total number of viable tumor cells. Totals are multiplied by 100 and displayed as integers. Intra-run repeatability was scored by three certified observers, combined precision was scored by two certified observers, and inter- and intra-observer precision studies were scored by three certified observers. Negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) were calculated for each study. Acceptance criteria (AC) were predefined, requiring a 95% confidence interval (CI) with a lower bound (LB) value of ≥ 85%. Agreement was based on the binary (positive/negative) PD-L1 expression status assigned to a stained slide by an observer.

All studies met the predefined AC. Intra-run repeatability of the assay achieved CI LB values of 94.3%, 97.1%, and 96.2% for NPA, PPA, and OA respectively. Combined precision achieved NPA, PPA, and OA CI LB values of 89.5%, 95.5%, and 93.5%, respectively. Inter-observer precision resulted in CI LB values of 90.8%, 93.9%, and 93.6% for NPA, PPA, and OA, respectively. Finally, intra-observer achieved NPA, PPA, and OA CI LB values of 94.4%, 96.4%, and 96.0%, respectively.
Overall, based on 95% CI LB values and the fulfilled AC, these studies demonstrate high precision and repeatability of PD-L1 IHC 22C3 pharmDx in the detection of PD-L1 expression in breast cancer (triple negative and non-triple negative, collectively). Further, these results feed into the growing body of research demonstrating CPS as a precise and reproducible scoring algorithm across multiple tumor types. In addition to strengthening confidence in the reliability of the device and scoring algorithm, these consistent results have a potential impact on breast cancer treatment; however, additional studies are required to confirm.

P3-02-01: Is Autologous Fat Grafting a Safe Option or a Risk of Cancer Recurrence in Women with Breast Cancer Who Carry Germline Pathogenic Variants?

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Background: Autologous fat grafting (lipofilling, LF) is widely accepted for breast reconstruction after cancer surgery (breast conservative treatment and mastectomy), due to its potential to improve aesthetic outcome. However, its long-term oncological safety in breast cancer patients with pathogenic germline variants (gPV) is still not well documented. This study aims to compare the incidence of local recurrence in breast cancer (BC) patients with gPV who underwent LF compared to those who received breast reconstruction without LF.
Methods: A retrospective, single-center, case-control study has been conducted, including BC women with gPV referred to the High Cancer Risk Clinic in southern Switzerland who underwent surgery followed by breast reconstruction with or without LF. Autologous fat was aspirated using a standard tumescent solution, yet neither centrifuged nor enriched. Patient demographic details, clinical information, reconstruction procedures, and fat grafting data were systematically collected.
Results: Among 897 women with BC referred for genetic counselling and testing, 132 carried heterozygous gPVs in the following genes: 44 (33%) in BRCA1, 39 (30%) in BRCA2, 1 (1%) in BRCA1 and BRCA2, 10 (8%) in CHEK2, 4 (3%) in PALB2, 8 (6%) in ATM, 1 (1%) in CDH1, 1 (1%) in PTEN, 3 (2%) in BRIP1, 3 (2%) in NTLH1, 2 (2%) in RAD51C, 2 (2%) in NBN and 1 (1%) in TP53. In addition, variants were identified in other cancer predisposition genes based on family history: 3 (2%) in MUTHY, 1 (1%) in CDKN2A, 2 (2%) in PMS2, 4 (3%) in CFTR, 1 (1%) in MSH6, 1 (1%) in MITF and 1 (1%) in SDHA. The median

age at cancer diagnosis was 42 years (range: 21-82). Out of the women included in the study, 54% underwent mastectomy, while the remaining 46% opted for breast-conserving surgery. A total of 25 LF procedures were performed, with an average of 2 sessions per patient (range: 1-4). Median follow-up was 84 months from the primary surgery (range: 12-408 months) and 36 months from the LF (range: 12-84 months). Thirty-seven cases of locoregional recurrence were observed, including 1 (4%, a BRCA1 triple-negative breast cancer not associated with ductal intraepithelial neoplasia) in the LF group and 36 (33.6%) in the no-LF group. Fourteen of these patients underwent mastectomy, and four had contralateral prophylactic mastectomy. For this group of patients, the results of the genetic tests were distributed as follows: 14 for BRCA1, 12 for BRCA2, 2 for ATM, 3 for CHEK2, 2 for MUTYH, 1 for NTLH1, 1 for NBN, 1 for PALB2 and 1 for TP53.
Conclusions: The current evidence does not support the opinion that the use of LF in reconstruction following mastectomy or conservative surgery in gPV carriers with BC significantly increases the risk of locoregional recurrence.

P3-02-02: Genetics and Breast Cancer: Insights and Challenges from Two Years of Analysis of the Private Health System in Brazil.

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Introduction: Approximately 10% of breast cancers are associated with hereditary genetic mutations, notably genes linked to moderate risk of breast cancer (CHEK2 and ATM), and high risk (BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, and TP53). Patients with pathogenic variants in these genes are potential beneficiaries of individualized strategies, and therefore the study of these genes is useful in managing affected families.
The aim of this study was to evaluate pre and post-genetic test data of patients with breast cancer, as well as the implications of these results on patient management and follow up.
Methods: An observational, cross-sectional, and descriptive study was conducted in a private hospital in Belo Horizonte, Minas Gerais, Brazil. Data were obtained retrospectively from breast cancer patients who underwent oncogenetic medical appointments between 01/01/2021 and 12/31/2023. Clinical information from pre and post-genetic test consultations were collected, including test results, treatment implications, and absences from post-test appointments.
Results: This study included all the 126 patients who received genetic testing indications from the oncogenetic specialist. Ages at breast cancer diagnosis ranged from 26 to 90 years. Epidemiological characteristics are summarized at table 1.Remarkably, only 55.6% of patients returned for the post-test consultation, with 14.3% of them having pathogenic results in one of these genes: BRCA1, BRCA2, TP53, CHEK2, and MUTHY (Chart 1). Treatment impacts are described in Table 3. Likely pathogenic results were identified in 2,85% of cases and variants of uncertain significance (VUS) in 45.7% (Table 2).

Five patients had pathogenic mutations in BRCA genes and were indicated for contralateral prophylactic mastectomy. One underwent breast-conserving surgery without contralateral prophylactic surgery. Bilateral prophylactic salpingo-oophorectomy was not indicated for one patient under 40 years old. Of the other four patients, three underwent oophorectomy. None used adjuvant PARP inhibitors (PARPi). The three patients with pathogenic TP53 mutations underwent contralateral prophylactic mastectomy and Li-Fraumeni syndrome screening. Patients with pathogenic mutations in MUTHY and CHEK2 were not candidates for risk-reducing surgery and received high-risk screening.
Discussion: Genetic testing could bring positive implications. A study published by Bae et al. showed that women at high-risk of breast cancer who underwent screening with mammography and MRI had better overall survival than those screened with mammography alone. Furthermore, the use of PARPi as adjuvant therapy increases progression-free survival, as well as overall survival in the early setting, which justify genetic testing in candidates for PARPi.
Confirming the impact of genetic counseling, in this present study, 90% of the patients who returned with pathogenic mutations received additional interventions due to the test results, with 80% being risk reduction surgeries.
Despite these findings, it is alarming that over 40% of our patients did not attend the post-test appointment. Considering that over 17% of our sample had a pathogenic or likely pathogenic mutation, it is concerning the number of patients that could potentially have their clinical management and counseling compromised.
A publication by Mansilla et al. also showed a low success rate for genetic counseling consistent with our data. This reflects a paradoxical trend between increasing test availability and low patient adherence to comprehensive genetic counseling. This phenomenon may be due to low patient understanding, and anxiety about the responsibility of sharing results with their relatives.
Conclusion: In a scenario of widespread genetic testing, and its positive effects, it is equally important to improve genetic counseling strategies, and implement close surveillance to maximize the benefits of oncogenetics.

P3-02-03: HEPARANASE LIQUID BIOPSY AND BREAST CANCER

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Background: Breast cancer is a complex disease with high clinical and genetic heterogeneity and represents a public health problem worldwide, as it is among the four main causes of premature death in most countries. The molecular profile of solid tumors may be established using surgical or biopsy tissue samples. However, tissue-based tumor profiles are subject to sampling bias, provide only a snapshot of tumor heterogeneity, and cannot be obtained repeatedly. Therefore, analyses of circulating nucleic acids, blood samples, or other body fluids, commonly called liquid biopsies, can also contain tumor-derived genetic information. Liquid biopsy represents an innovative method that has gained prominence in recent years, a significant change in how we diagnose and monitor various diseases,

especially cancer. Previous studies from our group showed that circulating lymphocytes obtained from patients with breast cancer have higher glycosidase heparanase (HPSE1) expression. Objective: We aimed to investigate the expression of HPSE1 in circulating T-lymphocytes collected from blood samples of patients with breast cancer compared to the control group (women not affected by neoplasms). Methods: To obtain the mononuclear cell fraction, T-lymphocytes were extracted using Ficoll-paque (Sigma-Aldrich) from the collected peripheral blood sample. Total RNA was extracted from T-lymphocytes with Trizol (Invitrogen), following the manufacturer's instructions. After obtaining the cDNA by reverse transcription, the expression of HPSE1 was evaluated by quantitative PCR. Results: A significant increase in the expression of HPSE1 was observed in T-lymphocytes obtained from breast cancer patients compared to unaffected individuals. The results also showed higher expression according to the different molecular subtypes of breast cancer (Luminal B, HER2 positive, and triple-negative). Conclusion: We conclude that expression analyses of HPSE1 using liquid biopsies allow a non-invasive and real-time assessment, favoring prospective studies where early diagnoses and personalized treatments can be developed.

P3-02-04: Phase II, Single Arm Trial of Denosumab's Effect on Breast Density and Breast Tissue Biomarkers

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Background: Receptor activator of nuclear factor-κB (RANK) and its ligand (RANKL) regulate bone metabolism, immune tolerance, immunity, and mammary gland biology. RANKL, induced by progesterone, prolactin, vitamin D3, PTHrP, TNF-α, prostaglandin E2, and interleukins, impacts osteoclast differentiation, lymph node formation, breast cell proliferation, and immune regulation. RANKL/RANK is essential for mammary stem and progenitor cell populations linked to progesterone and BRCA1 mutation-driven breast cancers. This pilot investigated RANKL inhibition with denosumab, a therapeutic anti-RANKL antibody, to assess effects on breast density in postmenopausal women. Methods: This single-arm, open-label pilot administered 120 mg of denosumab subcutaneously monthly for 6 doses to postmenopausal early-stage breast cancer patients. Patients may have received aromatase inhibitors. Those on SERMs were ineligible. Breast density was measured by MR imaging of the breast at baseline and after 6 months. Analysis was performed on 25 evaluable patients. The primary endpoint was the MRI-measured breast density (MRD) change from baseline to the end of study, approximately one month after last injection. A ≥5% relative decrease in breast density in ≥ 30% of participants was predefined to warrant a larger study. Results: Thirty postmenopausal women with a history of breast cancer received the intervention, 25 evaluable with 5 excluded (withdrew consent, image distortion, or ineligibility). All evaluable patients completed baseline and 6-month MRI measurements and received at least 5 doses of monthly denosumab. Median age was 60 years, median BMI was 28.5 kg/m2, and 44% were on aromatase inhibitors. Median baseline MRD was 16.2% (range 4.91-45.34%). Fourteen (56%) were characterized as

heterogeneously dense (BI-RADS c), one (4%) extremely dense (BI-RADS d), and ten (40%) as scattered fibroglandular by mammography. Excluding two high density outliers, baseline RANKL correlated with breast density (Spearman's rho = 0.51). All patients had undetectable RANKL levels after 6 months of denosumab. Relative MRD decreased by -8.19% (mean) and -6.34% (median) for the primary endpoint after 6 months. Fifteen subjects (60%, 95% CI= 42-75%) had a relative MRD decrease of ≥5%, with only two (8%) increasing. There was no significant correlation between changes in MRD and baseline MRD, age, BMI, or baseline RANKL levels. As a reference, a similar population of postmenopausal women on aromatase inhibitors (N=39) followed for 6 months, 21% had a decrease in MRD of ≥5%, 46% had no change, and 33% had an increase of ≥5%. The difference between DMAB and the ref group remained significant after multivariate adjustment (p=0.01). Denosumab was well tolerated, with no significant or unexpected toxicities. Seventeen doses were administered at home by an RN. Exploratory analyses of a subset of patients with available breast biopsies revealed potential correlations between change in MRD following denosumab treatment and baseline immune cell phenotypes including for example higher baseline RANKL+/CD8+ T cells in participants with greater decreases in MRD at 6 months (rho= -0.49, p= 0.10). Conclusions: In this pilot, therapeutic suppression of RANKL for putative breast cancer prevention was well-tolerated and could be administered at home. RANKL levels correlated with baseline breast density, and treatment for 6 months was associated with a reduction in breast density that favors effects of RANKL inhibition on breast tissue. Our results support further studies to define denosumab and RANKL's effects on breast density, including effects that may be mediated through a RANKL-positive breast immune microenvironment in postmenopausal women.

P3-02-05: Mitochondrial Metabolism-Related Features Guiding Precision Subtyping and Prognosis in Breast Cancer, Revealing FADS2 as a Novel Therapeutic Target

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Background: Breast cancer is one of the most common malignant tumors in women. Mitochondria are critical components within cells. Changes in mitochondrial metabolism play a crucial role in regulating tumor cells and further impacting clinical prognosis. However, their interplay in breast cancer remains a pending issue. This study aimed to reveal the relationship between mitochondrial metabolism-related genes and breast cancer by constructing risk features.

Methods: Breast cancer transcriptome chip data were obtained from TCGA and GEO, while mitochondrial gene data were downloaded from the MitoCarta3.01 database. The "ConsensusClusterPlus" package was used for clustering analysis, with subsequent processing for GSEA, GO, and KEGG pathway analyses. A prognosis model for breast cancer was established using Cox regression and LASSO algorithms. The CIBERSORT algorithm and MCPcounter algorithm were utilized to calculate each patient's immune cell infiltration level. Additionally, the expression of pivotal genes in breast cancer patient tissue specimens

and cell models was validated to verify their association with biological functions in breast cancer cells.
Results: We employed the LASSO regression algorithm to reveal mitochondrial metabolism-related genes in the TCGA BRCA dataset and identified four prognosis-related genes (MYH11, LTF, FADS2, and PSPHP1). These findings were further validated through analysis using the GEO dataset. Based on the four-gene prognosis model derived from mitochondrial metabolism, breast cancer patients with a high-risk score had shorter overall survival compared to the low RS patient group. Immunological analysis revealed that patients with higher risk scores were more likely to be unresponsive to immunotherapy but more sensitive to several conventional chemotherapies, suggesting the potential strategy of combining chemotherapy with immunotherapy to enhance the efficacy of current T cell-based immunotherapy. Univariate and multivariate Cox regression analyses validated the mitochondrial gene model as an independent prognostic indicator for predicting overall survival. They established a nomogram based on the mitochondrial gene model to predict the prognosis of breast cancer. Inpatient tissue validation, pivotal genes exhibited an expression trend consistent with the results of bioinformatic analysis. Finally, through cellular experiments, we verified the expression and function of the key gene FADS2, finding that FADS2 was highly expressed in breast cancer cell lines, and upon its knockout, the cell's invasion, migration, and colony-forming abilities were significantly reduced.
Conclusion: This work demonstrates that prognostic features based on mitochondrial-related genes are related to clinical outcomes, tumor progression, and genetic alterations. Our research findings may provide insights for developing new targets for breast cancer treatment, early intervention, and prognosis prediction.

P3-02-06: Prevalence of BRCA1/2 Mutations in Low ER Positive/HER2 Negative Breast Cancer: Clinico-Pathological Parameters and Comparisons with Unselected TNBC

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Introduction: Following the 2010 ASCO guidelines' establishment of a 1% ER positivity threshold, numerous studies have identified the clinical parallels between ER low positive & HER2 negative breast cancer (Low-ER) and triple-negative breast cancer (TNBC), noting an elevated BRCA1/2 mutation incidence in Low-ER cases. Despite NCCN guidelines advocating for genetic testing in all TNBC patients, Low-ER cancers have yet to receive similar consideration. This investigation aims to assess the prevalence of BRCA1/2 mutations and associated clinicopathological features in unselected Low-ER cases, and to compare the BRCA1/2 prevalence with that observed in unselected TNBC.
Methods: This multicenter, retrospective, non-randomized single-arm study analyzed data from January 2014 to December 2022 at Samsung Medical Center, Seoul National University Hospital, Asan Medical Center, and Gangnam Severance Hospital. The study included adult

females with primary Low-ER & HER2 negative breast cancer, excluding those without biobank samples, with mixed IHC subtypes, or with synchronous breast cancers of different IHC types. Buffy coat samples from biobanks were used to supplement cases without prior BRCA1/2 mutation tests. Comparative analysis with TNBC was based on data from a previous study by Park, W.K., et al, examining BRCA prevalence in TNBC patients from June 2008 to January 2016 at Samsung Medical Center[1].
Results: Of the 268 Low-ER patients, 94 had prior genetic testing, and 174 were assessed via buffy coat analysis. BRCA1/2 mutations were detected in 13.8% of Low-ER patients, with significant variation across age groups: 43.2% in patients aged ≤40, 51.4% in those aged 41-60, and 5.4% in patients over 60 years (p=0.002). The prevalence of BRCA1/2 mutations in Low-ER patients was not significantly different from that in TNBC patients, with 12.8% of TNBC patients carrying the mutations (p=0.666). Age-stratified BRCA prevalence in the TNBC group was 33.6% for patients ≤40 years, 59.8% for those 41-60 years, and 6.6% for those >60 years, with no significant differences from the Low-ER group (p=0.328, 0.868, and 0.729, respectively). In the Low-ER group, patients with a family history of breast or ovarian cancer had a significantly higher mutation rate (51.4%) compared to those without (15.3%), with an odds ratio of 5.851 (95% CI 2.796-12.243, p<0.001).
Conclusion: The study reveals that the prevalence of BRCA1/2 mutations in Low-ER patients is comparable to that in TNBC patients. This finding supports the extension of BRCA1/2 mutation testing guidelines to include Low-ER patients, similar to those for TNBC, to enhance treatment planning.
Reference
Park, W.K., et al., Long-term oncologic outcomes of unselected triple-negative breast cancer patients according to BRCA1/2 mutations. NPJ Precis Oncol, 2024. 8(1): p. 96.

P3-02-07: Breast Cancer Polygenic Risk Score and Patient Survival Outcomes Among Caucasians

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Introduction: Genome-wide association studies have identified many common, low-penetrance variants associated with breast cancer risk. This has led to the development of polygenic scores (PGS) to aggregate the effects of individual variants for risk prediction. However, the genetic determinants of breast cancer-related outcomes remain largely unknown. We hypothesize that breast cancer patients with high PGS are at an increased risk of experiencing a second breast-related adverse event, such as recurrence, contralateral breast cancer, or death.
Methods: The study is based on the Pathways Study, which is a prospective cohort study of breast cancer survivors enrolled shortly after diagnosis in 2006-2013 at Kaiser Permanente Northern California, with ongoing follow-up. Genome-wide genotype data were available

from 3,995 patients for calculation of 4 PGS, including PGS313, PGS4k, PGS5k and PGS6m. Scores were categorized into tertiles to investigate their associations with 7 survival outcomes including recurrence, contralateral second primary breast cancer, other second primary cancer, and death. Hazard ratios (HR) and 95% confidence intervals (CI) were derived from multivariable Cox proportional hazards regression models. The models were adjusted for age at diagnosis, race and ethnicity group, tumor stage, tumor grade, IHC subtype, surgery, radiotherapy, chemotherapy, and endocrine therapy.
Results: The median age at diagnosis was 60 (23.6-94.8) years and most (68%, n= 2696) patients self-identified as Non-Hispanic whites. Many women had estrogen receptor (ER)+ (83.4%), stage I and II tumors (89%), with 13.3% HER2+ tumors. The majority (60%) received lumpectomy and some type of adjuvant therapy (44.3% radiotherapy, 47.0% chemotherapy, and 74.6% received hormonal therapy). By December 31, 2021, the median follow-up time was 10.5 (0.2-14.2) years. In the overall cohort, there were 504 recurrences, 419 total second primary cancers (146 contralateral and 237 non-breast), and 762 deaths (352 breast cancer-specific). Among Non-Hispanic whites, there were 333 recurrences, 322 total second primary cancers (113 contralateral and 184 non-breast), and 558 deaths (224 breast cancer-specific).
In the overall cohort, breast cancer patients with high (T3) PGS313 had a higher risk of recurrence (HR 1.29, 95% CI: 1.02-1.61, p=0.04), all-cause death (HR 1.28, 95% CI: 0.96-1.31, p=0.03), total breast cancer events (HR 1.29, 95% CI: 1.04-1.54, p=0.02) and invasive breast cancer events (HR 1.34, 95% CI: 1.09-1.63, p=0.006) compared to those with low (T1) PGS313. Although breast cancer-specific death was observed to be higher among those with high PGS313 in the overall cohort, it was not statistically significant (HR 1.34, 95% CI: 0.99-1.73, p=0.007).
In the subgroup analysis, it was observed that Non-Hispanic White breast cancer patients with high (T3) PGS313 had similar higher risk of recurrence (HR 1.29, 95% CI: 1.06-1.83, p=0.04) but not contralateral breast cancer (HR 1.22, 95% CI: 0.76-1.94, p=0.58) or other second primary cancers (HR 1.01, 95% CI: 0.77-1.34, p=0.86). They also had a higher risk of all-cause death (HR 1.30, 95% CI: 0.99-1.41, p=0.04), breast cancer-specific death (HR 1.53, 95% CI: 1.08-2.15, p=0.03), and invasive breast cancer (HR 1.33, 95% CI: 1.06-1.71, p=0.02). The other three PGS were not associated with survival outcomes in either the overall or Non-Hispanic White cohorts examined.
Conclusions: Breast cancer patients with higher PGS313 scores were found to have an elevated risk of recurrence, invasive breast cancer events, and death. These results suggest that breast cancer PGS may have additional prognostic utility in patients diagnosed with the disease, indicating the need for further investigation.

P3-02-08: Incorporating Breast Radiomics and Body Mass Index to Improve Breast Cancer Risk Prediction in Minority Women

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Introduction: Obesity and increased breast density are both independent risk factors for the development of breast cancer. However, mammographic density and body mass index (BMI) are inversely related. We previously demonstrated that an artificial intelligence (AI)-based radiomics method, such as a convolutional neural network (CNN) applied to screening mammograms, can improve breast cancer risk prediction when combined with established clinical risk factors, particularly among Black and Hispanic women. We evaluated whether the addition of BMI and CNN would improve performance of the Breast Cancer Surveillance Consortium (BCSC) model, version 2, among racially/ethnically diverse women undergoing screening mammography.
Methods: We conducted a retrospective cohort study among women, age 35-74, who underwent screening mammography at Columbia University Irving Medical Center (CUIMC) in New York, NY between 2014 and 2018 and who had available BMI data and mammograms evaluable for CNN analysis. We extracted data from the electronic health record (EHR) on age, race, ethnicity, first-degree family history of breast cancer, prior breast biopsy results, mammographic density, height, weight, and BMI. We compared the predictive performance of two hybrid models against the traditional BCSC model using area under the receiver operating characteristics curves (AUCs) and the DeLong test. Hybrid model 1 added CNN risk scores (score 0-1) to traditional BCSC clinical risk factors, and hybrid model 2 added BMI and CNN risk scores to the BCSC clinical risk factors.
Results: Among 14,558 evaluable women, mean age was 56.2 years (SD, 9.6 years) with 27.2% non-Hispanic White, 35.4% Hispanic, 9.4% non-Hispanic Black, 4.5% Asian, and 23.5% other/unknown race/ethnicity. Almost half (43.6%) of women were obese. Within five years of the baseline mammogram, 99 women were subsequently diagnosed with invasive breast cancer. Compared to the performance of the BCSC model (AUC=0.656, 95% confidence interval [CI]=0.602-0.711), hybrid model 2 (BCSC + CNN + BMI) had a significant improvement in breast cancer risk prediction (AUC=0.695, 95% CI=0.643-0.747, p=0.031). In particular, adding CNN and BMI to the BCSC model significantly improved breast cancer risk prediction among non-Hispanic Black and Hispanic women. Compared to the performance of the BCSC model among non-Hispanic Black women (AUC=0.556, 95% CI=0.341-0.770), hybrid model 1 (BCSC + CNN) and hybrid model 2 performed better (AUC=0.838, 95% CI=0.732-0.944, p=0.033 and AUC=0.846, 95% CI=0.756-0.936, p=0.019, respectively). A similar pattern was observed for Hispanic women for the BCSC model (AUC=0.600, 95% CI=0.514-0.687), hybrid model 1 (AUC=0.661, 95% CI=0.572-0.750, p=0.059) and hybrid model 2 (AUC=0.673, 95% CI=0.588-0.758, p=0.031).
Conclusions: Among women undergoing screening mammograms, the addition of BMI and CNN risk scores to the BCSC risk model improved breast cancer risk prediction, especially among racial/ethnic minorities. The new BCSC model, version 3, now incorporates BMI with improved breast cancer risk prediction, especially among obese women. More accurate breast cancer risk prediction may improve personalized screening and prevention strategies for diverse populations.=

P3-02-09: Correlation between risk groups and molecular subtype of breast cancer with metabolic syndrome and ethnicity

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Background: Breast cancer (BC) is the most common malignancy among women worldwide. Comprehensive genomic profiling has been widely used to identify molecular biomarkers and signatures for developing novel therapeutic strategies. BC is a complex disease; besides the genomic and molecular characterization of tumors, racial/ethnic classification and body mass index (BMI) are key determinants of treatment outcomes. To explore gene-to-phenotype relationships, we obtained transcriptomic and clinical data from 978 patient samples comprising all four BluePrint® (BP) subtypes: Luminal A, Luminal B, HER-2, and Basal, representing ~25% of each genomic subtype. We investigated the association of biomarkers of inflammation, apoptosis, oxidative stress, autophagy, and ER stress in patients with BC and correlated them with BMI, menopausal status, and ethnicity.
Method: The FLEX registry (NCT03053193) enrolled 14000 patients with stage I-III BC across 90 institutions in the United States and stratified risk groups based on the 70-gene signature (MammaPrint®, (MP)) and molecular subtype based on the 80-gene signature (BP). We obtained transcriptomic (110 genes) and clinical data from 978 patient samples, representing ~25% of each genomic subtype. Principal component analysis (PCA) was conducted using the 'PCAtools' package in R to reduce the gene expression data's dimensionality and identify major variation patterns among the samples. Analysis of variance (ANOVA) was performed between groups using the first five principal components (PCs). The family-wise error rate was set at 0.05, using the modified Holm-Bonferroni approach.
Results and Conclusion: Of the 978 patients (Caucasians:81.7%; African Americans:2.58%; other races: 5.7%), the risk or genetic profile was as follows: MP: Ultra-Low = 76 (8%), Low = 176 (18%), High1 = 315 (32%), and High-2 = 411 (42%); BP: Luminal A 250 (26%), Luminal B = 250 (26%), HER2 = 228 (23%), and Basal = 250 (26%). The mean age was 58.84±13.04, the average BMI was 30.04±7.34, and the majority were postmenopausal (n = 717, 73%). The first five PCs accounted for 63.11% of the total variance of the dataset. These five PC loadings (variable contributes) were enriched for genes associated with various cellular mechanisms, including glucose metabolism, cellular growth, resistance to cell death, angiogenesis induction, oxidative stress handling, and epigenetic modifications. Interestingly, the pairs plot comparing PC1 through PC5 showed a certain degree of clustering and PC4 (total variance of 4.59%) indicated distinct clustering for each molecular subtype. The biplot for PC1 and PC4 showed a certain degree of separation when intrinsic molecular subtypes and risk of recurrence categories were used. SOD2, KLK5, KLK7, and IL8 showed a strong positive correlation with PC4, whereas GLI1 showed a strong negative correlation with PC4. The loading plot also showed a positive loading for SOD2, indicating a significant role of SOD2 in differentiating distinct molecular subtypes. Comparisons of PCs

with clinical variables showed that PC4 was significantly correlated with all baseline clinical variables, including age ($p < 0.001$), BMI ($p = 0.001$), race ($p < 0.001$), menopausal status ($p = 0.001$), molecular subtype ($p < 0.001$), and risk of recurrence ($p < 0.001$). Additionally, PC4 was also found to be significantly correlated with tumor size ($p < 0.001$), lymph node status ($p < 0.001$), and the presence of distant metastasis ($p < 0.001$).
In conclusion, our findings demonstrate that genes in PC4 play a crucial role in discriminating known molecular profiles when different risk categories, as it is significantly associated with baseline characteristics, and risk of recurrence. Further analysis should be conducted to explore genes that contribute to PC4, such as SOD2. These results highlight the potential of SOD2 as a prognostic biomarker and a therapeutic target in breast cancer.

P3-02-10: Evaluation of a polygenic risk score as a predictor of breast cancer, triple-negative breast cancer, and early-onset disease in Hispanic women.

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Background: Hispanic women in the U.S. have a lower incidence of breast cancer (BC) when compared to non-Hispanic white (NHW) women. However, Hispanic women diagnosed with BC tend to be younger, have more advanced disease at presentation, and have a higher risk of BC mortality compared to NHW women. Some studies have suggested that these differences may be due, in part, to a higher prevalence of aggressive subtypes, including triple-negative BC (TNBC). More accurate risk prediction methods are urgently needed to identify young Hispanic women with elevated risk of BC.
Incorporating polygenic risk scores (PRS) into clinical models can substantially improve risk assessment, but most PRS have demonstrated poor performance among non-European ancestries. We previously described a multiple-ancestry PRS (MA-PRS) based on 56 ancestry-informative and 329 BC-associated single-nucleotide polymorphisms (SNPs). MA-PRS predicts overall BC risk for diverse populations by characterizing genetic ancestry at each BC SNP and applying ancestry-specific SNP risks and frequencies.
Here, we evaluated the extent to which MA-PRS improves upon clinical factors for the prediction of overall BC, TNBC, and early-onset (< 50 years of age) disease in a large, independent cohort of self-reported Hispanic women.
Methods: We examined clinical and genetic records from self-reported Hispanic women referred for hereditary cancer testing from 8/22 – 9/23 and negative for pathogenic variants in BC-associated genes. The association of MA-PRS with overall BC, TNBC and early-onset disease was analyzed using multivariable logistic regression adjusted for personal and family cancer history, age, and genetic ancestry. Analyses were conducted within the full cohort and the subpopulation of patients < 50 years old. Odds ratios (OR) are reported per standard deviation (SD) with 95% confidence intervals (CI).
Results: 12,384 Hispanic women met the study eligibility criteria. A total of 2,071 (16.7%) were diagnosed with BC. Of those, 876 (42.3%) were diagnosed with early-onset BC, 196

(9.5%) were diagnosed with TNBC, and 86 (4.2%) were diagnosed with early-onset TNBC. Most (59.7%) of those diagnosed with BC had no family history of breast or ovarian cancer. MA-PRS significantly improved upon clinical factors for the prediction of overall BC (OR 1.63; 95% CI 1.53-1.73), early-onset BC (OR 1.62; 95% CI 1.49-1.76), TNBC (OR 1.42; 95% CI 1.22-1.67) and early-onset TNBC (OR 1.48; 95% CI 1.18-1.86). This effect of MA-PRS on risk stratification compares favorably to the 1.4 OR per SD reported in the current literature for mammographic density, which is widely recognized as an important risk factor.
Conclusions: MA-PRS substantially improved upon clinical factors for the prediction of overall BC, TNBC and early-onset disease in a large cohort of Hispanic women. Incorporation of MA-PRS into BC risk assessment has the potential to improve BC survival through more accurate identification of women at high risk.

P3-02-11: CDH1 genotype exploration in phenotype-first approach with hereditary lobular breast cancer syndrome

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Background: Pathogenic or likely pathogenic (P/LP) germline CDH1 variants confer a significant risk for diffuse gastric cancer (DGC) and lobular breast cancer (LBC) development within the known hereditary diffuse gastric cancer (HDGC) syndrome. Classically, LBC is part of this pleiotropic inherited cancer predisposition. However, in some circumstances, LBC can be the first (and unique) manifestation in P/LP germline CDH1 carriers, also in the absence of diffuse gastric cancer (DGC) manifestation. Recently, we introduced a newly concept, the so-called “hereditary lobular breast cancer” (HLBC) syndrome. HLBC is defined in this context by the presence of a P/LP CDH1 variant in either an isolated individual with early-onset LBC, or in women with bilateral LBC, or in a family with one or more LBC cases in first-degree or second-degree relatives, but no known DGC in either situation.
Objectives: The aim of this phenotype-genotype study was to assess the frequency of germline CDH1 variants in women with the HLBC phenotype fulfilling pre-established clinical criteria. Genomic inactivation was also analyzed in matched tumor samples in germline CDH1 variant carriers, and the association of genetic profiles with clinical-pathological data and survival. The BRCA1 and BRCA2 genes were also tested to verify a possible association (or exclusion) between CDH1 HLBC and the hereditary breast-ovarian cancer syndromes in these families. The main outcome was to define an accurate estimate of prevalence of germline CDH1 variants among patients with HLBC and the association of somatic sequence alteration with HLBC syndrome.
Methods: This cohort study was approved by the European Institute of Oncology ethical committee, and all available participants gave their written consent to be included in the study. The single-center, longitudinal, prospective cohort study was conducted from January 1, 1997, to December 31, 2021. Women with LBC observed at the European

Institute of Oncology, Milan, Italy, were included. Testing for germline CDH1, BRCA1, and BRCA2 genes was performed. Somatic and epigenetic profiling were assessed for germline CDH1 carriers, exploring intragenic loss of heterozygosity (iLOH), promoter CDH1 methylation and second somatic variant. Additional 324 genes were explored in tumor samples using the FoundationOne CDx assay. The Kaplan-Meier method and a multivariable Cox proportional hazards regression model were applied for overall and disease-free survival analysis.
Results: Of 5429 cases of primary LBC, HLBC phenotype was reported for 1867 (34.4%). A total of 394 women with LBC were available for genetic testing, among whom 15 germline CDH1 variants in 15 unrelated families were detected. Among these variants, 6 (40.0%) were P/LP, with an overall frequency of 1.5% (6 of 394). Of the 6 probands with P/LP CDH1 LBC, 5 (83.3%) had a positive family history of BC and only 1 (16.7%) had sporadic juvenile early-onset LBC (with age at diagnosis ≤45 ys). No germline BRCA1 and BRCA2 variants were identified in CDH1 carriers. An inactivating CDH1 mechanism (second hit) was revealed in 4 of 6 explored matched tumor samples (66.7%) in P/LP germline carriers. In HLBC tumor samples, structural alterations (iLOH and mutations) were identified with a higher frequency (86%), respect to epigenetic mechanisms (methylation). We noted a high accumulation of genomic aberrations in HLBC samples compared with sporadic LBCs. The P/LP CDH1 LBC variant carriers had a significantly lower age at diagnosis compared with the group carrying CDH1 variants of unknown significance or likely benign (42.5 [IQR, 38.3-43.0] vs 51.0 [IQR, 45.0-53.0] years; P=.03).
Conclusions: In this cohort study, P/LP germline CDH1 variants were identified in women not fulfilling the classic clinical criteria for HDGC screening, suggesting that detection of these variants may suggest a novel approach to test women with LBC with early age at diagnosis and/or positive family history of BC.

P3-02-12: Hereditary breast cancer risk assessment and referral at a high-volume, diverse mammography clinic

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Background: Despite treatment innovations and decreasing cancer mortality, Black and White patients (pts) in the United States have persistent inequities in breast cancer outcomes. Non-Hispanic Black women have the highest incidence rates of breast cancer between 20 and 39 years of age. The American College of Radiology (ACR) recommends breast cancer risk assessment for all women, particularly Black women, by the age of 25. In the St. Louis metropolitan area, pts residing in the North and Eastern regions with high Black populations are more likely to have late-stage breast cancer diagnoses.
Methods: In 2023, the Breast Cancer Equity Group at Siteman Cancer Center began a program to give on-site real-time screening mammography results and a hereditary cancer

risk assessment delivered by a breast health nurse navigator to pts in North County. During the under-30-minute waiting period from completion of the mammogram to the delivery of results, the nurse navigator asked pts 5 family cancer history questions developed by medical oncology, genetics, surgical oncology, and public health researchers based on NCCN guidelines. If they would meet NCCN criteria for genetic counseling and testing, they were referred to a high-risk breast clinic. We performed retrospective chart review on all pts screened to determine screening outcomes of high-risk vs normal-risk pts and which pts were previously flagged as high-risk in the electronic medical record (EMR).
Results:
Between September 2023 and April 2024, 154 pts were seen in the same day screening mammography and breast cancer risk assessment clinic, of whom 72.1% (111/154) self-identified as Black and 19.5% (30/154) were under the age of 50. Over one third of the pts coming in for a routine screening mammogram - 34.4% (53/154) - were determined to be high-risk for breast cancer, of whom 69.8% (37/53) were Black. All pts that screened as high-risk were offered referral to the high-risk breast clinic by the navigator. 75.5% (40/53) of the high-risk pts agreed to be referred to this clinic for further evaluation. In the high-risk group, 11.3% (6/53) of the pts had a mammogram at the appointment requiring follow-up, defined as BI-RADS 0, and 3.8% (2/53) were diagnosed with breast cancer. None of the pts requiring further imaging were lost to follow-up. In the normal risk group 3.0% (3/99) had abnormal mammograms and 1.0% (1/99) were diagnosed with breast cancer. In pts under the age of 50, 50% (15/30) screened as high-risk, 73.3% (11/15) of whom were Black. 75.5% (40/53) of the high-risk pts had relevant cancer family history data already documented in the EMR, but only 9.4% (5/53) had their cancer risk mentioned in the EMR by a physician. In this high-risk cohort, whose family cancer history was generally documented in the EMR, 54.7% (29/53) were overdue for a mammogram.
Discussion: While it is imperative that we understand the socioeconomic factors behind the ongoing racial inequities in breast cancer, it is also important to consider potential differences in cancer gene mutation prevalence between Black and White pts and their associated risks for cancer development at a young age. Our findings in this population highlight the need for more widespread genetic risk screening, as over a third of the pts screened positive as high familial risk for breast cancer. It may not be enough to rely on the EMR, as most of these pts had documented family cancer history but very few were flagged as high-risk. It is also key to consider risk screening at a younger age as per ACR guidelines, as 50% of the women under age 50 were classified as high-risk by family history yet over half were overdue for a mammogram. Our future implementation science projects will focus on disseminating this risk assessment to more clinics including primary care, bolstering the existing infrastructure to further support the high-risk breast clinic, and improving communication on screening benefits in the community to improve timely screening mammography.

P3-02-13: Global disparities in breast cancer screening programs and breast cancer mortality

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Background: Breast cancer screening is effective in reducing breast cancer mortality. Nevertheless, there is a lack of data on whether having a national breast cancer screening program has an impact on breast cancer mortality. In this study, we performed a comprehensive assessment of the associations between breast cancer screening programs and the mortality attributable to breast cancer in the 194 countries.
Methods: Global breast cancer screening data were collected from the WHO Global Health Observatory database. Correspondingly, breast cancer mortality data were procured from the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) 2019 database. Our analysis incorporated 48,960 data points embedded within 1632 cohorts, to estimate annual changes in breast cancer mortality at global, regional, and national levels from 2015 to 2019. We used a joinpoint regression model with a data-driven Bayesian information criterion method for calculating the annualized changes in breast cancer mortality. The significance level of the difference in mortality between countries with and without screening programs was assessed using bootstrap methods.
Results: Over the 2015-2019 period, about half of the countries (97 out of 194) reported implementing breast cancer screening programs that target the general population. Notably, countries with screening programs showed a significant annual reduction in age-standardized breast cancer mortality rates compared to countries lacking regular or consistent screening programs (mean difference: 1.18 [95% UI, 0.86-1.49]). In 2019, countries with breast cancer screening programs (18.6 deaths [95% UI 17.4-19.7] per 100,000) had 4.0 fewer deaths (95% UI: 2.0-6.1) per 100,000 population compared to countries without screening programs (22.6 deaths [95% UI 21.0-24.3] per 100,000). The reduction in mortality rates was particularly distinct among postmenopausal women aged 50-74 years, exhibiting a decline of 10.1 deaths (95% UI, 4.4-16.1) per 100,000 population in countries with screening programs. Regionally, during 2015-2019, breast cancer mortality rate in countries with regular screening programs was reduced by 1.27% (95% UI 0.13%-2.41%) per year in Sub-Saharan Africa and 0.85% (0.13%-1.57%) per year in Latin America and the Caribbean compared to the countries without such regular programs.
Conclusion: Countries with breast cancer screening programs experienced a significant reduction in breast cancer mortality. To effectively reduce breast cancer mortality on a global scale, it is imperative to encourage the adoption of national breast cancer screening programs and prioritize the expansion of screening coverage.

P3-02-14: Clinicopathogenomic analysis of Japanese metastatic breast invasive lobular carcinoma

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Objective: To analyze clinicopathogenomic data registered in C-CAT and to examine the genomic landscape of invasive lobular carcinoma and whether there is a significant difference between invasive ductal carcinoma (IDC) and lobular carcinoma ILC. Methods: From June 2019 to February 2024, 4084 metastatic breast cancer patients underwent cancer gene panel testing, were registered in C-CAT, and consent for secondary utilization was obtained (C-CAT Ver. 20240219). Of these, 3,114 were IDC (Luminal:1,578(50.7%), Luminal-HER2: 239(7.7%), HER2:152(4.9%), TN:908(29.2%)), and 210 were ILC (Luminal:142 (67.6%), Luminal-HER2:5(2.4%), HER2:3(1.4%), TN:45(21.4%)). RESULTS: Among ILC patients, 53.8% had CDH1 alterations, whereas only 1.9% of IDC patients had CDH1 alterations. CDH1 alterations were significantly more frequent in patients with ILC, as reported previously ($p < 0.0001$). Among ILC patients, 7.1% had ESR1 alterations, while 13.3% of IDC patients had ESR1 alterations ($p = 0.01$). The ratio of patients with high tumor mutational burden was significantly higher in the ILC group than in the IDC group (37/210 (17.6%) vs. 310/3114 (10.0%), $p<0.0001$). According to the alterations in the AKT pathway, 52.4% of ILC patients had PIK3CA alterations, whereas 34.5% of IDC patients had PIK3CA alterations ($p< 0.0001$). However, the AKT1 and PTEN did not differ significantly between the two groups. ARID1A alterations were significantly more common in the ILC group than in the IDC group (21/210 (10.0%) vs. 154/3114 (4.9%), $p =0.003$). MDM4 alterations were more common in the ILC group than in the IDC group (24/210 (11.4%) vs. 208/3114 (6.7%), $p =0.013$). Alterations in TP53, CDK12, GATA3, and FGFR1 were significantly more common in the IDC group than in the ILC group: 59.6% vs. 38.6% for TP53 ($p<0.0001$), 6.6% vs. 1.0% for CDK12 ($p=0.002$), 14.8% vs. 5.7% for GATA3 ($p=0.0004$), and 11.8% vs. 6.7% for FGFR1 ($p=0.03$). In the ILC group, the frequency of peritoneal metastases did not differ between the CDH1 alteration and non-alteration groups (21/113(18.6%) vs. 21/97(21.7%), $p=0.70$). However, ERBB2 non-alteration group were significantly more common peritoneal metastases than ERBB2 alteration group (39/174(22.4%) vs. 1/32(3.1%), $p=0.002$). Furthermore, the relationship between each alterations and overall survival was not significantly different when comparing overall survival with and without CDH1, ERBB2, and PIK3CA in the ILC group (CDH1:110 months vs 97 months, ERBB2:94 months vs 110 months and PIK3CA: 111 months vs 95 month, respectively, log-rank $p=0.821$, 0.183, and 0.672, respectively). Conclusions: This study revealed the genomic landscape of invasive lobular carcinoma in Japanese metastatic breast cancer. It is essential to understand the application of NGS-based genomic testing to support invasive lobular carcinoma.

P3-02-15: Combined Therapy of Progesterone Receptor Antagonist and COX-2 Inhibitor Prevents BRCA1-Deficient Mammary Tumorigenesis in Mice: A Novel Synthetic Lethal Strategy for Breast Cancer Prevention

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Background: we previously demonstrated that high-dose ulipristal acetate (UPA), a progesterone receptor (PR) antagonist, prevented tumors in a BRCA1-deficient mouse

model. For a clinically relevant translation, this study aimed to test the efficacy of low-dose UPA (5mg) alone and in combination with celecoxib (400mg), a COX-2 inhibitor, in Ad-K8-Cre Brca1f22-24/f22-24; Trp53f2-10/f2-10; R26Y (BPY) mice. This model allows tracing of tumor initiating cells (TICs) which are BRCA1; p53-deficient cells (YFP+) from initiation through premalignant stages and in tumors. We hypothesized that combination therapy enhances low-dose UPA efficacy.
Methods: 2-month-old female BPY mice received intraductal Ad-K8-Cre injections. 10 days later they were randomized into four groups (no-drug control, UPA, celecoxib, and combination; 12 mice/group), and monitored for tumor formation for 21-months. Treatments were incorporated into their diet (Inotiv inc.). Cancer preventive efficacy of drug treatment compared to the control was analyzed using the log-rank test (SAS v9.4) and survival analysis (R package). The coefficient of drug interaction (CDI) is calculated using tumor-free survival % endpoint. Premalignant glands from 1- and 5-month cohorts (3 to 4 mice/group/cohort) were analyzed using fluorescence-activated cell sorting (FACS) and RNA sequencing to identify drug-modulated genes and pathways (P adjusted <0.05).
Results: Combo-therapy (UPA + celecoxib) showed excellent tumor-preventive efficacy whereas monotherapy of each drug failed. At 16 months, tumor incidence was similar among monotherapy and control groups (55-58%), while the combination group showed 8% incidence (p=0.02; HR = 0.12, 95% CI = 0.03-0.55). At 21 months, tumor incidence was lowest in the combination group (33%), followed by UPA (64%), control (73%), and celecoxib (92%) (p=0.03; HR = 0.29, 95% CI = 0.09-0.93). Combo-therapy resulted in a 92% tumor-free survival rate at 500 days, significantly greater than the pure additive effect for monotherapy (36% [UPA] + 42% [celecoxib]), with a CDI of 0.06, indicating strong synergy (CDI <1). FACS analysis revealed that combo- therapy blocked the expansion of TICs (EpCAM+ YFP+), unlike monotherapy. Bulk RNA-seq analysis of TICs from the 1-month cohort showed that combo-therapy significantly increased luminal marker expression (Esr1, Pgr) and suppressed Tnfsf11 (RANKL), basal markers, and EMT genes. Single-cell RNA-seq of the 5-month cohort showed that luminal progenitors become dominant in TICs and combo-therapy upregulated the nonsense-mediated mRNA decay (NMD) pathway across multiple cell types and downregulated cancer-associated pathways (Wnt/β-catenin, TNFα via NF-κB, PI3K/AKT/mTOR, ECM organization) in epithelial cells.
Conclusions: The combo-therapy of UPA and celecoxib showed superior efficacy in preventing cancer in a BRCA1-deficient mouse model, demonstrating strong synergy. These findings provide novel insights into the biology underlying BRCA1-associated tumorigenesis and support the clinical potential of PR antagonists and anti-inflammatory agents as a novel synthetic lethal strategy for breast cancer prevention in high-risk women.

P3-02-17: tRF-1432–a novel target for reversing chemoresistance in breast cancer

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Chemoresistance is a significant challenge in the field of oncology and is the leading cause of treatment failure in breast cancer. Cancer cells could adapt to hypoxia microenvironment through metabolic reprogramming, while developing a strong drug resistance phenotype at the same time. However, the mechanism under which is still largely unknown. Efforts need to be done to identify novel targets and treatment modalities to address the complex issue of chemoresistance in breast cancer. In this study, we set our sights on an upregulated tRNA-derived fragment, tRF-1432, while we screened through high-throughput sequencing in breast cancer hypoxia models, especially in drug-resistant breast cancer cells/tissues. tRFs are a new category of small non-coding RNAs that occur when pre-tRNAs or mature tRNAs are selectively sheared by enzymes under certain pressure, and which play crucial roles in tumorigenesis. Further investigation on the biological roles and clinical value of tRF-1432 has been done. Overexpression of tRF-1432 is positively correlated with tumor cell proliferation and anti-apoptosis ability, and significantly increased chemoresistance of breast cancer in vitro and in vivo. Immunoprecipitation experiments confirmed the direct bond between tRF-1432 and RBMS1, knockdown of RBMS1 may deprive the tumor-promoting and chemoresistance properties of tRF-1432. Furthermore, in tRF-1432 overexpression breast cancer cells we observed a significant decrease of CCL3, a gene which could induce the transition in the phenotype of macrophages into a proinflammatory one. As a crucial member of tumor microenvironment (TME), macrophages participate in the mediation of therapeutic responses. Silencing tRF-1432 could facilitate M1 macrophage polarization and enhance the response to doxorubicin chemosensitivity in breast cancer. Mechanismly, tRF-1432 decreased the stability of CCL3-mRNA with the cooperation of RNA binding protein RBMS1, thereby reduced M1 macrophage polarization and promote the progression and chemoresistance breast cancer via CCL3-CCR5 signaling. In conclusion, our study clarified the role of hypoxia-induced tRF-1432 in breast cancer development and chemoresistance, and elucidate the molecular mechanism of which, aiming to provide a novel target for reversing chemoresistance in breast cancer.

P3-02-18: Multi omics reveals silencing of Argininosuccinate synthase and upregulation of nucleotide biosynthesis in tamoxifen resistance invasive lobular carcinoma

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Background: Invasive Lobular Carcinoma (ILC) constitutes a hormone receptor positive molecular subtype of breast cancer, that are distinct from Invasive Ductal Carcinoma (IDC), primarily due to E-cadherin loss, and slow proliferation rate. Clinical management of ILC patients presents significant challenges due to its advanced stage at diagnosis, resistance to conventional therapies, and late recurrence. Endocrine therapy remains the cornerstone for treating ILC patients; however, the emergence of endocrine resistance resulting in late recurrence poses a substantial clinical obstacle. ILC is an understudied subtype of breast cancer, where therapies tailored for IDCs are used to treat ILC. Understanding endocrine

resistance in ILC is thus of utmost importance as majority of these studies were conducted in IDC.
Objective: To elucidate the mechanisms underlying endocrine resistance in ILC.
Methods: We have developed tamoxifen resistant (TAMR) variants of ILC cell lines (MDA-MB-134-VI and SUM44PE). Both pairs of the parental and TAMR cell lines were subjected to Metabolomics and RNA-sequencing analyses. Growth kinetics was performed by viable cell counting over 120 hours. Gene knockdown was performed using shRNA technology. Promoter methylation was studied by Methylation-Specific PCR. Drug effects were studied using MTT assay. Human breast cancer patient data from TCGA_BRCA (The Cancer Genome Atlas) and the METABRIC Invasive breast Carcinoma cohort was used for survival analysis.
Results: TAMR cells exhibited a two-fold increase in IC50 for tamoxifen compared to the parental cells and significant increase in growth rate and migration. Overlap of metabolomic and RNA seq data revealed deregulation of alanine, arginine, and glutamate (AAG) metabolism, purine metabolism, and arginine and proline metabolism in both MB134-TAMR and SUM44-TAMR cells. Among the 15 commonly dysregulated genes within these pathways, downregulation of Argininosuccinate Synthase 1 (ASS1) significantly correlated with poor overall survival and distant metastasis free survival in ILC patients only if treated with endocrine therapy. Our study showed methylation-mediated silencing of the ASS1 promoter in TAMR cells and in ILC cells grown for long-term in estrogen-deprived media. Treatment of TAMR cells with a demethylating agent, decitabine (5-Aza-2'-Deoxycytidine), restored ASS1 expression. Knock down of ASS1 in parental cells increased IC50 for tamoxifen. Untargeted metabolomics revealed increased nucleotide intermediate levels in TAMR cells. Additionally, TAMR cells exhibited higher expression of several key enzymes in nucleic acid biosynthesis pathways, including PAICS, PRPS1, ADSS2, CAD, and DHODH. Activating phosphorylation of CAD (Ser1859), a key multienzyme protein in pyrimidine biosynthesis pathway was observed in TAMR cells. Pretreatment with decitabine or combined treatment with DHOD inhibitor Farudodstat, increased growth inhibitory effect of tamoxifen in TAMR cells.
Conclusions: Our study unveils ASS1 downregulation as a novel mechanism underlying acquired resistance to tamoxifen and establishes a metabolic link between ASS1 and nucleic acid biosynthesis. Methylation mediated silencing of ASS1 is prompted when estrogen signaling is blocked, diverting one of its substrates, aspartic acid, to nucleic acid biosynthesis and facilitating cell growth.
Significance: These findings offer potential therapeutic strategies to overcome tamoxifen resistance by either ASS1 demethylation or targeting nucleic acid biosynthesis, thereby improving treatment outcomes for ILC patients. Further validation of the combined therapy is warranted in diverse experimental models and patient samples.

P3-02-19: Simultaneous loss of BRCA2 and RB1 results in growth disadvantage but strong CDK4/6i resistance in HR+/HER2- breast cancer

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Background: Genetic instability and dysregulation of the cell cycle are hallmarks of cancer. In advanced HR+/HER2- breast cancer, the standard therapy involves cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) which directly target aberrant growth by inhibiting the CDK4/6-RB1-E2F axis. In combination with endocrine therapy, CDK4/6i substantially extend progression-free survival (PFS), however, therapy resistance is frequently observed in the clinical setting.
Inactivating mutations or loss of the breast cancer susceptibility gene 2 (BRCA2) have been associated with breast cancer for a long time and are observed in 30-40% of sporadic breast cancer cases. Additionally, patients with germline BRCA2 pathogenic variants (gBRCA2 PV) show decreased PFS on CDK4/6i combined with endocrine therapy. However, gBRCA2 PVs may not directly lead to CDK4/6i resistance. Instead, the proximity of the genes BRCA2 and retinoblastoma protein 1 (RB1) on chromosome 13 might result in simultaneous loss of both. Since RB1 is a direct downstream target of CDK4/6, its inactivation leads to significant resistance against CDK4/6 inhibition.
Methods: In the proposed project, we used a lentiviral-based CRISPR/Cas9 system to model the loss of BRCA2 and RB1 alone and in combination in HR+/HER2- breast cancer cell lines and examined the impact on cell proliferation and treatment sensitivity.
Results: The loss of RB1 resulted in increased proliferation, whereas the loss of BRCA2 did not affect growth rates. Interestingly, the combined loss of both genes led to significantly reduced proliferation. However, under therapeutic pressure, loss of RB1 was dominant and led to strong resistance towards the CDK4/6i Palbociclib. Nevertheless, Olaparib, a PARP inhibitor targeting DNA damage repair, remained effective in RB1- and especially in BRCA2-deficient cells where DNA damage repair mechanisms are already compromised.
Discussion: Our results indicate that the loss of RB1 and BRCA2 significantly impacts cell proliferation, with the inactivation of both genes leading to a disadvantage in cell growth. We hypothesize that this is due to the impaired ability to repair DNA double-strand breaks. BRCA2 is crucial for homologous recombination repair, while RB1 is involved in the non-homologous end joining pathway. We believe that the inactivation of both genes increases reliance on other error-prone repair pathways, such as single-strand annealing or microhomology-mediated end joining (MMEJ, also known as alternative end joining). This could also explain the heightened response to Olaparib, which, among other PARP proteins also targets PARP1, a component of the MMEJ repair pathway.
While simultaneous inactivation of both genes leads to growth disadvantage, potentially because of the diminished capacity to repair DNA-double strand breaks, our data suggests that they still retain a strong advantage under treatment pressure and that clones harbouring both mutations evolve during the time of CDK4/6i treatment.

P3-02-20: CDK4/6 inhibitor resistance in patients with ER+ breast cancer: Identification and characterization of resistance mechanisms in thirty-two XPDX models

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Background: Resistance to CDK4/6 inhibitors (CDK4/6i) has been well studied with several mechanisms identified, including loss of RB, FAT1, and ERa and altered expression of regulatory genes such as CCND1, CCNE1/2, and CDK2. Additionally, alterations responsible for resistance may be linked to duration of CDK4/6i treatment. Previously (AACR2023), we reported on seventeen ER+ breast XPDX models representing patients who responded to CDK4/6i for up to twelve months (RES12), and greater than one year (RES13+). To better understand if duration of clinical treatment correlates with unique resistance mechanisms, we established and characterized an additional fifteen breast XPDX models from patients post-CDK4/6i and compared molecular profiles and drug sensitivities in the panel of thirty-two models.

Methods: Seventeen breast models were previously established and characterized which included 8xRES12 and 9xRES13+. Fifteen new models were established from thirteen patients: eleven originated from fluid samples of which seven were designated as ductal (ST4137L, ST4322C, ST4322D, ST6023B, STM185, STM188, ST188B) and four as lobular carcinomas (ST4680D, ST6133, STM229F, ST383F); three were established from core biopsies (ST4534B, lymph node; ST5400, breast; STM354, liver), and one from circulating tissue (STM223B), all designated as ductal carcinomas. Of the fifteen new models, six were classified as RES12 and nine as RES13+. These models were passaged and challenged with palbociclib to confirm resistance. Receptor expression was determined by IHC and genomic analyses, including WES and RNAseq, were performed to identify mechanisms of resistance. For in vivo studies, CDK4/6i were dosed via oral gavage, once daily at 50 mg/kg; endpoints included tumor volume (TV) and time from treatment initiation (TTI) with %T/C values and tumor regression reported at study completion; a %T/C of ≤20 versus control was considered sensitive. Tumor regression (%T/C<0) versus Day 0 TV was also reported.

Results: Clinical time to progression (TTP) for RES12 (n=6) was four to twelve months and RES13+ (n=9) from thirteen to thirty-six months. All models, except ST4322C and ST4322D, retained ER expression in evaluated passages with similar histology compared with archival clinical samples. Sequencing identified several variants, including RB1 and/or PTEN loss or deletion, and increased gene expression in CCND1, CCNE1, and the PIK3CA/AKT pathway. PIK3CA mutations were reported in 7/15 models; mutations in RAF1, ESR1, FGFR and other genes were also identified across the panel, some more prevalent based on clinical TTP. Most models were resistant to fulvestrant and insensitive to some or all evaluated CDK4/6i treatments.

Conclusion: We have expanded our platform of breast XPDX from patients post-CDK4/6i treatment to thirty-two models and characterized each based-on duration of clinical treatment, molecular profiles, and drug sensitivities. We have also identified potential mechanisms of resistance based on CDK4/6i therapy and TTP. This platform is a valuable resource for developing novel therapies for CDK4/6i-resistant patients.

P3-02-21: Generation of Sacituzumab Govitecan Resistant Breast Cancer Models

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Treating triple-negative breast cancer (TNBC) presents a challenge due to its resistance to several conventional therapies, driven by its lack of expression of estrogen, progesterone, and HER2. Sacituzumab Govitecan (SG), an antibody drug conjugate (ADC) targeting Trop2, has recently been approved for treating TNBC and estrogen receptor positive (ER+) breast cancer after prior systemic treatments. It is largely undefined how cancer cells from different intrinsic subtypes become resistant to SG, therefore these studies focus on the development of model systems to define mechanisms of resistance to this targeted therapy. We hypothesize that SG resistance is driven by variation in the expression of Topoisomerase 1, Trop2, and other proteins involved in SG internalization. To test this hypothesis, we performed a set of studies that incorporated ER+ and TNBC patient-derived xenografts (PDXs). Bulk- and single-cell RNA-sequencing analyses from a set of 15 PDXs and some syngeneic, drug resistant pairs, found that most models had similar Trop2 RNA levels, however TNBCs had significantly higher Trop2 protein expression. Ex vivo dose-response analyses of PDX cells with SG found that TNBCs were more sensitive to SG at lower drug concentrations. In vivo testing of PDX tumors for SG response identified subsets of tumors that were resistant to SG and those that had varying levels of sensitivity. Further studies characterized SG response in several metastatic models, which will serve in identifying truly resistant models and will help to normalize any treatment effects from drugs that could potentially overcome SG resistance. Interestingly, some of the PDXs that were sensitive to SG became resistant after the PDX had acquired carboplatin resistance. Initial bulk RNA sequencing of PDXs with innate or acquired SG resistance have revealed differences in gene expression related to claudin genes, endosome formation, Topoisomerase 1 levels, and Trop2 localization. Ongoing studies are contrasting mechanisms of SG resistance with acquired insensitivity to other therapeutics and defining pharmacological approaches to inhibit SG refractory metastases.

P3-02-22: The role of circular RNAs in triple-negative breast cancer and chemotherapy resistance

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Background: Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related death among women worldwide. Triple negative breast cancer (TNBC) represents 15-20% of all incident breast cancers and is a highly aggressive subtype. Neo(adjuvant) chemotherapy (NAC) is standard for early stage TNBC; however, for those with residual breast cancer following NAC, recurrence is common and associated with few effective therapeutic approaches. Thus, identification of novel resistance

mechanisms and alternative treatment strategies remain an unmet clinical need. Circular RNAs (circRNAs) are a newly identified class of largely noncoding RNA molecules with covalently closed circular structures. Recent studies suggest that circRNAs play crucial roles in regulating tumor development, progression and chemoresistance. However, the role of circRNAs in mediating chemotherapy resistance remains unknown.

Methods: As a first step towards identifying circRNAs that participate in the development of chemoresistance, and to determine if targeting such circRNAs may be an efficacious strategy, doxorubicin-resistant (Doxo-R), paclitaxel-resistant (PTX-R), and double-resistant (DP-R) cell lines were generated from MDA-MB-231 cell lines. Human circRNA microarrays were utilized to profile the expression of approximately 14,000 known circRNAs in normal breast tissue, matched TNBC patient-derived xenografts (PDX) generated prior to and following NAC, and TNBC chemosensitive and chemoresistant cell lines. Top hits were validated using RT-PCR. Bioinformatic approaches were employed to identify circRNA-miRNA-mRNA networks regulated by differentially expressed circRNA transcripts. A reverse genetics approach was also employed using CRISPR to interrogate the contributions of over 4000 specific circRNA in driving the development of resistance to doxorubicin and paclitaxel.

Results: circRNA microarray profiling identified 429 and 310 transcripts differentially expressed in doxorubicin and paclitaxel resistant cells, respectively, compared to parental chemosensitive cell lines ($|FC| \geq 1.5$; p value < 0.05). Given that circRNAs can act as microRNA (miRNA) sponges, thereby inhibiting miRNA activity, we computationally identified circRNA-miRNA pairs, and assessed the abundance of mRNA transcripts predicted to be targets of paired miRNAs via RNA-seq. A differentially expressed circRNA-miRNA-mRNA network was further constructed. Dysregulated canonical pathways and gene ontology (GO) enrichment analysis were determined from these networks and revealed multiple GO term included negative regulation of miRNA transcription, miRNA and RNA metabolic process, and endoplasmic reticulum-associated degradation (ERAD) pathway. The CRISPR-Cas13 screen is ongoing at this time but results will be available prior to the meeting.

Conclusions: We have identified cancer specific circRNAs in TNBC cell lines, PDX models and patient tumors in the context of chemotherapy sensitive and chemotherapy resistant disease. Specific biological processes, pathways and signaling cascades have been implicated in the down-stream consequences of dysregulated circRNAs and in the potential development of resistance. Our ongoing studies seek to identify specific circRNAs sufficient to drive resistance to chemotherapy. Outcomes of these studies highlight the potential of specific circRNAs to serve as predictive/prognostic biomarkers and novel therapeutic targets for advanced forms of TNBC.

P3-02-23: Evaluation of ribociclib, abemaciclib and palbociclib resistance in ER+ breast cancer cells reveal novel therapeutic opportunities in ER+/HER2- breast cancers

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Backgrund: The cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) ribociclib, abemaciclib and palbociclib, have transformed the lives of patients with ER+/HER2− metastatic breast cancer (BC). However, most patients will eventually progress on treatment. The discovery of novel targets and optimization of therapy beyond CDK4/6i is an area of ongoing investigation. We conducted a comprehensive evaluation in CDK4/6i resistant BC cells and aimed to discover novel markers of resistance for therapeutic application.
Methods: The ER+/HER2− BC cell lines, MCF7 and T47D were chronically treated with increasing dose of ribociclib (R), abemaciclib (A) or palbociclib (P) over 8 months (0-600nM). CDK4/6i resistant cell lines (MCF7RR, MCF7RA, MCF7RP, T47DRR, TD7DRA, T47DRP) were isolated. CDK4/6i resistant and sensitive cells were evaluated for spheroid forming ability, cell cycle progression, apoptosis, cross resistance to other CDK4/6i, whole genome RNA sequencing, and cisplatin/volasertib (PLK1 inhibitor)/barasertib (Arora kinase B inhibitor) sensitivity. In addition, immunohistochemical evaluation of PLK1 and Aurora kinase B expression was completed in a clinical cohort of 4000 early-stage BC specimens.
Results: MCF7RR, MCF7RA, MCF7RP, T47DRR, TD7DRA, T47DRP cells manifested aggressive phenotypes with increased spheroid forming ability, progressed through G1/S phase of cell cycle despite CDK4/6i treatment and were resistant to apoptosis. Cross resistance to other CDK4/6is and cisplatin resistance was evident. Whole genome RNA sequencing revealed upregulation of several genes involved in cell cycle regulation, cell signalling, immune regulation, metabolic reprogramming and others across all resistant models compared to controls. Polo-like kinase 1 and Aurora kinase B were consistently overexpressed in MCF7RR, MCF7RA and MCF7RP cells compared to controls. Whereas CDKN4B and TGFβ were upregulated in T47DRR, TD7DRA, T47DRP cells compared to controls. MCF7RR, MCF7RA and MCF7RP cells, whilst resistant to CDK4/6i, remain sensitive to volasertib (PLK1 inhibitor) and barasertib (Aurora Kinase B inhibitor) therapy which was associated with G2/M cell cycle arrest and increased apoptosis. In clinical cohorts, PLK1/CDK4 and PLK1/CDK6 co-expression influence clinical outcomes in BC patients.
Conclusion: In ER+/ HER2− BCs, pathways to resistance to CDK4/6i therapy are complex and diverse. PLK1 or Aurora Kinase B targeting could be an attractive therapeutic strategy in certain CDK4/6i resistant ER+ BCs.

P3-02-24: Quantitative changes in hormone receptor- and Ki67-levels in response to short-term neoadjuvant endocrine therapy in breast cancer

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Background: A majority of breast cancers (BC) are hormone receptor-positive (HR+), expressing estrogen receptors (ER) and/or progesterone receptors (PR). Patients with HR+ BC benefit significantly from endocrine therapy. However, up to 25% of these patients develop endocrine-resistant disease, which remains a major cause of breast cancer mortality. Neoadjuvant endocrine therapy (NET) offers a valuable opportunity to identify favorable responders and those at risk for endocrine resistance. Quantitative image analysis of multiplex-stained tumors holds the potential for greater insights into these dynamics.
Methods: We conducted multiplex immunohistochemical staining for ER, PR, proliferation marker Ki67, and cytokeratin (CK) on 36 paired tumor specimens from our recently completed interventional clinical trial of short-term neoadjuvant endocrine therapy (NET) in patients with early-stage HR+/HER2- BC (NCT03219476). Optimized immunohistochemistry (IHC) protocols using OPAL reagents (Akoya) were performed on a Leica Bond Rx autostainer. ER, PR, and Ki67 levels in cancer cells within representative tumor regions of interest were quantified using QuPath image analysis software on Akoya PhenoImager scanned images. Pre- and post-NET data were analyzed using paired t-tests. Patient demographics and clinicopathological information have been published. Briefly, 36 patients with cT1-T3, cN0 HR+/HER2- BC were treated with ~4 weeks of NET prior to surgery. Median age was 64yrs (range 42-81), median pre- and post-NET tumor sizes were 1.3 cm (range 0.5-7.7) and 1.2 cm (range 0.09-4 cm), respectively. Six patients had pN1 at surgery, one had pN1mic disease, and one patient achieved a complete pathologic response (pCR).
Results: Post-NET, computed residual cancer cell Ki-67 positivity ≥10% was detected in 11 cases (11/36), indicative of endocrine resistance. Intermediate Ki-67 positivity (2.7–10%) was observed in 7 cases (7/36), while Ki-67 positivity was ≤2.7% in 18 cases (18/36), indicative of complete cell cycle arrest.
The Preoperative Endocrine Prognostic Index (PEPI) score, comprising post-NET tumor size, lymph node status, computed ER, and Ki67 positivity, was employed as a prognostic biomarker. A favorable prognostic PEPI score of 0 was observed for 9 patients, while 23 patients had an intermediate PEPI score of 1-3, and 4 patients had a PEPI score >4. All 9 patients with PEPI score of 0 and 6 patients with PEPI score of 1-3 had > 6.7-fold (85%) reduction in cancer cell %Ki67-positivity after 4 weeks of NET thus identifying a total of 15 patients with highly ER-dependent proliferation. The median reduction in cancer cell Ki67-positivity was 3.1-fold (67%) in the remaining 15 patients with PEPI score 1-3 and 1.7-fold (41%) in the 4 patients with PEPI score >4.
Single-cell analysis of Ki67-positive cancer cells demonstrated a significant reduction in the fraction of ER-positive proliferating cells following NET (median 6.3% post-NET vs. 41.5% pre-NET; $p<0.001$), indicating a shift towards ER-independent proliferation. Multiplex

single-cell metrics could be valuable in further distinguishing patients with tumors that respond to treatment from those at risk of developing endocrine resistance.
Conclusions: Short-term neoadjuvant endocrine therapy is a promising strategy to distinguish between tumors that are responsive to treatment and those with inherent or emerging endocrine resistance. The use of multiplex immunofluorescence and quantitative digital pathology offers the potential for more accurate identification of endocrine resistance. This approach may also help uncover molecular mechanisms that can be targeted by combination treatments, enhancing therapeutic outcomes for patients with hormone receptor-positive BC.

P3-02-25: CRISPR/Cas9 knockout of ALDH1A1 increases radiation response in triple-negative breast cancer

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Currently, there are no targeted therapies for triple-negative breast cancer (TNBC).1 While radiation therapy plays a crucial role in breast cancer treatment, its effectiveness against TNBC is hindered by acquired resistance to radiation over time. This highlights the need for more sensitive and specific therapeutic approaches.2 ALDH1A1 is associated with stemness and poor outcomes in TNBC and is a potential new target for treatment. ALDH1A1 contributes to chemoresistance in TNBC3, and we propose that it also promotes radiation resistance through both its antioxidant properties and regulation of cancer stemness. Previous studies have indicated that the radiation resistance observed in TNBC is linked to increased levels of ALDH1A14, suggesting it plays a protective role against radiation-induced cell death. In this study, we did a CRISPR/Cas9 knock out (KO) of ALDH1A1 in SUM 159 cells and derived single cell clones to screen for the effects of ALDH1A1 KO. Confirmation of ALDH1A1 KO was done at the genomic level by Sanger sequencing and at the protein level by western blotting analysis. The response of ALDH1A1 knockout clonal derived cells to radiation was analyzed using colony forming units (CFU), proliferation assays (Alamar blue), and Annexin V/PI staining. All the ALDH1A1 knockout clones analyzed showed enhanced radiation response with reduced proliferation, and colony formation units post-radiation, as compared to the parental cells. Furthermore, tumor initiation was measured in the parental cells and ALDH1A1 KO clones using the organoid culture system platform. Overall, ALDH1A1 KO clonal derived cells formed fewer tumoroids post-radiation, as measured by cell proliferation assays in a 3D culture system. Together these findings highlight the critical relationship between ALDH1A1 expression and radiation sensitivity, emphasizing the potential of ALDH1A1 as a target for improved treatment of TNBC.

P3-02-26: Sex-biased cancer cells induce endocrine therapy resistance and immune suppression in male breast cancer

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Sex differences in breast cancer（BC）lead to distinct clinical and molecular characteristics. Sex-associated heterogeneity relies intricately on the oncogenic properties of cancer cells and multicellular interactions in tumor microenvironments. Here we conducted a multiomic analysis encompassing single-cell RNA sequencing, spatial transcriptomics and large-scale histological analysis, combined with genetic and pharmacological perturbations to systematically develop a high-resolution and spatially resolved map of intratumoral expression heterogeneity in male breast cancer（MBC）. Among the malignant cells, we identify 9 consensus meta-programs (MPs, for example, estrogen response and interferon response), each consisting of dozens of genes that are coordinately upregulated in subpopulations of cells within MBC. Of these, MP5 exhibits significant male bias, characterized by aberrant activation of the estrogen receptor α (ERα) signaling pathway, inducing endocrine therapy resistance in MBC. Bidirectional interactions between MP5 malignant cells and immunosuppressive cancer-associated fibroblasts form an immune exclusionary microenvironment mediated by LRBA. Collectively, our study highlights the endocrine therapy resistant and immunosuppressive role of LRBA in MBC, and these findings may provide insights into the molecular features of MBC and facilitate the development of sex-specifical treatment strategies.

P3-02-27: circAXIN1 promotes EMT-mediated metastasis and doxorubicin resistance via Wnt/β-catenin pathway by regulating miR-486-3p/BCL11A axis in TNBC

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Circular RNAs (circRNAs) represent a novel type of regulatory RNA characterized by high evolutionary conservation and stability. CircRNAs are expected to be potential diagnostic biomarkers and therapeutic targets for various malignancies. However, the regulatory functions and underlying mechanisms of circRNAs in triple-negative breast cancer (TNBC) are largely unknown.
Using a high-throughput microarray assay, this study identified a novel circRNA, circAXIN1(hsa_circ_0005838), which was upregulated in TNBC tissues and cell lines. We found that circAXIN1 expression was increased in doxorubicin-resistance TNBC tissues and cells. Functionally, we demonstrated that circAXIN1 significantly promoted epithelial-mesenchymal transition(EMT)-mediated cell migration and invasion and enhanced doxorubicin resistance in vitro and in vivo. Mechanistically, RNA pull-down assay, RNA immunoprecipitation, and dual luciferase reporter assay disclosed that circAXIN1 was identified as a miR-486-3p sponge. We also found that BCL11A was a direct target of miR-486-3p, which functioned as an oncogene in breast cancer. Thereby, circAXIN1 upregulated BCL11A protein expression and accelerate TNBC progression. The rescue experiments also revealed that miR-486-3p overexpression or BCL11A knockdown partially reversed the regulatory functions of circAXIN1 on TNBC cell migration, invasion, and doxorubicin resistance. Furthermore, we showed that circAXIN1 promoted BCL11A expression via competitive interaction with miR-486-3p and then activated the Wnt/β-catenin signaling pathway. Additionally, in vitro and in vivo functional experiments demonstrated that circAXIN1/miR-486-3p/BCL11A axis promoted TNBC EMT-mediated metastasis and

doxorubicin resistance via activating the Wnt/β-catenin signaling pathway.
Collectively, our findings clarified a hitherto unexplored mechanism of the circAXIN1/miR-486-3p/BCL11A axis in EMT-mediated tumor metastasis and doxorubicin resistance in TNBC.

P3-02-28: A combination approach to target residual tumors via ferroptosis induction

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Background: Selection pressure from chemotherapy can result in small populations of resistant cells, even in tumors with apparent clinical response. These residual tumor cells can tolerate cytotoxic treatments, persist in a reversible, semi-dormant, diapause-like state, and ultimately drive tumor recurrence. Pharmacologically targeting these residual tumor cells has the potential to prevent or significantly delay tumor relapse, but it remains a clinical challenge.
Methods and Results: We report ferroptosis induction as a targeting strategy to eradicate residual tumor cells following chemotherapy. Through focused chemical library screening using an in vitro residual tumor model, we identified ferroptosis inducers that are effective against the residual tumor cells, but not the bulk proliferating tumor cell population. Ferroptosis induction eradicated residual tumor cells generated by treatments with various chemotherapeutic agents and radiation across multiple breast cancer subtypes. The hypersensitivity to ferroptosis induction in residual tumor cells is driven by elevated levels of ferrous irons, the catalyst of ferroptosis. To evaluate the therapeutic potential of ferroptosis induction in vivo, we established residual tumors in triple-negative breast cancer (TNBC) PDX models following treatment with doxorubicin and cyclophosphamide (AC). In TNBC PDX models, we demonstrated that ferroptosis induction with the single agent RSL3, a glutathione peroxidase 4 (GPX4) inhibitor, effectively eliminated the residual tumor cells and significantly delayed tumor recurrence post-chemotherapy. The time from initiation of treatment to recurrence for AC vs. AC+RSL3 groups was: 65.3+/-3.9 vs. 81.6+/-5.4 days, p=4.1E-06, for PDX-3107, and 81.4+/-4.9 vs. 102.9+/-12.3 days, p=1.8E-06, for PDX-3887. Importantly, simultaneous suppression of the two parallel ferroptosis defense mechanisms mediated by GPX4 and ferroptosis suppressor protein-1 (FSP1), through the combined use of RSL3 and iFSP1, further delays tumor recurrence in PDX models. The time to recurrence in AC vs. AC+RSL3/iFSP1 groups was 49.8+/-8.4 vs. 113.1+/-5.8 days, p=5.0E-09, for PDX-3204. Notably, combined treatment with RSL3/iFSP1 led to complete clearance of the residual tumors in 9 out of 11 mice bearing PDX-3887, with no tumor recurrence observed up to four months of follow-up.
Conclusion: Our study demonstrates that ferroptosis induction is an effective strategy for eradicating residual tumors and delaying tumor recurrence in TNBC following cytotoxic chemotherapy.

P3-02-29: The mechanism of acidic microenvironment promotes tumor-associated macrophages secreting glutamine to activate dual signaling pathways of mTORC1 and c-MYC in CDK4/6 inhibitor resistance of ER-positive breast cancer

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Resistance to CDK4/6 inhibitors in ER-positive breast cancer poses a clinical challenge, and the underlying mechanisms remain unclear. ER serves as a crucial regulator of glycolysis, promoting tumor progression and resistance by inducing microenvironmental acidification. We observed that an acidic microenvironment induces the polarization of tumor-associated macrophage (TAM) toward the M2 phenotype, leading to the secretion of glutamine. This activation of mTOR promotes resistance to CDK4/6 inhibitors in ER-positive breast cancer. Concurrently, glutamine promotes the upregulation of c-MYC in breast cancer cells, inducing lactate production and exacerbating microenvironmental acidification. We propose a scientific hypothesis: the acidic microenvironment drives TAM towards M2 polarization, secreting glutamine. This, in turn, activates both mTOR and c-MYC pathways, promoting resistance to CDK4/6 inhibitors and intensifying tumor microenvironment acidification. Through in vivo and in vitro experiments, we aim to elucidate the role of M2 macrophages in CDK4/6 inhibitor resistance under acidic conditions. We seek to clarify the molecular mechanisms by which M2 macrophages secrete glutamine, mediating CDK4/6 inhibitor resistance in ER-positive breast cancer and worsening microenvironmental acidification. Additionally, we aim to evaluate the sensitizing effects of targeting the acidic microenvironment and glutamine. Successful completion of this study holds the potential to provide a scientific basis for establishing novel approaches to overcome CDK4/6 inhibitor resistance in ER-positive breast cancer.

P3-02-30: PIK3CA hotspot mutations as biomarkers for prognosis and treatment prediction in ER+/HER2- postmenopausal breast cancer patients

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The PIK3CA gene, encoding the p110α catalytic subunit of phosphatidylinositol 3′-kinase (PI3K), is mutated in over 30% of breast cancers. Most mutations concentrate in the hotspots E542K, E545K, and H1047R, leading to hyperactivation of the PI3K/Akt pathway in vitro. In ER+/HER2- breast cancers, we found more than 40% of these mutations. Although PIK3CA is an oncogene, its mutations are linked to a good prognosis. This study explores the prognostic and predictive role of PIK3CA hotspot mutations in low-risk postmenopausal breast cancer patients randomized to receive tamoxifen or no systemic treatment, followed for over 25 years with extensive clinical records.

Mutation analysis was performed using formalin-fixed paraffin-embedded tumors with digital droplet PCR (ddPCR). PIK3CA mutations were detected in 40% of all patients and 45% of the ER+/HER2- subtype. The most frequent mutation was H1047R (23%), followed by E545K (17%) and E542K (6%). Significant associations were found between all hotspot mutations and low grade, PR positivity, HER2 negativity, and high expression scores of a PIK3CA-mutation-associated gene module. Additionally, all mutations were inversely correlated with multigene modules (IMMUNE1 and IMMUNE2), suggesting an immunosuppressive environment. PIK3CA mutations were often found in patients with ultralow risk of distant recurrences according to the 70-gene signature and lower expression of the proliferation marker Ki67.
PIK3CA mutations were associated with longer distant-recurrence-free intervals (DRFI) in all patients in multivariable analysis after adjusting for tumor size, receptor status (ER, PR, HER2), tumor grade, Ki-67, and tamoxifen (HR (95% CI)=0.53 (0.31-0.92)). The combination of PIK3CA mutations and/or low PTEN status also indicated better prognosis (HR (95% CI)=0.53 (0.30-0.92)).
PIK3CA mutations also indicated a good prognosis for patients with low or high risk of developing distant metastases by the 70-gene signature (HR univariate (95% CI)=0.5 (0.29-0.89)) compared to ultralow risk patients (HR univariate (95% CI)=2.5 (0.48-12.92)). Test for interaction P=0.13.
However, among low proliferating Luminal A subtype, PIK3CA mutations were associated with shorter DRFI after adjusting for size and grade (HR (95% CI)=5.7 (0.95-33.8)), compared to the highly proliferating Luminal A (HR (95% CI)=0.24 (0.06-0.98)). Similar results were found when analyzing the combination variables PIK3CA/PTEN or PIK3CA/pAKT in Luminal A.
PIK3CA mutations per se did not have a clear predictive value regarding tamoxifen response.
In conclusion, PIK3CA mutations predominate within the ER+/HER2- subtype, are associated with good clinical markers, and longer DRFI in all patients, especially among highly proliferative and high-risk tumors, but not within ultralow risk patients. Ongoing investigation aims to decipher other factors associated with PIK3CA mutations in patients with indolent tumors who present long-term relapses and may take advantage of new treatment strategies.

P3-03-01: Awareness of Rare Cancers: Hearing and Engaging Rural Communities (ARCHER-C)

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Background: Inflammatory breast cancer (IBC), a rare and NIH-designated cancer health disparity, is a highly aggressive form of breast cancer accounting for 7-10% of all breast cancer deaths. Furthermore, the unique presentation of IBC, lacking a solid mass and characterized by diffuse tumor cell spread, often leads to delays in diagnosis and treatment.

Reproductive risk factors and higher incidence rates among younger women and those from minoritized and marginalized populations, underscore the crucial role of primary care providers (PCP)—including physicians, physician assistants, and nurse practitioners. Social drivers of health (SDoH) further limit healthcare access for these communities. Recognizing that primary care practices are the initial contacts for navigating cancer diagnosis and treatment, we postulated that understanding barriers experienced by PCP and members of the public will foster engagement with large academic medical centers and breast clinics for improved diagnosis and management of IBC. In this study, we describe the development of a platform (ARCHER-C), designed to assess PCP and public knowledge, attitudes and health seeking practices related to IBC in the rural setting, as well as rare cancers more broadly.

Methods: Two surveys were developed with guidance from a Community Advisory Board, comprising members from academic institutions, urban and rural practices, topical experts, and patient advocates. PCP were recruited from FQHC and community practice settings within North Carolina. Mixed methods were used to analyze cognitive interview surveys administered to a convenience sample of participants recruited at-large from rural communities via email, social media; and direct recruitment from an urban practice serving primarily low-income, medically complex patients along with another clinic serving distinct rural communities. All responses were summarized using descriptive statistics.

Results: Data from public (n=34) volunteers self-reporting as Black (45%), White (30%), American Indian/Alaskan Native (24%), and residing in households earning under $50,000/yr (40%) along with PCP (n=30) were analyzed. Salient measures of IBC diagnosis, health disparity factors, referral and care coordination practices, COVID-19 impact, and continuing medical education (CME) were identified barriers at the PCP level. Furthermore, seventy-four percent of public participants had not heard of IBC; 38% did not identify being overweight as a risk factor; 38% were not aware of racial disparities; 50% recognized redness or thickening of the skin as symptoms; and even fewer recognized the characteristic “pitted” appearance of the skin. Participants noted worry about what the doctor may find, difficulty or fear talking to a physician, lack of insurance, transportation barriers, and religious reasons as contributing to delays seeking health care. Most importantly, 97% of the participants expressed their enthusiasm in sharing information about IBC and rare cancers with others, specifically family members and friends. In addition, majority expressed that adding visual aids and providing educational materials about IBC at the time of survey would be helpful.

Conclusions: This community-engaged model highlights the need for bidirectional provider- and public-facing research and education to reduce the burden of IBC and associated disparities. Strengthening the connection between primary care and specialized centers is essential for better diagnosis and management of IBC and other rare cancers, ultimately reducing health disparities. Funding in part by Duke Cancer Institute Community Outreach, Engagement, Equity Seed Grant (GRD, ANT) as part P30 CA014236, Duke MERITS Education Grant & Surgery Funds (GRD). Anh Tran and Gayathri Devi; Co-Senior Authors.

P3-03-02: Effects of socioeconomic status on access to next-generation sequencing in patients with metastatic breast cancer

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Background: Breast cancer is the most common cancer diagnosis in women. Metastatic breast cancer is difficult to treat and is a major cause of mortality related to breast cancer. Treatment includes therapeutic options that target specific molecular signals and pathways responsible for cancer growth and other malignant features. These advances have necessitated new biomarkers that can identify tumor molecular features to select the right patients who will maximally benefit from these therapies. Focused next-generation sequencing (NGS) on DNA isolated from the tumor tissue or circulating tumor DNA in the blood has quickly become standard of care to create actionable and personalized treatment plans. However, these tests are often expensive, limiting their clinical implementation. We hypothesized that limited access to these therapies increases health disparities in clinical oncology.
Objective: The aim of this study is to examine if neighborhood socioeconomic status (SES), as defined by area of deprivation index (ADI), as well as factors including race, ethnicity, and insurance status influence access to NGS testing in the Dallas area.
Methods: Data from 187 patients with recurrent MBC were obtained from the Dallas Metastatic Breast Cancer Study (DMBCS), a clinical database that was established in 2021 at a single academic medical system to track patient demographics, area deprivation index (ADI), race, ethnicity, treatments, and other variables that are not widely available in other national databases for MBC. Ethnicity was categorized into Hispanic versus non-Hispanic consistent with Office of Management and Budget census standards, and race was separated into White, African American, Asian American and Native Hawaiian/Pacific Islander. Commercial NGS testing was performed on patient tumor tissue or circulating tumor DNA from blood samples by Tempus and FoundationOne between the years 2014 through 2022.
Results and Discussion: Overall, 39% of patients in our dataset received NGS testing. Patients who are not Hispanic or Latino (n=140, OR: 3.99, 95% CI: 1.66-9.61) are about 4 times more likely to receive NGS compared to those who are Hispanic or Latino (n=42). Insurance, whether private (OR: 7.35, 95% CI: 2.29-33.08) or public (OR: 4.90, 95% CI: 1.55-21.81), also significantly increased the likelihood of receiving NGS testing compared to those without insurance. Interestingly, race was not a significant factor amongst White (n =131), African American (n=33), and Asian (n=8). Next, we sought to evaluate whether ADI would correlate with access to NGS testing. ADI measurements include education level, employment, housing quality, and income to rank neighborhoods by SES disadvantage; a higher quartile ADI equates to a greater disadvantage. Our data showed that patients in the lowest quartile ADI are 2.5 times more likely to have NGS testing compared to those in the highest quartile (OR: 2.54, 95% CI 1.07-6.20). These results suggest that NGS testing is disproportionately offered to insured patients with a higher SES, particularly those with insurance. Whether these discrepancies are inherent in clinical practice (e.g. physician

awareness) or from legitimate barriers related to access should be defined in future studies. However, identifying that these disparities in NGS testing exists promotes awareness and encouragement for clinicians to offer NGS more broadly.

P3-03-03: Disparities in Quality of Life Among Breast Cancer Survivors in the All of Us Research Program

Gagandeep Kaur, Jiayuan Wang, An D Truong, Hester Nguyen, Leah Puglisi, Carrie Costantini, Ritesh Parajuli, Hannah Lui Park

Background: Breast cancer is the most common cancer among women. Advancements in breast cancer treatment and early diagnosis have resulted in higher survival rates necessitating the importance of studying quality of life (QOL) among breast cancer survivors. QOL is an important endpoint in clinical trials as it provides insights into the overall well being and long-term outcomes of patients. Understanding QOL is also crucial for guiding treatment decisions, supporting survivorship care, and shaping healthcare policy. Previous studies have shown that racial/ethnic disparities in QOL exist, and factors affecting QOL have been investigated. However, inconsistencies remain regarding specific determinants, and a large-scale study examining disparities in QOL among breast cancer survivors in the U.S. has not been done.
Methods: We analyzed data from 2,022 female breast cancer survivors in the National Institutes of Health's All of Us Research Program database. QOL (scored from 1 to 5) was measured using survey response data in which participants answered the question: “In general, would you say your quality of life is – excellent (5), very good (4), good (3), fair (2), or poor (1).” Univariable and multivariable linear regression analyses were performed to identify demographic, socioeconomic and psychosocial factors associated with QOL. The multivariable analysis consisted of using Bonferroni correction and stepwise regression to adjust the p-values for multiple tests and to select the most statistically relevant variables contributing to QOL, respectively.
Results: The cohort was predominantly non-Hispanic White (84%), with the remaining participants being non-Hispanic Black (5.5%), Hispanic (3.1%), Other/Mixed (2.6%), and non-Hispanic Asian (1.6%). The average age was 70 years old. In the multivariable analyses, non-Hispanic Black (β= -0.33, 95%CI: [−0.49, −0.18], $p < 0.005$) and Hispanic (β= -0.38, 95%CI: [−0.58, −0.18], $p < 0.005$) survivors had lower QOL compared to non-Hispanic white survivors. For each additional year of age, the QOL score slightly increased by 0.01 units (95%CI: [0.009, 0.018], $p < 0.001$). Lower education (high school or lower) (β= -0.28, 95%CI: [-0.42, -0.14], $p < 0.005$) and lower household income levels (annual household income less than $25,000K) (β= -0.66, 95%CI: [-0.84, -0.48], $p < 0.001$) were also significantly associated with lower QOL. Interestingly, lacking confidence in filling out medical forms (β= -0.53, 95%CI: [-0.79, -0.28], $p < 0.005$) and feeling that medical providers were not listening (β= -0.22, 95%CI: [-0.29, -0.15 AZ], $p < 0.001$) were associated with lower QOL. Conversely, having assistance with daily tasks all or most of the time (β= 0.32, 95%CI: [0.19,0.45], $p < 0.001$) was linked to a higher quality of life.

Conclusion: In the national All of Us cohort, quality of life was associated with age, race and ethnicity, age, other socioeconomic factors, and psychosocial factors. Enhancing the availability of daily assistance and improving patient-provider communication can help mitigate some of these disparities. Our findings suggest that recognizing populations at risk and connecting them to resources with social work, support groups, and survivorship clinics will impact survivors' quality of life.\

P3-03-04: "Racial Disparities in Endocrine Response Pathway Activity in ER-Positive Breast Cancer: Insights from a Window of Opportunity Trial"

Diederick Keizer, Yvonne Wesseling-Rozendaal, Catherine Klein, Diana Kantrovich, Anna Weinand, Robert Babkowski, Mehran Habibi

Introduction: Mortality for hormone-receptor positive breast cancer is 19% higher among Black American women compared to White American women, despite having a lower incidence among Black women. Although these differences have been attributed to broader social barriers in accessing high-quality medical care, Jones et al. (Breast Cancer Research and Treatment, 2024 203:125–134) suggested different tumor biology is responsible for this outcome discrepancy. In this study, we utilized a novel technology to measure signal transduction pathway activity in breast tumors, generating new insights into the differences in endocrine response between patients of different races.
Materials and Methods: This single-institution window-of-opportunity study was conducted at Johns Hopkins University, Baltimore, USA. The study included 30 women with IHC-ER positive (>75%) and HER2 negative breast cancer who received neoadjuvant endocrine treatment (NAT) for two weeks. The participants comprised 13 White women and 5 Black women. Primary biopsies were subjected to signal transduction pathway analysis using the OncoSIGNal platform (InnoSIGN), which assessed the activity of four signaling pathways: estrogen receptor (ER), androgen receptor (AR), PI3K, and MAPK. A pathway was deemed active when the measured value, including its confidence interval, exceeded the 95th percentile of a previously established breast tissue reference range.
Results: The primary IHC-ER positive/HER2 negative breast cancer samples from Black women exhibited significantly lower ER pathway activity (43.0 ± 8.4) compared to those from White women (60.4 ± 11.2), with a p-value of 0.002. When these values were related to the breast tissue reference range, 92% of White women showed an active ER pathway, while only 20% of Black women had an activated ER pathway. Additionally, some patients in both racial cohorts displayed higher PI3K activity, with the highest PI3K activity observed in Black patients. There were no observed differences in AR and MAPK pathway activities between the races.
Discussion: This study reveals a significant disparity in ER pathway activity between Black and White women despite the fact all breast cancer samples being strongly ER-IHC staining positive. The lower ER pathway activity in Black women could partly explain their less effective response to endocrine therapy compared to White patients, as endocrine treatments specifically target the ER pathway. Furthermore, pathway analysis indicated

other potentially targetable pathways, such as PI3K, in patients with positive ER-IHC staining but inactive ER pathways.
Conclusion: The findings from this window-of-opportunity study highlight the need for larger cohort analyses to validate these initial observations and further investigate the clinical impact of these racial differences in breast cancer biology and treatment response.

P3-03-05: Impact of Comorbidities on Mortality, Length of Stay, and Hospital Charges in Breast Cancer Patients: A National Inpatient Sample Analysis

Kalaivani Babu, Srinishant Rajarajan, Kriti Dhamija, Vasuki Anandan, Charmi Bhanushali

Background: Breast cancer remains a significant public health challenge, particularly due to its association with high morbidity and mortality rates. Comorbid conditions, as quantified by indices like the Charlson Comorbidity Index (CCI), play a crucial role in influencing clinical outcomes, including mortality, length of hospital stay (LOS), and hospitalization charges. This study utilizes data from the 2021 National Inpatient Sample (NIS) to investigate these outcomes in breast cancer patients, aiming to provide insights for improving patient management and resource allocation.
Methods: We analyzed data from the NIS 2021 database, identifying breast cancer patients using the ICD-10 code C50. The primary outcome of interest was mortality, while secondary outcomes included LOS and total hospitalization charges. The analysis employed survey-weighted methods in Stata software to account for the complex survey design.
Mortality was assessed through overall rates and adjusted odds ratios (ORs) using logistic regression, accounting for variables such as age, sex, race, income quartile, CCI, hospital location, region, teaching status, and bed size. LOS and hospital charges were evaluated through mean calculations and adjusted regressions to identify the impact of demographic and hospital factors.
The Charlson Comorbidity Index was categorized as follows: 0 for no comorbidities, 1 for one comorbidity, 2 for two comorbidities, and ≥3 for three or more comorbidities.
Results: The study included a population size of 140,824.842 breast cancer patients. The overall mortality rate was 5.65% (95% CI: 5.36% - 5.94%).
Key findings from the logistic regression showed that the Charlson Comorbidity Index had a significant impact on mortality with an OR of 1.226 ($p<0.001$), indicating a higher risk of death with increasing comorbidities.
For LOS, the mean was 5.70 days (95% CI: 5.61 - 5.79 days). Significant factors influencing LOS included age (coefficient = 0.015, $p<0.001$), Black race (coefficient = 0.722, $p<0.001$), and the Charlson Index (coefficient = 0.301, $p<0.001$), indicating longer hospital stays with more comorbidities.
The mean total hospitalization charges were $74,939.30 (95% CI: $72,361.40 - $77,517.19). Significant factors affecting charges included Native American race (coefficient = $13,904.88, $p=0.002$) and the Comorbidities. The regression analysis indicated that each additional point in the Charlson Comorbidity Index was associated with an increase in

charges (coefficient = $2,609.05, p<0.001). Hospital characteristics also played a role, with teaching hospitals (coefficient = $8,097.84, p=0.004) and larger bed sizes (coefficient = $18,040.43, p<0.001) being associated with increased charges.
Conclusion: This study underscores the critical role of comorbidities in influencing the outcomes of breast cancer patients. Higher Charlson Comorbidity Index scores are associated with increased mortality, extended hospital stays, and higher hospitalization charges. The findings highlight the need for tailored management strategies to address the burden of comorbidities, aiming to improve patient outcomes and optimize healthcare resource utilization. The variability in LOS and hospital charges across demographic and hospital characteristics suggests potential areas for targeted interventions to enhance healthcare efficiency and patient care.

P3-03-06: Treatment and Survival Outcomes for Transgender and Gender-Diverse Patients with Breast Cancer from 2004–2022 in the National Cancer Database

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Introduction: Cancer disparities exist in transgender/gender-diverse (TGD) populations but there are limited data on the association of gender identity with breast cancer (BC) outcomes amongst TGD persons. Using the National Cancer Database (NCDB), we aimed to compare differences between TGD and cisgender patients with BC by examining 1) patient demographics and tumor clinicopathologic variables, 2) treatment characteristics, and 3) five-year overall survival (OS).
Methods: This retrospective cohort study queried the NCDB for TGD and cisgender patients diagnosed with BC from 2004–2022. Self-identified gender identity was abstracted from patient medical records when available. A random unmatched cisgender cohort was selected to explore differences in patient demographics, tumor clinicopathologic variables, and treatment characteristics compared to TGD patients. Next, a matched cisgender cohort was selected, matching on year of BC diagnosis, patient age, sex assigned at birth, race, Hispanic ethnicity, American Joint Committee on Cancer (AJCC) disease stage, and tumor receptor status to compare treatment and OS by gender identity. TGD patients with unknown sex assigned at birth were matched to cisgender female patients for the matched analysis. Chi-squared tests with Bonferroni corrections and multivariable logistic regression models were used to examine associations of variables with gender identity. Kaplan-Meier methods assessed five-year OS by gender.
Results: Of 4,376,089 patients with BC, 4,338,258 (99.1%) were female, 37,579 (0.9%) were male, and 252 (0.006%) were TGD. TGD patients had a median age of 51 years (IQR 42–61), most were non-Hispanic White (65.1%), had no medical comorbidities (78.6%), had health insurance (85.0%), and resided in the West (34.5%) or Northeast (23.8%) United States. Half (55.6%) of TGD persons were assigned female at birth, 20.2% were assigned male sex at birth, and 24.2% had an unknown sex assigned at birth. Of all TGD patients, 52.5% had

hormone-receptor positive (HR+)/HER2- tumors and 55.2% were node-negative.
Compared to a random unmatched cisgender cohort (n=252), TGD patients were younger (median age 51 years (IQR 42–61) vs 64 years (IQR 54–72), p<0.001) and had more persons assigned male sex at birth (20.2% vs 0.4%, p<0.001). While TGD patients more frequently had T2 tumors (24.2% vs 17.5%, p=0.008), there was no difference in disease stage between the TGD and cisgender cohorts (p=0.053). TGD patients with HR+ disease (n=201) received endocrine therapy less often than cisgender patients (58.7% vs 80.2%, p<0.001). On regression analysis, TGD patients were more likely to be diagnosed with BC at age <40 years compared to cisgender patients (OR 3.41; 95% CI 1.63–7.16, p=0.001) and had lower odds of receiving endocrine therapy for HR+ disease (OR 0.27; 95% CI 0.15–0.53, p=0.004).
Compared to a matched cisgender cohort (n=211), TGD patients with HR+ disease received endocrine therapy less often (58.5% vs 67.6%, p=0.04), a finding that remained significant on regression analysis (OR 0.50; 95% CI 0.30–0.82, p=0.032). Five-year OS was lower for the TGD cohort compared to the matched cisgender cohort (84.6% vs 91.7%, p=0.041) and persisted after excluding TGD patients with an unknown sex assigned at birth (n=154, 84.1% vs 93.9%, p=0.026).
Conclusions: Despite similar stage at diagnosis and tumor receptor status, TGD patients with BC were diagnosed at a younger age compared to their unmatched cisgender counterparts, were less likely to receive endocrine therapy for HR+ BC, and had inferior five-year OS compared to their matched cisgender counterparts. Strategic research is needed to elucidate the etiology of these disparities and develop targeted interventions to optimize treatment and outcomes for TGD persons with BC.

P3-03-07: Efficacy of Immunotherapy in Older Patients with Triple-Negative Breast Cancer: A Systematic Review

Marie Liu, Carrie Sha, Diana Lake, Jasmeet Singh, Mark Robson, W. Iris Zhi

Introduction: Immunotherapy (IO) has emerged as a promising treatment option shown to improve outcomes for patients with triple-negative breast cancer (TNBC), however, there exists a notable gap in our understanding of its efficacy within the older patient population. In this systematic review, we examined randomized clinical trials (RCTs) addressing the response to IO in older patients with TNBC.
Methods: We searched Medline ALL (Ovid), Embase (Elsevier), Cochrane Central (Wiley), ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform. The searches combined terms reflecting (1) the immune checkpoint inhibitors atezolizumab, durvalumab, and pembrolizumab; (2) breast cancer; and (3) RCTs. Results were limited to publications in English from 2013 to September 2023. Only articles that reported efficacy were included. Risk assessment was performed and overall risk of bias was graded. Two independent reviewers rated each study based on graded scales, and conflicts were resolved by a third reviewer.
Results: Our systematic review identified 18 full-text papers on 11 unique RCTs. Only four

RCTs presented results for patients 65 and older: KEYNOTE 355, KEYNOTE 522, IMPASSION 130, and IMPASSION 131. Of the first three RCTs, a total of 530 patients out of 2923 patients (18.1%) were 65 and older. The overall risk of bias was rated low or intermediate in all 11 RCTs.
In the early-stage TNBC setting, 131 of 1174 patients (11.2%) were 65 and older in KEYNOTE 522. The reported primary endpoint pathological complete response (pCR) was significantly improved in patients in the overall intention to treat (ITT) population in the first interim analysis (IO arm n = 401 patients; control arm n = 201 patients) who received pembrolizumab versus placebo (estimated treatment difference 13.6%, 95% CI 5.4 – 21.8%, p<0.001). Of the 602 total patients, 71 patients (11.8%) were 65 and older (IO arm n = 46; control arm n = 25), the treatment difference was 22.3% (95% CI -2.1 – 43.5%, p=N/A). At the fourth interim analysis (IO arm = 784 patients; control arm = 390), the co-primary endpoint event-free survival (EFS) in ITT was 84.5% at 36 months follow up, with hazard ratio (HR) 0.63 (95% CI 0.48 – 0.82, p<0.001). In patients 65 and older, the HR was 0.79 but not statistically significant (95% CI 0.40 – 1.56, p=N/A). In IMPASSION 031, atezolizumab in the early-stage setting was shown to have a significant improvement in the primary endpoint pCR in the ITT population (IO arm n = 165; control arm n = 168; rate difference of 17%, 95% CI 6–27%, one-sided p=0.0044).
In the metastatic setting, KEYNOTE 355 showed that for a total of 323 patients with Combined Positive Score (CPS) ≥ 10, median OS was 23.0 months in the IO arm compared to 16.1 months in the control arm (HR 0.73, 95% CI 0.55 – 0.95; p=0.0185) at median 44.1 months follow-up. In patients 65 and older with CPS ≥10 (n = 66), median OS was 28.3 months in the IO arm (n= 42) and 12.6 months in the control arm (n= 24) (HR 0.51, 95% CI 0.28 – 0.92, p=N/A).
In the metastatic setting, atezolizumab has not been shown to significantly improve OS in the ITT overall population or age-specific groups <65 or ≥65 in the final analysis of IMPASSION130.
Conclusion: There is a significant gap in data from RCTs evaluating the efficacy of IO in older patients with TNBC. The only FDA-approved IO in TNBC, pembrolizumab, was shown in the early-stage setting to improve pCR and EFS in older patients but was not statistically significant. In the metastatic setting, pembrolizumab significantly improved OS in older patients with CPS ≥10. Both studies had a limited number of patients 65 and older. No other studies reported statistically significant outcomes in patients ≥65. These results underscore the need for further research in older patients.

P3-03-08: Clinical Characteristics of Male Breast Cancer by Race and Factors Predicting Survival: An updated analysis

Jayasree Krishnan, Malak Alharbi, Guangwei Yuan, Kristopher Attwood, Arya Mariam Roy

Introduction Male breast cancer (MBC) is extremely rare and represents less than 1% of breast cancer (BC). While racial disparities in outcomes for female BC are well known, limited studies have investigated these disparities among MBC patients (pts). Some studies

indicate that Black race is associated with increased mortality in MBC, whereas conflicting results are observed in other studies. We aim to analyze the clinical characteristics of MBC by race and identify factors predicting survival in MBC pts. Methodology We queried the National Cancer Database for MBC pts diagnosed during 2004-2020. The demographic (age, race, education, income), clinicopathological (tumor stage, grade, nodal status, charlson Deyo comorbidity class (CDCC), hormone receptor status) and treatment characteristics (chemotherapy (CT), radiation(RT), hormone therapy (HT) and surgery (Sx)) were summarized by race. Statistical comparisons between racial groups were made using Kruskal-Wallis and Pearson chi-square tests, as appropriate. The overall survival (OS) by race was summarized using a Kaplan Meier curve. Multivariate analyses were conducted using the cox regression model to identify factors predictive of survival in MBC pts. Results A total of 23,758 MBC pts were identified, of which 80.5% (n=19161) were White (W), 13% (n=3054) Black (B), 3.5% (n=883) Hispanic (H), 1.5% (n=353) Asian (A) and 1.5% other race. The median age at diagnosis for W was higher compared to other races (67 y in W vs 64 y in A, 63 y in B and 60 y in H (P<0.001). Black pts were more likely to have higher comorbidity burden compared to other groups (CDCC 3+ W: 2.1%, B: 3.8%, A: 2.5%, H: 1.9%, all p<0.001). Black pts were also more likely to have stage IV disease (B: 10%, W: 6.5%, A: 5.7%, H: 7.2%, all p<0.001), larger (cT4 B: 8.9%, W: 5.6%, A: 5.7%, H: 6.3%, all p<0.001) and poorly differentiated tumors (W: 30%, B: 33%, A: 30%, H: 33%, all p<0.001) and node positive disease (B: 27%, W: 17%, A: 19% H:21%, all p<0.001). Whites had more estrogen (ER) (W: 89%, B: 85%, A: 84%, H: 85%, all p<0.001) and progesterone receptor (PR) (W: 81%, B: 74%, A: 77%, H:78%, all p<0.001) positive tumors while Black pts had more HER2 positive (W: 7.7%, B: 9.8%, A: 8.8%, H: 7.6%, all p<0.001) compared to other racial groups. Compared to White, other racial subgroups had more triple negative (W: 2.4%, B: 4.3%, A: 4.2%, H: 4.1%, all p<0.001) tumors. White and Hispanic MBC pts received less CT (W: 38%, B: 44%, A: 41%, H: 34%, all p<0.001) compared to Black and Asians. However, White pts received more HT compared to other racial groups (W: 61%, B: 55%, A: 54%, H: 53%, all p<0.001). The OS rates were lower in Black and higher in Asian pts compared to other racial groups (5 y OS: W: 73%, B: 67%, A: 81%, H: 78%). Univariate analysis showed that Black MBC pts had worse OS rates compared to Whites (HR 1.2 95% CI (CI) 1.09-1.24, P<0.01) however after adjusting for other covariates, no racial differences in survival were noted. Multivariate model showed that age (HR 1.04, CI 1.04-1.05, p<0.001), high comorbidity burden (CDCC3+) (HR 2.3 CI 1.88-2.90, P<0.001), TNBC subtype (HR 1.70, CI 1.35-2.14, p<0.001), poorly differentiated tumor (HR 1.35 (1.23-1.49, p<0.001) were associated with worse OS. Receipt of RT (HR 0.52, CI 0.28-0.98, P=0.04), CT (HR 0.73, CI 0.65-0.81, P<0.001) and HT (HR 0.79 CI 0.71-0.88, P<0.001) were associated with improved OS. Interestingly, compared with lumpectomy, pts who had mastectomy (HR 1.22 CI 1.03-1.45 P=0.025) and no surgery (HR 2.53 CI 2.03-3.17, p<0.001) experienced worse OS. Conclusion Greater comorbidity index, higher stage, poor differentiation, and triple negative subtype are associated with worse survival outcomes in MBC. Black MBC pts were observed to have large, node-positive, and aggressive tumors. In our updated analysis, after adjusting for socioeconomic and tumor characteristics, we did not observe any

significant disparities in survival outcomes in MBC based on race. Future research is warranted to develop strategies to improve the survival of MBC pts.

P3-03-09: Evaluating the impact of race, ethnicity, and neighborhood-level socioeconomic factors on invasive lobular carcinoma incidence and survival in the United States

Kathryn Demanelis, Neil Carleton, Margaret Q. Rosenzweig, Julia Foldi, Marija Balic, Stephanie Walker, Megan-Claire Chase, Adrian V. Lee, Steffi Oesterreich

Introduction: Invasive lobular carcinoma (ILC) accounts for ~10% of all female breast cancers (BC) diagnosed and is the most common special type of BC. Disparities related to race/ethnicity, urban-rural residence, and neighborhood-level socio-economic status (SES) influence the entire cancer control continuum from cancer development to diagnosis to survivorship. Presently, the impact of these potential disparity factors on ILC diagnosis and survival have not been extensively reported. The objective our analysis was 1) to evaluate the differences in ILC incidence and survival by these potential disparity factors and 2) to compare with no specific type (NST) BC using data from the Surveillance, Epidemiology, and End Results (SEER) registry.

Methods: We obtained access to the SEER 22 specialized dataset with census tract attributes (November 2022 submission) and included female BCs diagnosed from 2010-2019. Cases were specified as NST and ILC based on ICD-O-3 histologic codes: 8500 and 8520. The following disparity factors were examined race/ethnicity, neighborhood-level SES (quintiles derived from Yost Index), and urban-rural residence (USDA Rural-Urban Commuting Area codes). Age-adjusted incidences were computed, and joinpoint regression analyses evaluated the yearly change in NST and ILC incidence. Multivariable generalized Poisson models were employed to evaluate joint impact of the disparity factors on incidence as well as interaction between histology and each disparity factor. Kaplan-Meier survival curves were used to estimate overall and BC-specific (BCSS) survival. The Fine-Gray competing risk regression model was used to evaluate the impact of these potential disparity factors on BCSS, and models were adjusted for age at diagnosis, race/ethnicity, neighborhood SES, and stage at diagnosis. Stratified analyses were conducted by hormone receptor (HR)/HER2 status and age at diagnosis (<50 or 55+ years).

Results: From 2010-2019, 961,502 BC cases were included in our analysis, and 73.3% (age-adjusted incidence = 92.9 [95% CI: 92.6, 93.1] per 100,000) and 9.5% (incidence = 11.8 [95% CI: 11.7, 11.8]) were NST and ILC, respectively. Within ILC, 87% of cases were HR+/HER2-. ILC incidence increased by 2.3% (95% CI: 1.7, 3.0) per year from 2010-2019 while NST incidence increased by 1.7% (95% CI: 0.8, 2.6). Overall ILC incidence was 29%, 41%, and 53% lower in non-Hispanic (NH)-Black, Hispanic, and NH-Asian women compared to NH-White women, respectively, and these differences were substantially greater than what was observed for NST. ILC and NST incidence decreased by 13% and 6%

per decrease in neighborhood-level SES quintile, respectively. Across all disparity factors overall ILC incidence increased from 2010-2014 to 2015-2019 ($p < 0.001$ for all groups). Poisson modeling uncovered interactions ($p < 0.001$) between NST/ILC and each of these disparity factors, indicating a differential impact of these disparities on the likelihood of ILC diagnosis. For HR+/HER2- BC, 10-year BCSS was lower for ILC compared to NST (83% vs. 87%, $p < 0.001$), and the impact of race/ethnicity and neighborhood SES differed for NST and ILC (pinteraction < 0.05 for both). While HR-/HER2- (i.e., triple negative BC) compromised <2% of ILC cases, 10-year HR-/HER2- BCSS for ILC compared to NST was substantially lower for NH-black (55% vs. 69%) and Hispanic (58% vs. 74%) women as well as women residing in neighborhoods with the lowest SES (55% vs. 69%) and rural areas (51% vs. 73%).

Conclusions: Race/ethnicity and neighborhood-level SES are associated with ILC incidence and survival disparities, and these disparities are greater for ILC compared to NST. While ILC incidence is lower among racial/ethnic minorities, it has been increasing across all racial/ethnic groups, indicating that ILC can affect all women, regardless of race/ethnicity, age, and SES. Our work highlights that additional research and action is needed to address disparities in ILC diagnosis and treatment.

P3-03-10: Leverage GPT-4 and social media to understand health disparity for transgender patients with breast and gynecological cancers.

Kathryn Mishkin

Objectives: This study focuses on the often-understudied experiences of transgender individuals, specifically those assigned female at birth, navigating breast and gynecological cancer care. Leveraging social media and advanced AI (GPT-4), we explored their healthcare journeys, coping mechanisms, challenges, and the broader impact of their diagnoses.
Methods: User-generated discussions from major social media platforms (Reddit, X, YouTube) were collected, spanning 2012 to 2022. A combination of systematic searches, rule-based filters, and AI (GPT-4) was used to extract narratives specific to transgender patients. Pre-established themes were identified through manual analysis and literature review. Two independent researchers coded a subset of the data to ensure reliability, achieving substantial agreement (Cohen's kappa $\kappa = 0.93$). GPT-4 was then utilized to categorize these posts into distinct stages of the patient journey and the pre-established main themes. Emergent subthemes and perspectives identified by GPT-4 were manually validated.
Results: From over 30 million posts on social media, 1,235 were eligible for inclusion. The analyzed posts were distributed across various cancer indications: breast cancer (30.4%), cervical cancer (25.9%), endometrial cancer (34%), and ovarian cancer (9.7%). Three primary themes emerged: lack of awareness, access issues, and clinical challenges. Posts about clinical concerns focused on the burden of disease (87.1%), with emphasis on physical health. A concerning lack of healthcare system and provider awareness of

transgender cancer needs was highlighted in 65.7% of posts, with 16.5% expressing concerns about incorrect pronoun use. Nearly one-third of posts addressed financial issues (health insurance coverage limitations), while 71.9% addressed non-financial access issues, including concerns about trans-competent healthcare providers.
Conclusion: By utilizing social media data and AI, we captured experiences and disparities in breast and gynecological care faced by transgender individuals. Findings underscore the need for increased provider awareness, improved healthcare access, and inclusive clinical practices. Addressing these disparities and advocating for more equitable and empathetic cancer care is crucial, regardless of gender identity.

P3-03-11: Structural Racism and Aggressive Breast Cancer Biology

Neha Goel, Alexandra Hernandez, Susan Kesmodel, Michael Antoni, Steve Cole

Objective: To determine the association between objective (geospatial) and subjective (perceived) measures of structural racism and aggressive breast cancer tumor biology, defined using validated social adversity-associated transcription factor (TF) activity.
Background: Structural racism is associated with shorter breast cancer recurrence-free survival, independent of individual, tumor, and treatment characteristics, suggesting potential unaccounted biological mechanisms by which structural racism influences tumor biology. The field of social genomics offers a mechanistic approach to study the influence of social adversity on tumor biology through validated TFs of social adversity.
Methods: We quantified TF-binding motif prevalence within promoters of differentially expressed genes for 147 tissue samples prospectively collected on protocol. Covariate-adjusted multivariable regression analyzed objective measures of structural racism using the validated Index of Concentration at the Extremes and subjective measures of perceived discrimination using the Expanded Everyday Discrimination Survey with validated TFs of social adversity and aggressive biology—pro-inflammatory activity [nuclear factor-κB (NF-kB) and sympathetic nervous system (SNS) activity [cyclic 3′-5′ adenosine monophosphate response element-binding protein (CREB)], controlling for age, race/ethnicity, BMI, stage, grade, tumor type (TNBC, etc), and neoadjuvant therapy.
Results: Increasing objective structural racism and perceived discrimination were associated with SNS activation (up-regulated CREB) and aggressive tumor biology (up-regulated NF-kB).
Conclusions: To our knowledge, in the largest human social genomics structural racism study, objective measures of structural racism and subjective measures of perceived discrimination were significantly associated with TFs of aggressive biology and SNS activation, implicating SNS activation as one potential mechanism behind structural racism-associated survival disparities. We have previously shown that these TFs correlate with worse clinical outcomes such as shorter survival and higher Oncotype DX scores further reflective of aggressive tumor biology. These findings remain to be validated in a national cohort.

P3-03-12: Social Determinants of Health (SDOH) and Z Code Utilization among Patients with Breast Cancer (BC)

Nehanda Jones, Adam Blair, Elizabeth Lipschultz, Jacob Koskimaki, Brooke Smither, Robert Miller

Background: Previous studies show that SDOH play an important role in BC stage, treatment, and outcomes. Disparities in BC overall survival (OS) by SDOH are well documented. Z codes were introduced into International Classification of Diseases, 10th edition in 2015 to identify reasons for clinical encounters when circumstances other than a disease or injury are recorded as diagnoses, but uptake has been slow. Herein we report Z codes Z55 – Z65, which specifically identify SDOH impacting health care utilization, for BC patients (pts) in CancerLinQ (CLQ). Methods CLQ is a health data platform representing ~7M pts across 100+ US oncology practices and cancer centers. In this retrospective cross-sectional analysis, CLQ pts diagnosed (dx) with BC actively managed in 2021 were identified and pt diagnosis codes (PDC) evaluated. Descriptive statistics and multi-variate logistic regression analysis were performed, estimating statistically significant proportions (%) and adjusted odds ratios with corresponding 95% confidence intervals [aOR (95% CI)] for associations between independent demographic variables and Z code reporting. BC pts with >1 Z code reported were compared to BC pts with no Z codes. Results We evaluated 8,134,071 BC PDC representing 236,115 BC pts actively managed in 2021. BC pts were 77% Non-Hispanic White (NHW), 16% Non-Hispanic Black (NHB), with Asian and Hispanic/Latino BC pts comprising <6%. BC pts dx age <40 were 3%, 12% dx age 40-49, 29% dx age 50-59, 25% dx age 70-79, with 10% dx age ≥ 80. 52% of BC pts were married, 33% divorced/widowed, with 15% single. BC pts were largely female (99%) and urban (86%). Of the BC PDC assessed, 7,069 were Z code PDC representing 7,624 BC pts, 3.2% of CLQ BC pts actively managed in 2021. Among BC pts represented, 50.9% had 1, 13.4% had 2, 6.3% had 3, 14.6% had 4, and 14.7% had 5+ Z codes reported. The 5 most frequently reported Z code groups were: Z63 (35%) - Support group & family circumstances Z59 (27%) - Housing & economic circumstances Z65 (16%) - Other psychological circumstance Z60 (11%) - Social environment Z56 (5%) - Employment NHB, Asian, and Hispanic/Latino BC PDC had greater odds of Z code reporting compared to NHW, with Hispanic/Latino BC PDC having almost 2x greater odds of Z code reporting [1.8 (1.64, 1.94)]. An inverse relationship was observed between dx age and aOR of Z code reporting, with BC PDC of pts dx age <40 having the greatest odds [1.4 (1.24, 1.58)] and BC PDC of pts dx ≥80 having the lowest [0.8 (0.76, 0.92)]. Divorced/widowed PDC [1.7 (1.60, 1.78)] and single PDC [1.6 (1.53, 1.75)] BC PDC had greater odds of Z code reporting compared to married BC PDC. Rural BC PDC [0.9 (0.81, 0.94) had lesser odds of Z code reporting compared to urban BC PDC. Conclusion CLQ BC Z code utilization in 2021 (3.2%) exceeded previously reported disease-agnostic studies (1.3% - 1.9%). Z codes indicative of factors related to socioeconomic status (SES), employment, and social isolation represent 94% of all Z codes reported among BC PDC in this analysis. This finding is consistent with previous studies of SDOH among BC pts. Hispanic/Latino, divorced/widowed, and single BC PDC had the greatest odds of Z code reporting, with rural BC PDC among the lowest odds. The

association of poorer quality care and survival among rural pts is well-known. Similarly, previous studies of women dx age <40 experiencing negative SDOH were more likely to receive chemotherapy, less likely to take endocrine therapy if indicated, and suffered worse OS. Thus, the inverse relationship observed between dx age and Z code reporting as well as low odds of reporting in rural settings should be further studied in future analyses which include BC stage, treatment patterns, and outcomes. Greater Z code reporting by providers could uncover SDOH trends impacting BC diagnosis, healthcare utilization, treatment, and outcomes.

P3-03-13: Ethnic and Geographic Disparities in Mucinous Breast Cancer

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Introduction: Mucinous breast cancer (MBC) is a rare subtype of invasive breast cancer which accounts for one to four percent of overall invasive breast cancer in the United States. The clinical features of MBC have not been fully understood due to limited patient data. Here, we report ethnic and geographic disparities in patients with MBC.

Method: Mortality and overall survival rates (OS) were obtained from the Surveillance, Epidemiology, and End Results (SEER) Stat Database 17-registry (2000-2021). The 7th edition of American Joint Committee on Cancer (AJCC) staging was used due to the unavailability of differentiation grade data in the 8th edition of AJCC staging. Patient subgroups were divided into ethnicity (White, Black, Asian American and Pacific islanders; AAPI), household income (>=$75,000 vs <$75,000 per year), age of diagnosis (25-45 vs 45-65 vs 65+ years), and geographics (metropolitan vs nonmetropolitan area). Propensity score matching (PSM) was used to reduce the effect of confounding factors such as surgery, chemotherapy, radiation therapy, stage, grade, hormone receptor status (estrogen receptor; ER and progesterone receptor; PR), and human epidermal growth factor receptor 2 (HER2) status. Univariate and multivariate Cox regression analyses were used to obtain independent prognostic factors. Patients with duplicate record, missing information regarding confounding factors were excluded in the analysis.

Result: Overall, 5,219 patients were included in the final analysis. The independent prognostic factors were age of diagnosis, race, stage, differentiation grade, hormone receptor status (ER and PR), HER2 status, metastases (bone, brain, liver, and lung), surgery, chemotherapy, radiation, income, and marital status. In ethnic subgroups, AAPI showed better OS than in White before and after PSM (before PSM, HR 0.50, 95% CI, 0.41-0.62, p<0.01; after PSM, HR 0.54, 95% CI, 0.42-0.69, p<0.01). White and Black patients did not reveal significant differences in survival. (HR 1.03, 95% CI, 0.88-1.21, p=0.74). After PSM, White patients had an OS benefit than in Black (HR 0.56, 95% CI, 0.44-0.71, p <0.01). In geographic subgroups, patients in the metropolitan areas did not show significant difference compared to patients in the nonmetropolitan areas before PSM (HR 0.87, 95% CI, 0.74-1.02, p=0.09). However, patients in the metropolitan areas exhibited better OS compared to patients in nonmetropolitan areas (HR 0.61, 95% CI, 0.48-0.77, p<0.01) after

PSM. In income subgroups, patients with household income >=$75,000 revealed better OS than those who have income less than $75,000 before PSM (HR 0.82, 95% CI, 0.74-0.91, p<0.01). However, those groups did not show statistical significance in OS after PSM (HR 0.89, 95% CI, 0.80-1.00, p=0<0.01).
Conclusion: Overall, mortality and OS varied among different ethnic groups and regions, though additional studies are warranted for Native Americans or Alaskans with a larger patient dataset. In regards to primary prevention, an early intervention could be especially beneficial for patients in rural areas while MBC is known for its good prognosis. Furthermore, several possible confounding factors such as alcohol or smoking could be further controlled if they are included in the SEER dataset in the future. In addition, the use of AJCC 8th edition with differentiation grade data is warranted in the future.

P3-03-14: Concordance of Self-Reported Demographic and Clinical Variables with Data from the State Cancer Registry among Breast Cancer Survivors of Hispanic Descent

Maria Eduarda de Azevedo Daruge, Eunkyung Lee

Background: Epidemiological research studies and population-based registries use various methods to gather accurate patient data. However, these methods can still be affected by inaccurate reporting. Reliable population-based data is crucial for public health surveillance, representing the source population, and allocating resources to address cancer disparities. This study aimed to compare the information reported in a survey to the data collected by the state cancer registry in order to assess its reliability.
Methods: The Hispanic Breast Cancer Study aimed to enroll Hispanic women with breast cancer using the Florida Cancer Data System (FCDS) patient recruitment process, to examine differences in health-related quality of life through self-reported surveys. After obtaining approval from the Florida Department of Health Institutional Review Board, FCDS provided patient contact information and clinical data for eligible participants. To be eligible, women had to be at least 20 years old, diagnosed with breast cancer at least six months prior, reported to FCDS, not pregnant or lactating, self-identified as Hispanic, and lived in Central Florida. Concordance analysis was carried out to measure the agreement between the self-reported survey data and the information in the cancer registry. This analysis included correlation coefficients for continuous variables such as current age and birthplace, and kappa values for categorical variables like Hispanic origin/ethnicity, race, and laterality.
Results: A total of 364 women completed the surveys. The women's current age showed a strong correlation (r= 0.99, P< 0.001), indicating a high level of agreement between their self-reported age and the age reported by the FCDS. There was a moderate correlation for birthplace (r=0.43, P< 0.001). The agreement for Hispanic origin and race was low (kappa for Hispanic origin= 0.19; κappa for race= 0.05), while the agreement for laterality was deemed acceptable (kappa= 0.52).
Conclusions/Implications: The study's varying concordance levels emphasize the pressing

need for better data collection systems. The low concordance for race and Hispanic origin underscores the significance of this issue. Improving the quality of health data could lessen patient burden during surveys and will be vital in addressing cancer disparities among Hispanic breast cancer survivors.

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P3-03-15: Racial differences in incidence of depression, anxiety, and insomnia among breast cancer patients on endocrine therapy

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Introduction: Endocrine therapy in breast cancer patients is asscociated with increased risk of developing mood, anxiety, and sleep disorders. Studies have shown that there are lower rates of diagnosis and treatment of mood disorders and anxiety disorders for African Americans and Hispanics than for Caucasians. This study explored whether there are racial differences in incidence of depression, anxiety, and insomnia in breast cancer patients on endocrine therapy.
Methods: We performed a retrospective, propensity score-matched cohort study using the TriNetX Analytics Network database, which includes de-identified electronic health records from over 70 healthcare organizations and 101 million individuals. We included adult female breast cancer patients treated with endocrine therapy from January 2010 to June 2023. We excluded patients with metastatic disease. We also excluded patients with a prior history of depression, anxiety or insomnia. We matched patients on predetermined variables including age, breast cancer-directed therapy, and comorbidities. We compared incidence of diagnosis of depression, anxiety and insomnia among patients in different race/ethnicity subgroups (Hispanic, Black, White, and Asian) either on aromatase inhibitor (AI) or tamoxifen within the first year of initiation of endocrine therapy.
Results: We identified 93,606 and 33,031 patients on AI and tamoxifen, respectively. We matched 23,002 patients in both AI and tamoxifen cohorts. The incidences of depression are 11.65 vs. 12.22 per 1000 patient-years (p=0.64) on AI vs. tamoxifen, respectively; the incidences of anxiety are 17.43 vs. 15.73 per 1000 patient-years (p=0.13) on AI vs. tamoxifen, respectively; the incidences of insomnia are 6.52 vs. 5.26 per 1000 patient-years (p=0.07), on AI vs. tamoxifen respectively. Compared to matched White counterparts, Black patients (n=8946) on AI have a lower risk of depression (HR=0.74, p=0.03), a lower risk of anxiety (HR=0.60, HR<0.01), but no significantly different risk of insomnia. Compared to matched White counterparts, Hispanic patients (n=3472) on AI have a higher risk of insomnia (HR=1.97, p=0.02), but no significantly different risk of depression or anxiety. Compared to matched White counterparts, Asian patients (n=3795) on AI have a lower risk of depression (HR=0.18, p<0.01), a lower risk of anxiety (HR=0.30, p<0.01), but no significant difference in insomnia. Compared to matched White counterparts, Black patients

(n=2675) on tamoxifen have a lower risk of depression (HR=0.74, p=0.05), a lower risk of anxiety (HR=0.68, HR=0.03), but no significant difference in insomnia. Compared to matched White counterparts, Hispanic patients (n=1709) on tamoxifen have no significantly different risk of depression, anxiety or insomnia. Compared to matched White counterparts, Asian patients (n=1884) on tamoxifen have a trend towards lower risk of depression (HR=0.56, p=0.06), a lower risk of anxiety (HR=0.33, p<0.01), but no significant difference in insomnia.
Conclusion: Depression and anxiety are diagnosed at a lower incidence in Black and Asian patients compared to White patients on endocrine therapy. This may reflect lack of access to care, under-recognition, or cultural differences in minority patients, and further studies are needed to identify the causes of these differences.

P3-03-16: Molecular stromal signature to predict Ductal Carcinoma in Situ (DCIS) progression

Niki Prekete, Michael Allen, Eleni Maniati, Louise Janet Jones

Ductal Carcinoma in Situ (DCIS) is a pre-invasive breast carcinoma, where the cancer cells proliferate within the breast duct. Because the ducts are not connected to the circulatory or the lymphatic system, DCIS does not constitute a lethal disease. Due to the implantation of the mammographic screening programme, the percentage of women diagnosed with DCIS is increasing. These women who are diagnosed with DCIS are currently treated as if they were going to progress to invasive cancer, as there is no way to predict the progression of the disease and stratify patient treatment. Analysis of the neoplastic cells of DCIS has not revealed genetic changes linked to the risk of progression, the scientific interest has been turned to the tumour microenvironment.
This study hypothesised that changes that take place in the stromal compartment surrounding DCIS ducts can promote the invasive capacity of the carcinoma and as a result promote the progression from DCIS to invasive disease. The study aimed to discover alterations in the stromal area of normal, pure DCIS and invasive carcinomas that may indicate risk of future progression and create a signature to distinguish the progressive and the non-progressive DCIS.
A cohort of four patients with matched clinicopathological characteristics who were previously diagnosed with invasive breast cancer and did not have neoadjuvant treatment was selected. RNA sequencing was performed on samples from the invasive, DCIS and normal breast areas of these patients (n=12). Although the samples contained the whole tissue, they were selected to represent more the microenvironmental compartment of each disease stage.
The analysis of the RNA sequencing samples led to the identification of 50 genes, that showed a stepwise increase (n=18) or decrease (n=32) in expression from normal, pure DCIS or invasive cases. The majority of the pathways and processes the genes of the signature involved were connected to elements of the microenvironment, such as collagen degradation and crosslinking, avβ3 integrin, and biological processes including T-cell

activation. Interestingly, the RNA sequencing results in these whole tissue samples showed a much higher number of differentially expressed genes between the Invasive Tumour vs DCIS comparison than in the DCIS vs Normal comparison suggesting that the whole tissue DCIS is more similar to the Surround than in the invasive tumour disease stage. Gene targets; FN1, VCAN and MMP11 were validated through Immunohistochemistry or RNAScope in an external sample cohort comprised of normal, pure DCIS and DCIS with invasion cases. Finally, the genes were combined in a signature and an in silico analysis of public datasets that included independent normal, DCIS and invasive cases from patients also validated our polygenic risk score.
This study has managed to identify a panel of genes showing altered expression between normal, pure DCIS and invasive cancer tissues. The results emphasised the importance of the tumour microenvironment in disease progression. The findings were then combined in a multigene signature associated with prognosis and risk of progression on public datasets.

P3-03-17: Genetic Profiles and Precision Medicine in Adolescent and Young Adult Chinese Women with Breast Cancer: A Comprehensive Study

Qing Hao, Fei Pang, Chengyi Wang, Hongrui Zhou, Aodi Wang, Hui Chen

Background: Breast cancer is a major health concern worldwide, especially among Adolescent and Young Adult (AYA) women who face unique biological and clinical challenges. AYA breast cancer patients often present with aggressive subtypes, requiring specific treatment considerations such as fertility preservation and long-term survivorship. This study aims to analyze the genetic characteristics of AYA Chinese women with breast cancer, focusing on genomic alteration profiles and their correlation with clinical outcomes, to provide precise treatment guidance.

Patients and Methods: We analyzed tumor and matched blood samples from 202 young Chinese breast cancer patients (≤39 years) diagnosed between 2014 and 2024. Targeted next-generation sequencing was conducted on formalin-fixed paraffin-embedded (FFPE) or fresh tumors. Personalized ctDNA assays were used for Minimal Residual Disease (MRD) monitoring to detect early recurrence and guide treatment. Experiments were performed in a CAP/CLIA-certified laboratory.

Results: The study cohort had a median age of 35 years (range 18-39). Among them, 48.02% (97/202) were in stages I-II, 42.08% (85/202) in stages III-IV, and 9.9% (20/202) had unclear staging. Molecular subtypes included 28.71% (58/202) HER2+, 32.67% (66/202) ER+/PR+/HER2-, and 28.22% (57/202) triple-negative breast cancer (TNBC). The top 5 mutated genes in TNBC were TP53 (86%,49/57), PIK3CA (14%,8/57), RB1 (14%,8/57), PTEN (10.5%,6/57), and CDKN2A (8.8%,5/57). For HER2+ patients, they were ERBB2 (86%, 50/58), TP53 (64%,37/58), PIK3CA (28%,16/58), CCND1 (10.3%,6/58), and BRAF (5.2%,3/58). ER+/PR+/HER2- patients had TP53 (36%,24/66), PIK3CA (35%,23/66), CCND1 (20%,13/66), PTEN (12.1%,8/66), and AKT1 (7.6%,5/66). Among the 58 HER2+

cases, 49 have ERBB2 somatic gene amplification, while 5 in 49 co-occuring point mutations. Germline testing was performed on 197 patients, with 11.67% (23/197) showing pathogenic/suspected pathogenic mutations in BRCA1/2. A case study of a 34-year-old HER2+ patient demonstrated the value of precision medicine and MRD monitoring. MRD testing detected recurrence two months earlier than imaging. Treatment with the HER2-targeted drug DS-8201 led to complete response, maintained over four consecutives negative MRD tests.

Conclusions: Our findings underscore the diverse molecular subtypes and significant genetic mutations among young Chinese breast cancer patients, highlighting the necessity of personalized treatment strategies. Incorporating precision medicine and MRD monitoring can significantly enhance early recurrence detection and improve overall treatment outcomes. Future research should focus on validating these findings and developing tailored strategies in larger cohorts.

P3-03-18: Hormone receptor status and PD-L1 expression prevalence in breast cancer when assessed with PD-L1 IHC 22C3 pharmDx and Combined Positive Score

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Background: Programmed cell death-ligand 1 (PD-L1) is of great interest in breast cancer (BC). PD-L1 IHC 22C3 pharmDx (SK006) is an approved companion diagnostic assay for patients with triple-negative breast cancer (TNBC). Multiple sub-types of BC are identified by the binary expression status (positive/negative) of human epidermal growth factor 2 (HER2) and the hormone receptors (HRs) estrogen receptor (ER) and progesterone receptor (PR). The ability of the assay to reliably identify PD-L1 expression in TNBC (ER-/PR-/HER2-) tissues was previously demonstrated. However, the sensitivity of the assay on HR+/HER2- specimens (including ER+/PR+, ER+/PR-, and ER-/PR+; non-TNBC) is not as well-known. The data presented here demonstrates the ability of the assay to reliably identify the dynamic range of PD-L1 expression on non-TNBC specimens when assessed using the Combined Positive Score (CPS).

Methods: Primary and metastatic BC specimens, sourced from multiple anatomical sites, were prepared and stained according to the assay's Instructions for Use (IFU). The PD-L1 IHC 22C3 pharmDx immunohistochemical assay uses Monoclonal Mouse Anti-PD-L1, Clone 22C3, for detection of PD-L1 protein in formalin-fixed, paraffin-embedded tissues. Stained sections were scored using CPS, which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. CPS totals are displayed as integers with a maximum CPS of 100. Additional cut sections from the same specimens were also prepared and stained using Agilent ER, PR and HER2 reagents, where positivity or status was based upon the IFU for each reagent. Each

specimen was then grouped into non-TNBC or TNBC sub-types. Prevalence was assessed to demonstrate that the assay will detect the target molecule (PD-L1 protein) in BC specimens across the dynamic range of PD-L1 expression (CPS 0–100). For the purposes of this abstract, scores were reported according to the CPS ≥ 1 and CPS ≥ 10 cutoffs.

Results: Prevalence data of Agilent's internal BC tissue bank was evaluated. At the time of the assessment, two categories of BC specimens and their totals were grouped: 149 non-TNBC and 184 TNBC. When examining the CPS ranges for the non-TNBC specimens, 55/149 (36.9%) were found to have a CPS ≥ 1 and 17/149 (11.4%) had a CPS ≥ 10. When examining the CPS for the TNBC specimens, 115/184 (62.5%) were found to have a CPS ≥ 1 and 57/184 (31.0%) had a CPS ≥ 10. For all specimens of each cohort (non-TNBC and TNBC), the assay identified PD-L1 expression across the dynamic range. This analysis indicates that the dynamic range of PD-L1 expression is present across all BC tissues regardless of HR status, though a higher prevalence is noted in TNBC specimens at the CPS ≥ 1 cutoff.

Conclusions: Overall, the assay detected PD-L1 protein across the dynamic expression range (CPS 0–100) for both non-TNBC and TNBC specimens. In this small specimen cohort, retrospective analysis demonstrated that HR status may correlate to a difference in PD-L1 prevalence in HER2- breast cancer specimens. This analysis further demonstrated that PD-L1 IHC 22C3 pharmDx can identify PD-L1 expression in the dynamic range within non-TNBC specimens, specifically. Further studies should be conducted within different combinations of HR status and PD-L1 prevalence to expand on this data, to address gaps in knowledge, and to learn if HR status may inform PD-L1 immune checkpoint inhibitor therapy benefit for BC patients.

P3-03-19: Somatic Mutation Profile of Metastatic Breast cancer in Brazilian Patients using Next Generation Sequencing

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Background: Advances in next-generation sequencing (NGS) offers multigene mutational profile that provides comprehensive genetic information on breast cancer molecular pathology and increases the likelihood of identifying mutations that may be potentially susceptible to targeted treatments. There are limited data on the molecular epidemiology of metastatic breast cancer in low- and middle-income countries. This study analyzed the clinical-pathological characteristics and mutational profile of the population using NGS tests from two oncologic centers at Brazil, with a focus on actionable mutations that could be targeted and that resulted in a change of cancer treatment. Methods: We conducted a retrospective analysis from 2018 to August 2023 of patients with metastatic breast cancer (MBC) who received NGS in two centers at Brazil. We enrolled patients from São Paulo and Brasilia. Results: In total 78 NGS from patients with MBC were analyzed, including 52 with

luminal phenotype, 07 who were HER- 2 positive, and 19 triple negative breast cancer. The population was predominantly female (97,4%). Median age was 40,5 years. Most of the patients, 88.46% had recurrent disease and 11,54% de novo metastatic breast cancer. The NGS samples were mostly from a metastatic site (67,95%). Liquid biopsy was applied in 8,9% of the cases, while 91,1% were from tissue biopsy most from liver (19,23%), followed by lymph nodes (19,23%), primary breast nodule (14,10%), lungs (12%) and bones (5,3%). A total of 127 gene mutations were found. The clinically relevant mutation found were in TP53 (42,31%), PIK3CA – AKT1- PTEN pathway (41,05%) distributed as 32,05% in PIK3CA, 1% in AKT1 and 8% in PTEN loss, ESR1 (17,95%), ERBB2 mutations (11%), BRCA 1/ 2 mutations (6%) and ATR/ATM/PALB2 (5%). The molecular alterations, such MSI high (1,28%) and TMB (mean value 3 mut/Mb) predictive of response to immunotherapy were rare. For the HR positive tumors mutations at PIK3CA (35,1%), TP53 (28,6%), ESR1 (25%) were the most prevalent. In contrast, the triple negative tumors (24,36%) had a higher incidence of TP53 (76,9%) and a low prevalence of PIK3CA/AKT/PTEN mutation (1,2%). HER 2 population (8,9%) the most prevalent mutations were found in ERBB2 (100%), and TP53 (50%). Of the 78 NGS, 34 had genomic alterations that were targetable by medications approved in FDA, EMA and/or ANVISA (regulatory agency in Brazil). The findings of the NGS resulted in a change of treatment in 17,95%. Nine patients received Alpelisib. Three patients received anti-HER2 therapies, 02 of them were luminal subtype and 01 was HER 2 positive breast cancer. One patient was treated with Olaparib for BRCA 2 somatic mutation, luminal subtype. The median survival of the general population was 218 days. The median survival stratified by change of treatment was 218 days for the group with genomic findings and treatment modification while the median survival was 162 days for the group without genomic findings. But the difference wasn't statistically meaningful (p = 0,35) according to long Rank analyses. Conclusion: The increased accessibility of the NGS may lead to a greater number of potential therapeutic implications. The prevalence of the mutation was different between the intrinsic subtypes of cancer. The mutations in PIK3CA/AKT/PTEN pathway and ESR1 can be targetable after iCDK4/6 inhibitor progression, underlyng the importance and necessity of a NGS in the treatment of HR positive metastatic breast cancer.

P3-03-20: A multicenter retrospective review of clinical-pathological characteristics associated with survival outcomes in a large cohort of metaplastic breast cancer patients.

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Background: Metaplastic breast cancer (MpBC) is a rare and aggressive subtype of breast cancer (BC) that is frequently triple negative (TN) and relatively chemo resistant. MpBC is a heterogeneous group of invasive BC in which cancer cells demonstrate both epithelial and mesenchymal differentiation. Although MpBC is generally treated similarly to invasive ductal carcinoma, these tumors tend to be refractory to chemotherapy (CT) despite the greater incidence of high grade and TN cancers. In this analysis, we examined the clinical

and pathological characteristics associated with worse prognosis in patients with MpBC.
Methods: Patients (pts) treated for MpBC at Miami Cancer Institute (2017-2021; identified by COTA real-world Analytics® platform) and Mayo Clinic (1997-2021; identified by Epic slicer dicer tool) were retrospectively reviewed after IRB approval. Pt demographics, pathologic characteristics, staging, treatment, and outcomes data were collected. Kaplan-Meier method was used to estimate disease-free survival (DFS) and overall survival (OS) at 1, 5, and 10 years (yr) post diagnosis. Univariable and multivariable Cox regression models were used to examine the association between pt/tumor characteristics and outcomes (DFS and OS). All tests were two-sided with p value <0.05 considered statistically significant. The analysis was done using R4.2.2.
Results: We identified 204 pts with MpBC with a median 2.5 yr of follow up from the time of initial diagnosis. Median age at diagnosis was 60.5 yr, and 162 pts (79.4%) were post-menopausal. At presentation 175 (94.6%) had early-stage disease, 160 (84.7%) had tumor size less than 5 cm, and 156 (81.7%) were node negative on imaging. Family history was positive in 48% of pts and 10 (12.2%) had a positive germline mutation. Tumors were ER <1% in 163 (81.5%), ER 1-10% in 17 (8.5%), and HER2 negative in 174 (91.6%). Distant metastatic disease was noted in 21 pts (10.3%). 95 pts (50.8%) had lumpectomy, 90 pts (48.2%), had mastectomy, and 127 (68.3%) had sentinel node biopsy. At surgery, 119 (64.3%) had pT1/T2 tumors and 117 (63.2%) had pN0 tumors and 106 (66.2%) received adjuvant radiation. Spindle cell histology was seen in 43 pts (22.5%), followed by mesenchymal in 51 pts (26.7%) and squamous cell histology in 33 pts (17.3%). Of the total cohort, 57 (28.5%) received neoadjuvant CT and 6 (11.1%) of these pts achieved a pathologic complete response (pCR). pCR did not predict for improved survival outcomes in this group of pts. In the entire cohort, 5-yr DFS was 62.0% (95% confidence interval [CI] 54.2%-70.9%) and 5-yr OS was 79.7% (95% CI 73.0%-86.9%). In multivariate analysis, presence of distant metastatic disease (HR 5.55, 95% CI 1.85-16.69; p=0.002) and spindle cell histology predicted statistically significant worse mortality (hazard ratio [HR] 3.67, 95% CI 1.76-7.67; p<0.001). Increasing stage also predicted statistically significant worse OS (HR 2.17, 95% CI 1.29-3.62; p=0.003).
Conclusion: In this large, predominantly early-stage cohort of pts with MpBC, higher stage at diagnosis, distant metastatic disease, and spindle cell histology predicted worse OS. PCR rates following neoadjuvant CT was low, consistent with the reported literature, confirming the relative chemo resistant nature of MpBC. These results underscore the need for focused research efforts to develop novel therapeutic strategies to improve outcomes, particularly in MpBC with spindle cell histology and pts with distant metastasis.

P3-03-21: EFFECT OF REGULATORY T CELL INFILTRATION ON PATHOLOGICAL COMPLETE RESPONSE RATES WITH NEOADJUVANT THERAPY IN LOCAL AND LOCALLY ADVANCED HER2 POSITIVE BREAST CANCER

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Aim: In our study we aim to reveal the effect of the intratumoral Treg infiltration on the pathological complete response and to find out other clinicpathologic and cellular variables effective on pathologic complete response
Patients and Method: Fifty-five patients were included, aged 18 years and over, who were treated and followed up in the Medical Oncology Department of Antalya Education and Research Hospital between January 2015 and January 2022. Paraffin blocks of biopsy and operation tissues were eavaluated by two different pathologists reviewing immunohistochemical staining with CD4, CD25 and FOXP3. The effect of tumor infiltrating T cell (Tregs and Th cells) quantitiy on pathological complete response is evaluated.
Results: 55 local and locally advanced HER2 positive breast cancer patients were included but 45 patients' data (median age 50) were analyzed. While 34 patients had a pathological complete response (pCR) after neoadjuvant treatment, 11 patients had failed to achieve pCR. Th and Treg infiltration after NACT did not differ between early stage and locally advanced stage diseases. Intratumoral and peritumoral Th infiltration abundancy were observed to be lower in patients with a pCR than in those without a pCR. Similarly, in patients with a pCR, Treg infiltration abundancy in the intratumoral and peritumoral area was found to be lower compared to non pathologic complete responders. There is no TREG or Th cellular abundancy difference between intratumoral and peritumoral microenvironments. But increased intratumoral Treg infiltration increased the risk of non PCR status by 10.8 times.
Discussion and Conclusion: Variety of immune cells in the tumor microenvironment may be related with good or poor cancer outcomes via the activation or inhibition of immune surveillance.
Our study is the first to evaluate Th and TREG cellular abunbancy changes after neoadjuvant chemotherapy (NACT) in Her2+HR negative local or locally advanced breast cancer patients. High Treg infiltration after NACT is also found to be an independent predictor of risk of pCR failure.

P3-03-22: CK2 in breast cancer and associated clinical characteristics

Nicholas Wilson, Isabelle Kreber, Cristian Figueroa

Background: In breast cancer, aberrant casein kinase (CK2) signaling has been linked to invasiveness, metastatic potential, and prognosis. Here we explore how CK2 expression in breast cancer is associated with tumor stage, response to therapy, and prognosis.
Methods: Data analysis was performed with data from The Cancer Genome Atlas (TCGA). R version 4.2.2 was used for analysis. Student's t-test, Welch's t-test, Wilcoxon test, and Pearson and Spearman correlations were used to analyze continuous variables. Bonferroni correction was used to adjust p-values to reduce the risk of type I error for multi-group analysis. Analysis of survival and treatment response was performed using Kaplan-Meier Plotter (https://kmplot.com/) and ROC Plotter (https://rocplot.com/) which draw breast cancer sample data from TCGA, Gene Expression Omnibus (GEO), and other repositories.
Results: Using TCGA data, we examined the relationship between CK2 subunit expression and clinical stage, TMN staging, and hormone receptor positivity for intraductal carcinoma

(IDC). We found that mean expression levels of CSNK2A1, CNSK2A2, and CSNK2B were greater in stage II and stage III IDC than stage I ($p < 0.05$ for all). Looking at the T stage of TNM (tumor size), mean expression levels of CSNK2A1, CNSK2A2, and CSNK2B were greater in T2 than T1 ($p < 0.05$ for both). Mean CSNK2A2 and CSNK2B expression was 43% and 28% greater in ER negative vs ER positive IDC ($p < 0.001$ for both). Mean CSNK2A1, CSNK2A2, and CSNK2B expression was 7%, 36%, and 24% greater in ER negative vs ER positive IDC ($p = 0.012$, $p < 0.001$, and $p < 0.001$). Mean CSNK2A1 and CSNK2A3 expression was significantly greater in HER2 positive cancer than HER2 equivocal or HER2 negative IDC ($p < 0.002$ for all).
KM Plotter was used to explore associations between CK2 subunit expression and overall survival in breast cancer of varying clinical characteristics. In lymph node positive cancer, CSNK2A1 overexpression was associated with worse survival (Hazard Ratio, HR: 1.87, 95% Confidence Interval, CI: 1.14-3.05, $p = 0.011$). CSNK2A3 was also associated with worse survival (HR: 1.93, CI: 1.2-3.1, $p = 0.006$). In lymph node negative cancer, CSNK2A1 and CSNK2A3 overexpression were associated with worse survival (HR: 1.69, CI: 1.13-2.54, $p=0.010$ and HR: 2.38, CI: 1.5-3.76, $p < 0.001$). In ER positive cancer, CSNK2A3 was associated with worse survival (HR: 1.52, CI: 1.07-2.18, $p = 0.020$). In ER negative cancer, CSNK2A1 and CSNK2A3 overexpression were associated with worse survival (HR: 9.66, CI: 1.22-76.31, $p = 0.008$ and HR: 3.51, CI: 0.97-12.77, $p = 0.042$). In HER2 negative cancer, CSNK2A1 and CSNK2A3 overexpression were associated with worse survival (HR: 1.78, CI: 1.27-2.49, $p < 0.001$ and HR: 2.27, CI: 1.59-3.25, $p < 0.001$). In cancer treated with chemotherapy, CSNK2A1, CSNK2A3, and CSNK2B overexpression were associated with worse survival (HR: 3.18, CI: 1.65-6.12, $p < 0.001$, HR: 2.97, CI: 1.54-5.71, $p < 0.001$, and HR: 2, CI: 1.07-3.72, $p = 0.026$).
ROC Plot analysis found that CSNK2A1 was a weak classifier of pathological complete response to FAC (fluorouracil, doxorubicin, and cyclophosphamide) (Area under curve, AUC = 0.651, $p < 0.001$) and FEC (fluorouracil, epirubicin, and cyclophosphamide) (AUC = 0.648, $p < 0.001$).
Lastly, CK2 subunit expression was compared with that of the 21 genes from the Oncotype DX breast cancer assay. CSNK2A2 had significant mild negative correlations with many sex hormone-related genes. CSNK2B had significant mild to moderate positive correlations with many proliferation-related genes, internal control genes, and sex hormone-related genes.
Conclusions: This study demonstrates that CK2 subunit expression associates with a variety of clinical tumor characteristics, response to therapy, and prognosis in patients with breast cancer. CK2 may be useful as a prognostic biomarker and therapeutic target in breast cancer and warrants further exploration.

P3-03-23: Targeting of SEMA7A Signaling Improves Response to Endocrine Therapy in ER+ BC

Rachel Steinmetz, Traci Lyons, Lyndsey Crump

Estrogen receptor-positive (ER+) breast cancer (BC) comprises over 70% of all breast cancers and is the leading cause of BC-related deaths in women worldwide. Despite available therapies against ER+ BC, recurrence inevitably arises, primarily due to therapeutic resistance. Semaphorin 7a (SEMA7A) is a biomarker associated with poor prognosis and endocrine therapy resistance for BC patients. Survival analyses of ER+ BC patients on endocrine therapy confirms very high early recurrence in patients with SEMA7A-high tumors. Thus, our objective is to establish novel treatment strategies to improve outcomes for patients with ER+ SEMA7A-high BC. We are investigating the mechanisms by which SEMA7A promotes resistance to endocrine therapy and its potential as a therapeutic target for ER+ BC. In the normal mammary gland, SEMA7A binds integrins and activates pathways including PI3K/AKT signaling, which promotes tumor cell survival and multi-drug resistance. Using reverse phase protein array we found that SEMA7A-overexpressing (OE) MCF7 cells have increased expression of pAKT and integrins ITGB1 and ITGB4 compared to controls cells. Our mechanistic CoIP studies revealed that SEMA7A binds directly to ITGB1 and ITGB4 on ER+ tumor cells. This novel finding suggests that SEMA7A signals through multiple ECM/integrin mediated pathways that have been associated with treatment resistance (as reviewed by Bissel and colleagues). Further, SEMA7A-integrin binding activates AKT via PI3K stimulation, as well as downstream signaling activated by pAKT. Our preliminary studies in mice show that SEMA7A OE tumors respond poorly to fulvestrant, compared to controls. Therefore, we sought to overcome the promotion of fulvestrant resistance by SEMA7A by inhibiting SEMA7A signaling via PI3K inhibitors (PI3Ki) or our novel anti-SEMA7A monoclonal antibody (SmAb). In vitro, SEMA7A OE cells are sensitive to PI3Ki alpelisib (1uM, Novartis) and GCT-007 (0.5uM, Global Cancer Technolgoy), while control cells are not. Importantly, PI3Ki sensitized SEMA7A-high tumors to endocrine therapy in vivo (n=5/group). Additionally, since SEMA7A has known immunomodulatory roles and we have shown that SmAb restores anti-tumor immunity, we sought to determine the effect of SmAb on tumor growth and fulvestrant resistance in an immune competent ER+ model. Therefore, utilized a novel syngeneic ER+ preclinical model comprised of TC11 murine tumor cells (a generous gift from Linda Schuler), which express SEMA7A, and are derived from FVB/N mice. Our results demonstrate that direct inhibition of SEMA7A via SmAb significantly reduces tumor growth rate in SEMA7A+ tumors. Results were validated in a second syngeneic ER+ model (SSM2 tumors, 129Sv-E mice; a generous gift from Alexander Borowsky). Interestingly, SmAb-treated tumors exhibit increased ER expression, suggesting a potential mechanism by which SmAb could sensitize endocrine-resistant tumors to fulvestrant. Our future studies will investigate therapeutic combinations and dosing regimens of fulvestrant and SmAb to overcome resistance to fulvestrant in SEMA7A+, ER+ tumors. Our studies suggest that patients with ER+SEMA7A+ tumors should be considered candidates for PI3K/SEMA7A-targeted clinical therapies or for direct and/or co-targeting with our novel anti-SEMA7A based therapy.

P3-03-24: Assessment of cell signaling pathway activity and expression of MKI67, ESR1 and ERBB2 as profiling tool for selection of targeted therapy in breast cancer

Yvonne Wesseling-Rozendaal, Dianne van Strijp, Eveline den Biezen, Anke Pierik, Robert Babkowski

Background: Assessment of hormone receptor status and HER2 is important in determining treatment options for breast cancer patients. The current standard of care relies on biomarker testing by immunohistochemistry (IHC) staining and fluorescence in situ hybridization (FISH).
Visual interpretation of staining intensity leads to semi-quantitative scoring and introduces an analytical error due to intra- and inter-observer variability. There is an acknowledged discordance in IHC signal in the low positive ER IHC cases and 1+ HER2 IHC cases. Furthermore, increasing numbers of studies have demonstrated that presence of the receptor on IHC is not always associated with activation of the related signaling pathway. We propose using quantitative and dynamic tumor characterization for ER and HER2 using mRNA expression combined with functional pathway activity profiling.

Methods: While immunohistochemistry measures protein levels of the breast cancer markers, here we measure the RNA of these markers instead. Gene expression levels of MKI67 (coding for the Ki-67 protein; proliferation marker), ESR1 (coding for the estrogen receptor protein), and ERBB2 (coding for the Human Epidermal Growth Factor Receptor 2 protein) were measured for 373 FFPE breast samples (303 tumors and 70 healthy normals). mRNA expression levels are measured using RT-qPCR and normalized to a set of housekeeping genes as part of the OncoSIGNal qPCR pathway test (InnoSIGN) for 7 pathways (ER, AR, PI3K, MAPK, Hh, Notch and TGFβ).

Results: Normalized mRNA expression is interpreted for each marker in relation to selected reference tissue cohorts. The distribution of MKI67 expression levels in primary, untreated breast cancer samples (n=188; 95% confidence interval (CI) [-6.2, -1.3]) was compared to the distribution of normal epithelial breast tissue samples (n=70; 95% CI [-9.6, -3.3]) and, although partially overlapping, were significantly different (p=1.47e-23).
ESR1 expression levels showed a clear separation (p=7.8e-27) between IHC-ER positive (n=89, ≥50% staining; 95% CI [-3.2, 1.8]) and IHC-ER negative (n=69, <1% staining; 95% CI [-10.9, -4.5]) breast cancer samples.
ERBB2 expression levels showed a clear separation (p=2.1e-11) between HER2 positive (IHC3 & FISH positive; n=20; 95% CI [1.9, 6.1]) and HER2 negative (IHC 0 / FISH negative; n=69; 95% CI [-3.5, 1.6]) breast cancer samples. HER2 low positive samples lie in between those clearly defined strongly positive and strongly negative groups: IHC 0/1/2 & FISH positive (n=14) ERBB2 expression 95% CI ranges [-1.5, 1.2]. Similarly, samples with FISH equivocal result (IHC 0/1/2; n=12) have a 95% CI ranging [-1.5, 1.0].

Conclusion: The OncoSIGNal test provides a robust and quantitative method to characterize

low ER and HER2 1+ IHC breast cancer samples. In addition to detecting and quantifying the relevant cell signaling pathways, the mRNA markers can be used to elucidate the results of low ER and HER2 1+ IHC staining, enabling the identification of patients who may benefit from targeted therapy.

P3-03-25: High expression of microRNA-486 levels can be a prognostic biomarker for better survival in ER-positive breast cancer patients

Yoshihisa Tokumaru, Emiri Sugiyama, Makoto Takeuchi, Manabu Futamura]

Background: Preclinical in vitro studies have shown that microRNA-486 (miR-486) functions as a tumor-suppressive miRNA. However, to the best of our knowledge, no study has investigated the clinical relevance of miR-486 in ER-positive breast cancer patients. We hypothesized that miR-486 expression in breast cancer is associated with its immune microenvironment and can be a prognostic biomarker.
Material and Methods: A total of 2042 patients' data were extracted from publicly available databases, The Molecular Taxonomy of Breast Cancer International Consortium (METARBRIC), The Cancer Genome Atlas (TCGA), and GSE57897. The clinicopathological and survival data, as well as transcriptome data, were included in these cohorts. The survival analysis and gene set enrichment analysis (GSEA) was conducted by comparing miR-486 high-expressing and low-expressing tumors, divided by the median cutoff. The association between the miR-486 expression and tumor immune microenvironment was assessed by xCell.
Results: The expression levels of miR-486 were suppressed in human samples. The expression levels of miR-486 were significantly lower in tumor tissues compared with normal breast tissues in both TCGA cohorts and GSE57897. These results were in same line with the notion that it is a tumor-suppressive miRNA ($p<0.001$ and $p=0.003$, respectively). To our surprise, miR-486 expression was not associated with cancer staging in any TCGA cohort subtypes. High miR-486 tumors demonstrated better disease-specific survival (DSS) and overall survival (OS) compared with low expressing in ER+/HER2- breast cancer patients ($p=0.044$, and $p<0.001$), which was not the case for the other subtypes in METABRIC cohort. Interestingly, high miR-486 tumors significantly demonstrated lower expression of MKI67, one of the most commonly used parameters for cell proliferation, correlated with miR-486 expression in ER+/HER2- ($p<0.001$) but not in other subtypes. This was also the case in immune-related gene sets; interferon-alpha response, interferon-gamma response, complement, and complement that enriched to high miR-486 tumors in ER+/HER2- only. Also, in the same line, Immune Score was significantly higher in ER+/HER2- ($p<0.001$). These results implied its association with the tumor immune microenvironment of ER+/HER2- breast cancer. Given these results, we next hypothesized that a high miR-486 tumor would infiltrate many immune cells. As expected, Anti-tumor immune cells, such as M1 macrophages, Dendritic cells, and Natural killer T cells, were all elevated in high miR-486 tumors in the ER+ /HER2- subtype. Given its association with survival outcomes, we cannot help but speculate that the better outcome of ER+/HER2-

breast cancer is associated with a tumor suppressive effect on the tumor immune microenvironment, which leads to improved survival outcomes.
Conclusion: High miR-486 levels in tumors were associated with a tumor immune microenvironment and better survival; thus, it can be a candidate for a prognostic biomarker in ER+/HER2-patients.]

P3-03-26: Long-Read Nanopore Sequencing for the Identification of Gene Fusion-Derived Breast Cancer Neoepitopes

Skylar Henry, Felipe Batalini, Yining Zhang, Eric Wilson, Abhishek Singharoy, Jin Park, Karen Anderson

Neoepitopes, which are unique HLA-binding peptides produced by cancer cells, are potential cancer-specific targets for T cell-based immunotherapies. However, there are ongoing challenges for the selection and prioritization of neoepitopes for therapeutics. First, next generation sequencing (NGS) is primarily used to select the somatic mutations and insertion/deletions that lead to neoepitopes, but this can require extended time periods and centralized equipment and data processing to retrieve results. Second, current HLA prediction algorithms for neoepitope binding rely on sequence-based methods with limited biochemical data for many diverse HLA alleles. To address these issues, we evaluated the use of nanopore long-read sequencing for rapid neoepitope detection. First, we optimized methods for upstream tumor RNA processing and cDNA synthesis to obtain a depth of coverage that provides confidence in sequence calls, given estimated stochastic error rates of 4%. We developed systems for quality control of RNA length and quality that are needed for accurate neoepitope mapping. Using the cell line MDA-MB-231 and six resected breast tumor samples, we demonstrate detection of known chromosomal fusions by nanopore cDNA-PCR sequencing, as well as unique fusions not detected by next-generation RNA-Seq (Illumina). We then developed a custom prediction computational pipeline (NeoTarget) to prioritize the neoepitope repertoire across HLA-class I alleles. This pipeline incorporates HLA-Inception, a deep convolutional neural network for neoepitope prediction based on molecular dynamic modeling of peptide-MHC electrostatic forces. We then demonstrate the detection and prediction of fusion-derived neoepitopes from two organoid PDX models, one pancreatic adenosquamous carcinoma and one cholangiocarcinoma. Overall, these methods can be applied to rapidly identify and prioritize neoantigens for personalized neoantigen-based immune therapies.

P3-03-27: Klotho proteins and small non-coding RNAs as potential prognostic markers and therapeutic targets for human triple-negative breast cancer and canine mammary carcinoma.

Luciana Mayer Kluppel, Klementina Fon Tacer, Fernanda Rosa, Tanja Plavec, Tana Srava, Vladimira Erjavec, Joseph Emmons, Heather Tilman, Alicia K. Olivier, Danijela Herga

Background: In addition to humans, dogs are one of the few animal species that also develop breast cancer, referred to as canine mammary carcinoma (CMC). CMC is the most prevalent tumor detected in intact female dogs. In contrast to laboratory rodents, CMC develops spontaneously and doesn't require genetic engineering or xenograft models. Furthermore, at a cellular level, mammary carcinoma in dogs is very similar to human breast cancer tumors. The high mortality rates of human breast cancer and CMC, along with the limited treatment options for aggressive triple-negative breast cancer (TNBC), highlight the urgent need for early diagnostic markers and new therapeutic targets for these diseases. A family of proteins involved in metabolism and aging, called Klotho, has been found to have an important role in carcinogenesis. αKlotho and βKlotho proteins act like tumor suppressors and are repressed by microRNAs (miRNAs) during oncogenesis. However, the role of γKlotho in cancer remains to be fully elucidated. Our previous studies suggest that γKlotho is activated in TNBC and acts as an oncogene. However, the molecular underpinning of its functions, expression regulation, and expression in CMC is not known. Here we aimed to determine the expression of γKlotho in TNBC and CMC, and its correlation with miRNA signature to understand its function and regulation in both species. Methods: RNA and miRNA were isolated from tumors and benign mammary tissues from female dogs (n=31) and two CMC cell lines (CF41.Mg and CMT U27) and subjected to mRNA and miRNA expression quantification using RT-qPCR, RNA, and microRNA sequencing. Results: Our data suggest that similarly to TNBC, the mRNA expression of γKlotho is significantly upregulated in CMC compared to normal mammary gland ($\mu = 0.007 \pm 0.0007$ AU, min=0, max=0.045) compared to normal tissue ($\mu = 0.0003 \pm 0.0003$ AU, min=0, max=0.0007), suggesting a role of γKlotho in the oncogenesis of both aggressive diseases. Conclusions and future work: γKlotho may represent promising novel therapeutic targets or stratifying markers for aggressive CMC and TNBC. Female dogs may represent a great preclinical model for potential therapeutic interventions of γKlotho-positive tumors, holding the potential to improve the therapeutic alternatives for both human and canine patients. Further analyses are underway to understand the molecular mechanisms of regulation by miRNA and molecular action.

P3-03-28: Integrative Bioinformatics Approach Reveals Potential Liquid Biopsy-based Biomarkers for Breast Cancer Metastasis Monitoring

Hideki Hideki, Cynthia Jinno, HyoYoung Kim, Jina Kim, Sunao Tanaka, Takeo Fujii, Sungyong You, Hideki Furuya

Background: Despite recent advances in screening and therapeutic strategies, breast cancer remains the first leading cause of cancer death among females. The 5-year survival rate is high as 95% at localized stages but drops significantly to 31% once the cancer metastasizes. Therefore, developing predictive tools of metastatic recurrence is an unmet clinical need to design preventive clinical trials. In this study, we explored potential protein-based biomarkers associated with metastatic recurrence in the patients with non-metastatic breast cancer who underwent curative intent local and systemic therapies and further

assessed their functions using publicly available dataset to identify potential biomarker candidates to further develop blood-based protein signatures for a future study.
Methods: This study used a public dataset (GEO ID: GSE102484), which is a microarray dataset that contains primary tumor samples (n = 683) sourced from subjects with (n = 95) and without (n = 582) distant recurrence (metastasis) of breast cancer (met and non-met, respectively). Subjects with stage IV tumor (n = 6) were excluded from this study. Our approach began with two sets of differentially expressed genes (DEGs). The first set of DEGs are from all the samples in the dataset regardless of breast cancer subtypes, while the second set of DEGs are from triple negative breast cancer (TNBC) samples only. To identify subjects with TNBC, basal-like breast cancer samples were selected using PAM50 algorithm with genefu R package. To select potential blood biomarker candidates, we developed an integrative bioinformatics pipeline. Briefly, 1) Differential expression analysis between met and non-met were performed using limma R package. We then selected DEGs with log2 fold change ≥ 0.58 and false discovery rate (FDR) < 0.1, 2) Genes with housekeeping/hematopoietic markers were removed, 3) The genes that can be detectable in human bloods were selected using the human secretome database from the Human Protein Atlas (HPA), and 4) The functional enrichment analysis of gene ontology (GO) and pathways were performed using topGO and pathfindr packages, respectively. This pipeline was applied to the two sets of DEGs separately.
Results: The differential expression analysis of all breast cancer samples identified 134 significantly upregulated genes in met and the selection process with the pipeline resulted in 12 marker candidates including CD93, HPRT1, MMP1, EPHB4, HSPD1, MAP4, CCDC80, MMP11, SAFB2, CALD1, CRISP3, and GAPDH. Subsequent GO enrichment analysis identified functions associated with these markers including angiogenesis, calcium ion binding, and extracellular matrix and structure organizations. Nucleotide metabolism was observed significance in pathway. We then focused on the TNBC samples from the dataset and identified 162 significantly 162 significantly upregulated genes in met from TNBC. The functional enrichment analysis of the 162 genes revealed significant enrichment of GO biological processes in met, including regulation of apoptotic process, angiogenesis, and calcium ion binding. We also found that FoxO signaling pathway, mTOR signaling pathway, and MAPK signaling pathway significantly enriched in met. Out of the 12 marker candidates from the 134 upregulated genes, 8 marker candidates including CALD1, CD93, EPHB4, SAFB2, HSPD1, MAP4, CCDC80, and HPRT1 were also found in TNBC.
Conclusion: Through an integrative computational analysis, we identified 12 markers associated with breast cancer metastasis, of which 8 were specifically linked to metastasis in TNBC. These markers are involved in diverse functions and pathways that contribute to the metastatic process in breast cancer. Future studies will focus on validating these biomarkers using liquid biopsy samples from breast cancer patients, potentially offering a less invasive diagnostic approach.

P3-03-29: Calcification characteristics in women with benign breast disease and the risk of subsequent breast cancer: a case-control study

Merle van Leeuwen, Sandra van den Belt-Dusebout, Jia Ning Zhuchen, Shannon Doyle, Petra Kristel, Lennart Mulder, Jayakrupakar Nallala, Pieter Westenend, Nick Stone, Esther Lips, Ritse Mann, Jelle Wesseling

Background: Benign breast disease (BBD) is commonly detected in women participating in breast cancer screening programs and comprises a diverse group of lesions. The clinical significance of BBD lies in its association with an increased risk of developing breast cancer, which depends on the histological subtype. Calcifications, frequently observed in mammographic screenings, are critical in the detection and diagnosis of both benign and malignant breast conditions. While many calcifications are benign, some patterns are indicative of ductal carcinoma in situ (DCIS) or invasive breast cancer (IBC). Better characterization of breast cancer risk among women with BBD, considering both clinical and molecular findings, can improve surveillance, early diagnosis, and survival. This study aims to identify mammographic and chemical characteristics of calcifications that are associated with subsequent development of DCIS or IBC.
Methods:
A matched case-control study was conducted of women diagnosed with BBD at the Netherlands Cancer Institute and Albert Schweitzer Hospital between 2004 and 2023. Cases (n=65) were women with BBD who developed ipsilateral DCIS or IBC ≥ 6 months after a first BBD diagnosis, whereas controls (n=244) were BBD patients who did not develop subsequent ipsilateral DCIS or IBC during the follow-up (FU) duration of their matching cases. Additionally, controls were matched based on the year and age at the time of BBD diagnosis. Patient characteristics (e.g. age at diagnosis, vital status) and characteristics of both the benign lesions and subsequent malignant lesions were extracted from pathology reports using text searches and Palga codes. Mammographic lesion types (e.g. calcifications, masses, architectural distortion, asymmetries) were extracted from radiology reports. Qualitative mammographic features including calcification morphology and distribution were extracted from mammograms by two researchers and a trained radiologist. Quantitative mammographic features including breast density score and calcification cluster size and number will be extracted using TRANSPARA 2.0, an radiology artificial intelligence decision support system. In a subset of cases (n = 29) and controls (n=59) chemical characteristics were measured using infrared and Raman spectroscopy.
Results : The baseline comparison of mammographic qualitative features comprised 65 cases and 244 matched controls.Median age at BBD diagnosis was 51 years (range 35-80). Median FU from BBD diagnosis to DCIS or IBC was 6 years (range 1-17). Most cases and controls had non-proliferative BBD (89% and 94%) rather than proliferative BBD. Among cases, 19 developed DCIS while 46 developed IBC. While cases and controls showed comparable proportions of mammographic lesions, calcifications were more prevalent among cases (48.0% vs. 34.0%), approaching statistical significance (p = 0.06). Significant differences in calcification morphology were observed (p = 0.009), with cases more likely to display fine pleomorphic calcifications (23 % vs. 8.5%). The distribution of calcifications

was similar between cases and controls (p = 0.49). Multivariate-adjusted conditional regression models showed an odds ratio (OR) of 1.7 (95% CI: 0.9-3.0) for the association between calcification presence and DCIS/IBC development, albeit with considerable uncertainty. Presence of suspicious calcification morphologies (amorphous, fine pleomorphic, linear) suggested an OR of 3.0 (95% CI: 0.9-10.0) compared to benign morphology.
Conclusions: The trends observed in this study suggest potential prognostic value of calcification morphology in women with BBD. Additional results on quantitative mammographic features and chemical characteristics of cases and controls will be presented at the conference.

P3-03-30: Molecular landscape of breast cancer in pre- and postmenopausal women

Sameer Udhane, Pawan Noel, Fadel Alyaqoub, Ariane Kemkes, Cynthia Flannery, Nishitha Therala, Angela Deem, Jean-Paul De La O, Gargi Basu

Background: Breast cancer (BC) in younger women is often more aggressive and diagnosed at a later stage. The choice of endocrine therapy (ET) for women with hormone-receptor positive (HR+) BC varies based on whether she is premenopausal (preM) or postmenopausal (postM): tamoxifen is usually selected for preM women, while postM women receive aromatase inhibitors (AIs). Approximately 20-30% of advanced HR+ BCs will develop ET resistance and progress on first-line therapy. Whether at progression or in the advanced/metastatic setting, comprehensive genomic profiling (CGP) may provide subsequent-line treatment options, including molecularly matched therapies. Evidence suggesting preM women with HR+ tumors have poor persistence with ET underscores the importance of identifying targets for later-line therapy to improve clinical outcomes for these younger patients. This study investigates the difference in biomarker alterations in preM and postM BC.

Methods: A retrospective analysis of BC samples analyzed with the OncoExTra® assay, which identifies somatic mutations and gene fusions by whole exome DNA sequencing and whole transcriptome sequencing, was performed. Because menopausal status was not available, patients were classified as preM or postM based on age: patients aged ≤50 years were considered preM, and those >50 years were considered postM. The frequency of alterations in preM and postM HR+ BC was determined separately for samples collected from primary and metastatic sites. Potential associations between alterations and subgroups were evaluated using Fisher's Exact Test and the Benjamini-Hochberg False Discovery Rate procedure.

Results: We analyzed 2,573 BC samples with non-missing age, including 772 (30.0%) samples from preM women and 1,801 (70.0%) samples from postM women. Most samples were HER+/HER2- (1,721 (66.9%)), with smaller proportions of HER2+ (369 (14.3%)),

triple-negative BC (TNBC; 377 (14.7%)), and not otherwise specified (106 (4.1%)) samples. In HR+/HER2- BC, alterations in several genes were more frequent in preM compared to postM samples, including: BRCA1 (3.4% vs 0.6%), TP53 (28.5% vs 21.1%), IGF1R (5.3% vs 1.5%), MYC (3.6% vs 1.3%), PPM1D (3.8% vs 1.4%), and GATA3 (19.5% vs 12.2%) (P<0.01 for all comparisons). Conversely, alterations in the following genes were significantly higher in postM compared to preM HR+/HER2- BC samples: alterations in PIK3CA that serve as an FDA-approved companion diagnostic biomarker (CDx; 45.0% vs 35.5%) and PIK3CA non-CDx alterations (7.3% vs 4.5%) as well as ESR1 (8.8%% vs 5.1%), MAP3K1 (11.4% vs 7.4%), and CDH1 (18.5% vs 11.3%) alterations (P<0.05 for all comparisons). In HER2+ BC, ESR1 alterations (5.1% vs 0.7%) were more frequent in postM compared to preM BC, while ERBB2 alterations were more frequent in preM compared to postM BC (79.9% vs 69.8%) (P<0.05 for both comparisons). In TNBC, BRCA1 (18.8% vs 3.6%) and KIT (3.9% vs. 0.0%) alterations were significantly higher in preM compared to postM samples (P<0.01 for both comparisons). BRCA1, IGF1R, and PPM1D alterations predominantly occurred in metastatic preM BC samples (P<0.01 for all comparisons). Also, PIK3CA alterations predominantly occurred in primary postM BC, including both CDx (32.0%) and non-CDx (5.5%) alterations. ESR1 alterations, including mutations, fusions, and amplifications, were present in 21.8% of metastatic postM BC tumors, while ESR1 CDx mutations were found in 15.9% of these tumors.

Conclusion: Our analysis uncovered therapeutically relevant differences in biomarker alterations between preM and postM BC. The higher frequency of ESR1 alterations in HR+/HER2- BC samples from postM women may indicate a resistance mechanism to AIs, as postM women with HR+ BC are commonly treated with this class of drugs. Additionally, the frequency of BRCA1 alterations was significantly higher in TNBC as well as HR+/HER2- preM BC. [CR1] [JH2]
I would eliminate this last sentence. This is intuitive and seems self-serving from a CGP-company. [CR1]agree -- at a minimum suggest replacing "increases" with "may have the potential to increase"

P3-04-01: Extracting Chromatin Signatures from Breast Cancer Using Open Chromatin Guided Machine Learning

Sakuntha Gunarathna, Regina Nguyen, Aerica Nagornyuk, Motoki Takaku

Cell-free DNA (cfDNA) is increasingly recognized as a promising non-invasive biomarker that reflects the genetic and epigenetic profiles of originating cells. Cancer cells release cfDNA fragments characterized by distinctive nucleosome positioning patterns, which could be harnessed for cancer prediction. However, a comprehensive understanding of cfDNA's patterns and its application in developing predictive computational models for cancer remains incomplete. To explore this, we isolated cfDNA from plasma samples with the aim of establishing a simple protocol to extract unique genomic signatures enriched in breast cancer patients. Our analysis confirmed the enrichment of cfDNA in regulatory open

chromatin regions, demonstrating tissue-specific attributes. By focusing on open chromatin loci relevant to breast cancer and immune cells, we identified 2,804 genomic regions that exhibited differential enrichment in cfDNA from breast cancer patients compared to healthy individuals. To further confirm the significance of these differentially enriched genomic loci, we conducted an XGBoost machine learning model using publicly available data to test if they are sufficient to distinguish breast cancer cfDNA patterns. The model demonstrated high prediction accuracy, achieving an overall accuracy of 85.29% and a 3-fold cross-validation score of 84.43%, outperforming models trained on randomly selected genomic regions. Furthermore, by extending the analysis of open chromatin regions to include all previously defined ATAC-seq peaks in luminal breast cancer and CD4 positive T cells, our optimized machine learning model demonstrated an even higher accuracy score of 92.06%, with a 3-Fold Cross Validation score of 89.04%. Importantly, the established XGBoost model provides interpretable outputs that enable the extraction of critical genomic regions essential for cancer prediction. Our findings highlight the potential of cfDNA as a non-invasive screening tool for cancer detection. Moreover, our approach demonstrates an effective strategy for identifying specific genomic loci that distinguish cancer patients from healthy individuals, thus paving the way for the development of a promising non-invasive diagnostic tool.

P3-04-02: Advanced Breast Cancer by Region in the Hawaii and Pacific Islands Mammography Registry

Dustin Valdez, Arianna Bunnell, Nusrat Zaman Zemi, John Shepherd

Background: Despite recent advances in early detection and treatment, breast cancer remains a major cause of morbidity and mortality among women in the U.S. Notable racial/ethnic differences in incidence and survival have been described [1]. For example, Native Hawaiian women have the highest breast cancer incidence in Hawaii despite their favorable reproductive patterns. Japanese American women now experience the same breast cancer risk as non-Hispanic White women, although the incidence in Japan is still lower. Further, the percentage of breast cancers that are advanced are considerably higher in Asian American Women in Hawaii and the Pacific compared to the US mainland, 15% versus 9%.

We present the Hawaii and Pacific Islands Mammography Registry (HIPIMR) which contains over 100,000 unique women undergoing breast imaging in the state of Hawaii. Included are demographic, geolocation, risk factor information, and cancer outcomes obtained through linkage with the Hawaii Tumor Registry (HTR) and Hawaii State Department of Health and Vital Records (HSDHVR). In this study, we asked if advanced-stage breast cancer rates differed by region within each island and may be associated with determinants related to access and acceptance of breast health screening.

Methods: Women who participate in mammographic screening in the HIPIMR were grouped into the following regions: Big Island, Maui, Kauai (Molokai, Lanai), 4 divisions of Oahu using zip code groupings (central, Honolulu, leeward, windward) and unknown

region. Chi-squared tests of independence and pairwise comparisons with Bonferroni correction were then performed to identify differences between the regions at a significance level 0.05. Invasive cases were represented as a percent of advanced stage (3 and 4) to all cases.
Results: Currently the HIPIMR contains 124,475 unique women with visits from 2009 to 2024, and of those 5511 women had invasive cancer during this period. Percent advanced stage rates were Big Island 35.71%, Maui 21.8%, Kauai (Molokai, Lanai) 43.86%, 4 divisions of Oahu using zip code groupings (central 31.72%, Honolulu 35.15%, leeward 38.15%, windward 32.13%) and unknown region 40.37%. The pairwise comparisons showed a significant difference ($p<0.05$) between Honolulu and (Big Island, Kauai (Molokai, Lanai), windward and unknown regions. Also, the unknown region was significantly different than (Honolulu, Central, and Leeward). No significant differences were found between other pairs.
Conclusion: Within the HIPIMR, the advanced breast cancer rate is not uniform across Hawaii, with Kauai (Molokai, Lanai) experiencing the highest advanced cancer rate of 43.86% while the lowest rate was Maui at 21.8%. After pairwise comparisons, the advanced cancer rate of Honolulu significantly differed from many of the more rural islands such as Big Island, and Kauai (Molokai, Lanai). Further research must be done to understand the risk factors associated with causing some regions to experience higher advanced cancer rates.
References:
1. Dietze, E.C., et al., Triple-negative breast cancer in African-American women: disparities versus biology. Nature Reviews Cancer, 2015. 15(4): p. 248-254.

P3-04-03: Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water and Breast Cancer Risk among Women in the Military

Celia Byrne, Michelle S. Mellers, Lynsey L. Lewis, Barbara J. Fuhrman

For several decades, breast cancer rates have increased among younger women under age 50. Previous studies indicated women serving in the U.S. Military had higher incidence of early onset breast cancer than expected based on the U.S. population, but the reasons remain unclear. High levels of Per- and Polyfluoroalkyl Substances (PFAS)), known endocrine disrupting chemicals, have been found in drinking water on a number of US military bases. PFAS are in many industrial and consumer products, but the use of Aqueous Film Forming Foam (AFFF) for firefighting since the 1970's is considered the major source of contamination on military installations. We assessed the association of duration assigned to a military installation reported to have high drinking water PFAS levels with breast cancer rates using data from a nested case-control study among active duty women in the military.
Through the Department of Defense (DoD) Cancer Registry, we identified 832 women diagnosed with (711) invasive breast cancer (BC) or with (121) ductal carcinoma in situ (DCIS), who joined the military since 1990 and were diagnosed between January 1, 2000

and December 31, 2022, while on active duty. We selected 856 matched controls from active duty women who had no reported breast cancer diagnosis at the time of the cases' diagnosis, joined the military in the same year, were born in the same year, and of the same race/ethnicity. In this unique study sample 85% of cases are under the age of 45 and 56% are non-White. In a 2020 report to Congress, the DoD identified 22 military installations where the drinking water PFAS levels were above the 2016 EPA guidelines of 70 ppt. Among the women in this study, 46.8% of the cases and 43.7% of the controls were assigned to one of these 22 installations at some time prior to the cases' year of diagnosis. To estimate the rate ratio for breast cancer associated with time assigned to the identified installations, we calculated the odds ratio (OR) with 95% confidence intervals (CI) using a logistic regression model that controlled for the matching factors.
Compared to women never assigned to any of the identified 22 installations, those assigned for three or more years had an OR = 1.45 (95% CI: 1.09-1.96) for invasive BC or DCIS. The increase in the rate was slightly higher for invasive BC only (OR = 1.52; 95% CI: 1.11-2.07), for those diagnosed with invasive BC before the age of 45 (OR= 1.79; 95% CI:1.27-2.53), and for those diagnosed with invasive BC before 2016, when PFAS contamination was likely higher (OR = 2.02; 95% CI: 1.27-3,21). While assignment to a base with high levels of PFAS in drinking water does not necessarily determine individual exposure levels, these findings might in part provide an explanation for the previously reported higher rates of breast cancer among women serving in the U.S. Military.
Disclaimer: The views expressed are those of the authors and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences or the Department of Defense.

P3-04-04: The Butyrate Transporter SLC5A8 Selectively Inhibits Breast Tumor Metastasis

Sonia Batan, Jabunnesa Khanom, Sabarish Ramachandran, Selvakumar Elangovan, Nanditi N. Thangaraju, Subha Sundaram, Snigdha Ganjikunta, Breanna Kennedy, Vadivel Ganapathy, Puttur D. Prasad, Muthusamy Thangaraju

Introduction: The mammary gland is a dynamic organ that undergoes significant developmental changes during pregnancy, lactation, and involution. The process of involution is a highly orchestrated series of molecular and physical events that can be divided into two distinct phases. (Lund et al., 1996). Accumulation of milk in the alveolar lumen (milk stasis) is required to initiate the first phase during which the secretory cells begin to enter apoptosis. Here we provide genetic and molecular biological evidence to shows that the short-chain fatty acid Butyrate (BTR), a significant component in milk, contributes to milk stasis-induced apoptosis in the mammary gland, and SLC5A8, a BTR transporter, is obligatory for this effect. Slc5a8 deletion in mice is associated with delayed mammary gland involution, susceptible forto chemical, xenograft, and syngeneic transplant, and predisposes to early onset of mammary tumorigenesis and accelerated lung metastasis driven by genetically engineered mouse models of breast and lung cancers.

Objective: To establish the functional link between mammary gland remodeling and its relevance to mammary tumor growth (primary) and distant metastasis.
Methods: All animals were housed and handled according to approved protocols established by the Georgia Health SciencesAugusta University (GHSUAU) Animal Care and Use Committee and NIH guidguidelinesance's. For the measurement of HDAC activity, A commercially available HDAC assay kit (BioVisionBio Vision) was used. Statistical analysis was done using one-way ANOVA followed by Bonferroni multiple comparison test. The software used was Graph Pad Prism, version 5.0. A p-value <0.05 was considered statistically significant.
Results: SLC5A8 is a tumor suppressor, and its expression is silenced in many human cancers, including breast cancer (Thangaraju et al., 2006a; Babu et al., 2011). Here we analyzed Slc5a8 expression at various stages of mammary gland development. Slc5a8 expression was evident at mRNA and protein levels in the virgin mammary gland; it was induced marginally during pregnancy and lactation. Slc5a8 was primarily expressed in the lumen-facing apical membrane of the mammary ductal epithelium. deletion of Slc5a8 in mice leads to a delay in mammary gland involution. We also analyzed the expression of involution markers (Stat3, p53, Bax, BMF, survivin, and IGFBP-5) and apoptotic cell death in wild wild-type (Slc5a8-/-) and Slc5a8-knockout (Slc5a8-/-) mice mammary glands at different stages of involution. pStat3 expression was increased dramatically on Inv d1 and d2 in wild- type glands but to a much less extent in Slc5a8 -/- glands. Further, the expression of p53 and Bax (pro-apoptotic genes), BMF (anoikis inducer), and IGFBP-5 (inhibitor of IGF-induced prosurvival signaling) were significantly increased in wild wild-type glands compared to Slc5a8-/- glands. The anti-apoptotic protein survivin was significantly reduced on Inv d1, d2, and d3 in wild wild-type glands, but this reduction was not observed in Slc5a8-/- glands. Slc5a8 transports into cells the HDAC inhibitors butyrate, pyruvate, and propionate into cells the. (Thangaraju et al., 2009). These inhibitors are selective for HDAC1 and HDAC3. Butyrate, a significant component of breast milk, promotes differentiation in normal cells but induces apoptosis in highly proliferative cells and cancer cells. Slc5a8 induces apoptosis in lactating mammary epithelial cells during involution through modulation of HDAC expression and activity. Butyrate, a substrate of Slc5a8, plays a critical role in promoting mammary gland involution through HDAC inhibition and death receptor activation. Administration of exogenous butyrate induces precocious mammary gland involution in mice. Next, we wanted to exploretested whether if the deletion of Slc5a8 plays any a role in mammary tumorigenesis. The biological changes that occur in mammary epithelial cells during pregnancy/lactation and involution are similar in many respects to those associated with tumor development and tumor regression, respectively. Deletion of Slc5a8 in mice is associated with early onset of mammary tumor formation, accelerated lung metastasis, and decreased overall survival. Finally, we wanted to analyze whether if mammary gland-specific overexpression of Slc5a8 SLC5A8 would influence mammary gland involution and mammary tumorigenesis. Mammary gland-specific overexpression of Slc5a8 SLC5A8 induces precocious mammary gland involution and protects from MMTV-Neu-driven mammary tumorigenesis.
Conclusion: Slc5a8 is a tumor suppressor in the mammary gland, and butyrate is essential

for its tumor-suppressive function. Mammary gland-selective overexpression of Slc5a8 SLC5A8 in mice enhances mammary gland involution and protects against breast cancer.

P3-04-05: Expression analysis reveals clues to the molecular mechanisms driving breast cancer risk in women with benign breast disease

Yasminka Jakubek Swartzlander, Hongyu Gao, Jia Ji, Natascia Marino, Rana German, Michele L. Cote

Introduction: A previous diagnosis of benign breast disease (BBD) is associated with an increased risk of breast cancer, although the molecular mechanisms underlying this association remain unclear.
Methods: We conducted a pilot study to investigate differences in expression between breast tissue donated from women with a history of BBD and women without a history of BBD enrolled in the Komen Tissue Bank (KTB). Briefly, the KTB recruits healthy, cancer-free participants to voluntarily donate breast tissue cores collected via needle biopsy. Additionally, detailed risk factor information is collected at the time of donation, and participants are followed for subsequent breast cancers. Of the 186 samples available for analysis, we selected 27 samples from women with BBD at the time of donation (cases) and 27 samples from women without BBD at the time of donation (controls), matched on age, body mass index category (BMI), and menopausal status. Existing transcriptomic data came from a previous study from Marino et al (GEO accession GSE164641) which utilized Illumina's HiSeq4000 to analyze frozen breast tissue cores from healthy, breast-cancer free donors. We utilized the Edge R 4.2.1 software for analysis of raw count data (RNA). In addition to the expression analysis by BBD status, a subset of samples (n = 133) that were NOT included in the differential expression analyses were analyzed to conduct an association analysis between differentially expressed genes identified in the matched subset and Tyrer Cuzick breast cancer 10-year risk scores. These analyses were adjusted for BMI and age with Tyrer Cuzick risk score as the dependent variable.
Results: There was a similar distribution of age, body mass index (BMI), and menopausal status in the case and control groups. We removed 1 control sample (age=52, BMI=34, postmenopausal), because the log10 normalized mean counts was near 0; all other samples had similar distributions of log10 normalized mean counts. A differential expression analysis was conducted and 2 genes (Carboxypeptidase B1; CPB1, and Shieldin Complex Subunit 3; SHLD3) were significantly overexpressed (FDR < 0.05) in breast tissues from women with BBD (log fold-change=2.5 for CPB1 and 0.56 for SHLD3). Additionally, we performed pathway enrichment analyses and observed upregulation of genes involved in the innate and adaptive immune response, cell cycle, as well as Hippo and TGF-beta signaling pathways in women who had BBD. To further investigate the potential utility of CPB1 and SHLD3 gene expression as markers for breast cancer risk, we conducted association analyses between transcripts per kilobase million (TPM) normalized expression of CPB1 and SHLD3 and the 10 year Tyrer Cuzick breast cancer risk score. We observed an association between SHLD3 expression and breast cancer risk score (p=0.007). SHLD3 plays

a critical role in DNA double strand break repair pointing to a potential increase in the rate of DNA damage in breast tissues from women with BBD compared to those without BBD. For CBP1, we did not observe an association between expression and breast cancer risk score (p=0.17).
Conclusions: We identified gene expression differences in healthy breast tissue from women with and without a prior BBD diagnosis. Specifically, SHLD3 may serve as a potential biomarker of breast cancer risk in women with a history of BBD and was also associated with higher Tyrer Cuzick scores in women who did not have BBD. Our results indicate that activation of immune, cell cycle, and signaling pathways known to be involved in cancer initiation and progression are upregulated in healthy breast tissue from women with BBD who have an elevated lifetime risk of developing breast cancer. These findings suggest that tissue-based markers of risk can be identified even in healthy breast tissue years prior to a cancer diagnosis.

P3-04-06: Genomic and transcriptomic profiling of BRCA mutation carrier tissues reveals the landscape of early pathogenesis of BRCA1/2-associated breast cancer

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Women who harbor germline heterozygous mutations of BRCA1 or BRCA2 have a high risk of breast cancer. Our previous study showed that patient-derived, ostensibly normal BRCA2mut/+ luminal progenitor (LP) cells are more prone to exhibit sub-chromosomal copy number variations and associated DNA damage relative to non-carriers, potentially reflecting early breast tumorigenesis. Clinically assessable biomarkers for early pathological changes of BRCA1/2 mutation in LP cells remain unknown. Single-cell RNA sequencing (scRNAseq) of LP cells of BRCA1/2mut/+ carriers revealed enrichment of KIT expression (KIT+) and subsequent transcriptional factor activations were observed in LP cells of BRCA1/2mut/+ carriers relative to non-carriers. Moreover, pathway analysis uncovered that KIT+ BRCA-mutated LP cells were enriched in pathways involving DNA binding transcription activator activity and oxidative phosphorylation. These gene signature profiles were recapitulated in bulk RNA-seq of BRCA2 mut/+ carrier LP cells. Collectively, our preliminary data suggest such analyses may identify potential biomarkers for near-term risk prediction that reflect early pathogenesis of BRCA1/2-associated carcinogenesis. Following further validation via tissue microassay analysis and clinical studies, our findings may eventually assist patients and clinicians in decision making regarding the timing and necessity of preventive surgeries for BRCA1/2 mutation carriers.

P3-04-07: Modeling BRCA1 and BRCA2 Mammary Tissues and Estrogen Positive Breast Cancer Using iPSC-Derived Organoid Models

Simon Gayther, Alyssa Okimoto, Subash Dhungana, Kate Lawrenson, Xiaojiang Cui, Nur Yucer

Germline BRCA1 and BRCA2 mutations (BRCA1/2mut) predispose women to all subtypes of breast cancer (BC) - ER positive, ER negative and triple negative disease. The cumulative risks in women by age 80 years for all breast cancer (BC) range from 45-75% in BRCA1mut carriers and 41-70% in BRCA2mut carriers compared to the general population (~13% lifetime risk of BC for women in the USA). Many aspects of the underlying biology of these genes in subtype-specific BC development remain unknown. Specifically, although BRCA1 & BRCA2 interact in the same DNA double-strand break repair pathway and BRCA1mut & BRCA2mut both cause basal, triple negative BC, BRCA2mut are more likely than BRCA1mut to cause luminal like ER positive BC. We hypothesize that exposure of normal mammary tissues to estrogens causes ER positive BC in women with BRCA2mut compared to women with BRCA1mut.

We differentiated induced pluripotent stem cells (iPSCs) from subjects with and without BRCA1/2mut into models of normal mammary tissues using a patient-specific iPSC-derived 3D mammary organoid system and studied the neoplastic phenotypes of organoids with and without estrogen (E2) exposure. The iPSC model system enables self-renewal and differentiation into multiple cell type lineages. Intrinsically, this allows cells to self-organize into different cell partners to study the differential effects of specific genetic and/or environmental risk factors on neoplastic growth. Thus, iPSC-derived, mammary models can reproduce the status of germline genetic mutations associated with the development of breast cancers.

We first developed a protocol to generate iPSC derived mammary models from multiple female subjects with and without BRCA1mut or BRCA2mut. None of the BRCA1/2mut carriers had a history of BC and all had had mastectomy for BC prevention because of their BRCA1/2mut status. Immunofluorescent cytochemical staining (ICC) confirmed that all models stained for lineage specific markers (CK18, mammaglobin, CDH1), confirming their mammary tissue status. iPSC derived mammary organoid models from both BRCA1 and BRCA2 heterozygous mutation carriers conferred a neoplastic phenotype reminiscent of a ductal carcinoma in situ (DCIS), a proposed precursor of BC (i.e., organoid models retained the wildtype copy of BRCA1 or BRCA2) compared to BRCA wildtype (BRCA1/2WT) controls, which retained both copies of both genes (confirmed by whole genome sequencing). This suggests haploinsufficiency contributes to the phenotype in both BRCA1 and BRCA2 carriers.

We will present the data after differentiation of BRCA1mut, BRCA2mut and BRCAWT iPSC derived mammary models and then exposed to 150 picomol E2 treatment. The goal was to evaluate if mammary organoids from BRCA2mut carriers and quantify if they are more sensitive to E2 exposure than BRCA1mut and BRCAWT models. This may suggest that they are more likely to develop into BRCA2mut driven ER positive BC, which would suggest a hormone-dependent neoplastic transformation in BRCA2mut carriers reflecting the ER

positive subtype. These finding could present opportunities for reducing mortality due to ER positive BC by driving BRCA2mut cells to an ER positive BC phenotype: This tumor subtype is know to have improved survival and therapeutic response through hormonal dependent treatments such as Tomoxifen.

P3-04-08: Redefining Bravery: A New Paradigm for Helping Young Women Understand Their Risk for Early Onset Breast Cancer

Ally Moehring, Ginny Kincaid, Nancy O'Reilly, Carolyn Headley, Temeika Fairley

Breast cancer in young women is more likely to be hereditary and is often aggressive and difficult to treat. The Centers for Disease Control and Prevention (CDC)'s Bring Your Brave (BYB) campaign helps young women and health care providers understand risks for breast cancer using the power of testimonials, storytelling, and medical education. Our panel session will discuss how CDC is using audience-centric approaches to share information about breast health and breast cancer with women under the age of 45.

Message Development

The BYB campaign: 1) offers clear, tangible steps to understanding and managing early breast cancer risks; 2) tailors messaging for all communities; and 3) equips women and HCPs with tools to make informed decisions about their health. CDC conducted formative research to develop and assess impact of messages and resources aligned with these goals. We will discuss the process used to develop, disseminate, and test effectiveness of these communication tools.

Entertainment Education

CDC used the power of pop culture and entertainment to deliver messages about breast health and breast cancer to young women.

An analysis of TV storylines (2017-2020) revealed that most depictions of breast cancer featured older white women and end-of-life scenarios, while women of color and positive outcomes were rarely shown in entertainment. Based on these results, CDC partnered with Hollywood, Health, and Society to reach writers and creators in the TV and gaming industries to tell a different story. CDC's efforts have resulted in multiple storyline placements in TV shows and a mobile game integration.

Family Conversations

When young women are the first in their families to learn they have an increased risk for hereditary breast or ovarian cancer (a BRCA1 or BRCA2 gene mutation) they are met with the task of telling other family members about their risk. The process of sharing this information with family members can be difficult while they are coping with their own diagnosis.

CDC partnered with the National Association of Chronic Disease Directors to develop a suite of resources to support family conversations about hereditary breast and ovarian cancer risk. These resources include stories of young previvors or survivors sharing their experiences and discussing risk information with family members. An interactive simulation program was developed to allow users to practice these conversations.

Provider Education
CDC partnered with the American College of Obstetricians and Gynecologists (ACOG) to create a comprehensive, two-part CME course to educate health care providers about early onset breast cancer, including risk factors, signs, and symptoms. Since 2020, there have been 3,670 course registrations, 3,083 course completions, and 20,622 CME/CEUs awarded. Pre/post-test results show a +21% knowledge change for Part I of the course (risk factors), +12% change for Part II (health disparities, genetic counseling and testing, risk reduction, epidemiology, and treatment). 57% of respondents reported an intent to change their practice based on learnings.

P3-04-09: Comprehensive Analysis of Rare Variants Associated with Genetic Predisposition to Non-BRCA Familial Breast Cancer Among Arabs

Ehsan Ullah, Hikmat Abdel-Razeq, Sana Bentebbal, Abdullah Shaar, Nehad Alajez, Mohamad Saad, Julie V. Decock

Background: Familial breast cancer represents 5-10% of breast cancer cases, whereby the prevalence of germline mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 (gBRCAm) varies greatly among ethnic groups. In Jordan, pathogenic gBRCAm have been observed in over 10% of high-risk breast cancer patients tested as per the NCCN (National Comprehensive Cancer Network) guidelines. Almost half of pathogenic/likely pathogenic variants detected are non-BRCA1 or BRCA2 genes. Hence, the underlying genetic factors contributing to familial breast cancer remain unknown.
Methods: This study aims to identify cancer risk variants and assess the performance of existing breast cancer polygenic risk scores (PRS) in a Middle Eastern cohort. We performed whole genome sequencing analyses on germline DNA of 180 patients with familial non-BRCA breast cancer (King Hussein Cancer Center, Jordan), and 6000 healthy subjects from the Qatar Precision Health Institute.
Results: Whole genome analysis identified 88,783 single nucleotide variants that are pathogenic/likely pathogenic or are predicted to have a high impact on protein sequence. Approximately 12% of variants were found in cancer-associated genes, of which 74 were classified as pathogenic/likely pathogenic and high-impact variants. Five variants (in MSH3, XPA, PALB2, TP53, and RAD51) were only observed in patients and not in healthy control subjects, suggesting that they might be associated with cancer risk susceptibility. In addition, we found 37 pathogenic/likely pathogenic and high-impact variants in non-cancer related genes, which were only observed in patient samples and could be of interest for further study as novel genetic variants associated with an increased risk of developing breast cancer. Performance analysis of 120 existing breast cancer PRSs revealed that 84% of scores could discriminate breast cancer patients from controls. The four best-performing PRS (PGS003759, PGS003738, PGS003398, and PGS000510) demonstrated AUCs ranging from 0.664 to 0.702, indicative of a good performance in our cohort.
Conclusions: In-depth genomic analysis of non-BRCA familial breast cancer patients from Jordan identified several rare variants in cancer-related genes as well as in novel non-

cancer-related genes. Four distinct PRSs derived from European ancestry cohorts demonstrated a good performance in our cohort, suggesting an added clinical validity in patients of Arab ancestry. Our findings highlight the need for additional studies across ancestries to identify common and population-specific genetic variants that may predispose women to familial breast cancer.

P3-04-10: Integrated germline and tumor whole genome sequencing to identify novel hereditary breast cancer genes in women with very early onset breast cancer

Lily Owens, Qihong Zhao, Simone McInerny, Maia Zethoven, Theresa Wang, Na Li, Paul James

Background: Early onset breast cancer (EOBC), commonly defined as BC occurring in women < 45 years, accounts for about 7% of all new female BC cases but because of the much worse clinical outcome compared to post-menopausal BC, they account for 15% of BC deaths. Given the very early age at onset and the fact that many of these women have a strong family history of breast and other cancers, it is highly likely a clinically important proportion are driven by high-risk monogenic genetic factors. Despite testing for known hereditary breast cancer (HBC) genes in these women, the causative gene for the majority of EOBC cases is not identified. Consequently, comprehensive germline genetic analysis of women with EOBC is likely to identify novel HBC genes that to date have been elusive when studying women with later onset BC where causes other than high-risk genes will confound the genetic analysis. This study used whole genome sequencing (WGS) to assess the presence of rare pathogenic variants in the germline of women with and extreme phenotype of breast cancer, who had tested negative for known HBC genes.
Methods: Forty-four cases of female BC diagnosed under the age of 35 (32 aged 26-30, 6 diagnosed ≤ 25 years) where both germline and tumour DNA was available for WGS were identified from the Variants in Practice Study. All patients were referred for germline genetic testing and returned a negative result for pathogenic variants in all known HBC genes. WGS was conducted on matched germline and tumor DNA to identify potentially pathogenic germline variants in novel HBC genes as well as assessing for evidence of bi-allelic inactivation in the tumor.

Results: No large deletions or translocation within or in the vicinity of any of the known HBC genes were identified and 8 rare variants identified in known HBC genes were excluded due to the absence of bi-allelic inactivation in the respective tumours. Genome-wide analysis of the WGS data identified 593 rare (<0.001 allele frequency in GnomAD) loss of function (LoF) germline variants across 519 genes. 439 genes were excluded from further analysis based on loss of the LoF allele in at least one tumor leaving 81 variants in 80 genes among 28 individuals where there was loss of the wild-type allele in all the tumours. Few of the 80 candidate genes function in DNA repair or genomic maintenance pathways nor any reported role in cancer predisposition. Only one of the 80

genes, ARFGEF1, had LoF variants identified in two carriers with each of the others only observed in just a single case. ARFGEF1 is a guanine nucleotide exchange factor involved in intracellular protein trafficking and cellular organisation, that is a possible tumour suppressor gene that is somatically mutated in some breast cancer.
Conclusion: Despite selection of BC cases with similar very early onset disease and therefore likely to be due to high-risk monogenic genetic factors, there remained a high degree of genetic heterogeneity similar to previous observations in later onset high risk BC cases. Assuming novel HBC genes conform to a two-hit mechanism, many genes were able to be excluded as candidates because of loss of the LoF variant allele in the tumor. A putative BC predisposition role for ARFGEF1 is supported by the observation of 2/44 case carriers and consistent biallelic inactivation the tumors.
Overall our results identified multiple potential candidate genes of interest, however, despite extreme phenotype of very early onset BC, did not identify a cohesive or recurring underlying genetic cause of BC in this cohort emphasising the extreme genetic heterogeneity that exists in the missing heritability of BC.

P3-04-11: Perceptions, Attitudes, and Education of Oncology Health Care Providers Regarding Genetic Testing and Counseling in a Resource-Restricted Country.

Lulwa El Saket, Allison L. Cirino, Eugene Wong, Sarah Spinette, Perman Gochyev, Bayan Altalla, Sarah Abdel Razeq, Hikmat Abdel Razeq

Access to cancer genetics services in Jordan is limited due to limited genetic counseling workforce, genetic testing options, and absence of formal genetic counseling training programs. As such, non-genetic counseling healthcare providers (NGCHP) are required to fill this gap and are the primary providers of genetic counseling services. Understanding their perspectives and experiences is crucial to bridge the accessibility gap of genetic counseling services in Jordan. This study assessed NGCHP's perceived utility, comfort, and familiarity with genetic testing and counseling as well as their past genetics training and preferences for continuing education regarding genetic counseling/testing.
An online questionnaire was created based on similar studies and input from the study team. Questions included: provider demographics, experience, knowledge, perceptions/attitudes, comfort, education regarding genetic counseling and testing, and hypothetical cancer genetic testing scenarios per National Comprehensive Cancer Network Guidelines. Oncology healthcare providers in Jordan were recruited through the King Hussein Cancer Center, the Jordan Oncology Society, the Jordanian Hematology Association, and LinkedIn. Knowledge was assessed by scoring the responses to questions about hypothetical patient scenarios based on National Comprehensive Cancer Network guidelines. Comparative (linear regression, Pearson, and Spearman correlation) and descriptive analysis were performed using Excel and Stata.
There were 33 participants: 88% were male (n = 29,) and 61 % were physicians (n = 20) with 5 years or more of experience. Majority (n=29, 88%) of participants strongly or

somewhat agreed that genetic testing is relevant to their current practice and 32 (97%) strongly or somewhat agreed that it would be increasingly useful in the future. Most participants were either very or somewhat comfortable with aspects of genetic counseling. However, there was no statistically significant correlation between level of comfortability and either years of experience (Spearman's rho= 0.096) or age (P=0.886). The fewest participants were very comfortable with interpreting test results (n=18, 54%) or choosing the correct test (n=18, 54%). When asked specific questions related to patients' eligibility for germline genetic testing, participants
scored 76% or below in the knowledge section. Breast cancer knowledge scores were lowest for questions related to male breast cancer (40%) and triple-negative disease (60%), emphasizing the need for education and training to improve the identification and counseling of eligible patients for genetic testing. Despite minimal formal training NGCHPs are familiar with aspects of the genetic test process. However, there remains a gap in knowledge and how often they are offering or ordering genetic tests suggesting the need for dedicated genetic counseling services in resource-restricted countries.

P3-04-12: Quantitative evaluation and assessment using the automated volumetric breast density measurement software Volpara Density in Japanese women

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Background: Breast cancer screening is being conducted to reduce death from breast cancer, and only mammography screening has been proven to reduce breast cancer mortality. One of the most common causes that reduce the efficacy of mammography screening is a dense breast. The problems with a dense breast are the lower ability to detect breast cancer and the higher incidence of breast cancer. Therefore, evaluating the breast components with objectivity and reproducibility is important. However, in Japan, the composition of the breast is still assessed visually, and this assessment has intra- and inter-observer variability. Although an automated analysis software is widely used to evaluate breast composition in Western countries, there are few studies in Japanese women and limited data are available. Therefore, this study was performed to objectively evaluate the breast density of Japanese women.
Materials and methods: We used the fully automated volumetric analysis software Volpara Density, which performs three-dimensional analysis based on the raw data before imaging. From each set of raw data, the volumetric breast density (VBD), which is the most reliable measure to evaluate dense breasts, was quantified (%) and graded as follows:. a, <3.5% = fatty; b, ≥3.5% and <7.5% = scattered; c, ≥7.5% and <15.5% = heterogeneous ; and d, >15.5% = extremely dense. This study included 23,447 mammograms that were performed at a cooperative research facility between February 2021 and September 2022.
Results: The total number of female participants was 8,350. The median age was 49 years. The most common age group was 40–49 years (36.0%), followed by 50–59 years (33.0%).

The median VBD by age was 21.9% for 30–39 years of age, 19.9% for 40–39 years of age, 15.4% for 50–59 years of age, 13.1% for 60–69 years of age, 11.9% for 70–79 years of age, and 10.5% for 80–89 years of age, and it decreased with age. There was a clear negative correlation between VBD levels and age. We then examined the relationship between breast density and body mass index. We found that the median VBD was 23.9% for women with a body mass index <18.5 kg/m2, 18.6% for ≥18.5 and <25 kg/m2, 9.6% for ≥25 and <30 kg/m2, and 6.1% for >30 kg/m2. This finding indicated that VBD decreased as the body mass index increased. Furthermore, we investigated the effect of the previous childbearing status on VBD. In women aged in their 40s, there was no difference in VBD between those with and those without a history of childbearing. However, in women aged in their 50s and 60s, VBD was significantly lower in those with a previous childbearing history than in those without a history of childbearing.
Conclusion: VBD determined using an automated volumetric analysis appears to be higher in Japanese women than in Western women. Volpara Density software is useful to evaluate breast density in Asians.

P3-04-13: Characterization and Comparison of Breast White Adipose Tissue Inflammation in Nigerian and African American Women with Primary Breast Cancer

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Background: Obesity raises breast cancer recurrence risk and mortality in pre-and post-menopausal women. Most obese individuals harbor breast white adipose tissue inflammation (WATi), which is a central driver of obesity-related breast cancers. The prevalence of WATi and its association with obesity varies by race/ethnicity and has not been well characterized in low- and middle-income countries. Here we assess the prevalence of breast WATi and contributing factors, including body mass index (BMI), body composition, clinical characteristics, and blood biomarkers in a cohort of Nigerian women with primary breast cancer. To assess the potential impact of extrinsic factors, we also compared findings in the Nigerian cohort to a cohort of African American (AA) women with breast cancer.
Methods: We collected non-tumorous breast tissue and fasting blood from 97 consecutive Nigerian breast cancer patients and 82 AA women undergoing mastectomy for breast cancer treatment at Obafemi Awolowo University Teaching Hospital (OAUTH), Nigeria, and Memorial Sloan-Kettering, New York, respectively. We identified breast WATi by CD68 immunohistochemistry to highlight macrophages encircling a dysfunctional adipocyte that forms a crown-like structure of the breast (CLS-B). We measured the total adipose area using NIH Image J software to report the severity of inflammation as CLS-B/cm2. In the Nigerian cohort, we measured circulating levels of lipids, insulin, glucose, C-reactive

protein, and adipokines in fasted blood samples. Body composition was measured by bioimpedance analysis. Associations between WATi and clinical variables were analyzed using univariable logistic regression, Wilcoxon rank sum, and Pearson's Chi-squared tests to compare the two cohorts. Among patients with WATi present, the relationship between BMI and the severity of inflammation was assessed using Spearman's rank coefficient rho.
Results: In the Nigerian cohort, 90 women were evaluable (median age 52, interquartile range [IQR] 45-59; median BMI 27, IQR 24-31). Breast WATi was present in 32 (36%) of the Nigerian cohort. Higher BMI (OR: 1.19, 95% CI: 1.07-1.33, p <0.001), increased total body fat (OR: 1.09, 95% CI: 1.04-1.16, p<0.001), increased trunk fat (OR: 1.18, 95% CI: 1.07-1.32, p<0.001), hypertension (OR: 3.20, 95% CI: 1.19-8.88, p = 0.022), higher fasting plasma glucose (OR: 1.16, 95% CI: 1.01-1.39, p = 0.041), and lower circulating adiponectin (OR: 0.03, 95%CI: 0.00-0.56, p = 0.019) were associated with WATi presence on univariate analysis. In the AA cohort, 82 women were evaluable (median age 49, IQR 44-55; median BMI 30, IQR 27-35). Breast WATi was present in 57 (70%) of AA women. Compared to the Nigerian cohort, AA women had higher BMI (p < 0.001) and higher prevalence of breast WATi (p < 0.001).
Conclusion: In this Nigerian cohort, increased adiposity and metabolic syndrome were associated with adipose inflammation – a risk factor for breast cancer recurrence. Compared to AA women with breast cancer, the Nigerian cohort had a lower BMI and a lower rate of inflammation. Further studies are needed to characterize lifestyle differences to inform culturally-specific prevention and intervention strategies.

P3-04-14: Case report of Breast cancer in a transgender PALB2 pathogenic variant carrier taking hormone therapy

Jessica McMillan, Douglas Riegert-Johnson

Background: About one and a half million individuals in the United States identify as transgender or non-binary. Many patients undergoing male to female transition are prescribed gender affirming hormone therapy (HT). Individuals who also carrying pathogenic variants(PVS) in breast cancer susceptibility traits (BRCA1/2, PALB2 and others) likely have their already high risk for breast cancer increased by gender affirming HT. The aim of this study is to report a rare case of breast cancer in a PALB2 PV carrier undergoing gender affirming HT.
Case presentation: A 40 year old patient, assigned male at birth, presented to her primary care providers with a right breast mass. The patient had been taking gender affirming HT for approximately two years (oral estradiol and spironolactone daily). Diagnostic mammogram and targeted ultrasound were completed confirming a large dense lobulated mass in the retroareolar right breast measuring 6 x 6 x 7 cm with prominent right axillary lymph nodes. Pathology of right breast and axillary lymphnodes reported invasive ductal carcinoma (IDC), grade 2, ER-positive (96% staining), PR-weakly positive (4% staining), her 2/Neu-negative (1+ by IHC). Whole body CT was negative for distant metastasis. The patient received 4 cycles of dose dense Adriamycin Cytoxan (AC) as neoadjuvant therapy

followed by right breast skin sparing mastectomy with sentinel node biopsy. Final pathology was grade 3 invasive ductal adenocarcinoma with negative margins and one of two lymph nodes had macrometastases (TMN staging ypT3 (6 cm), ypN1a (1/2 sn). Patient completed adjuvant postmastectomy radiation 5000 cGy in 25 fractions and started tamoxifen.
She was referred to Clinical Genomics for evaluation. Family history is limited as she is estranged from her family. The patient consented to genetic testing and a PALB2 pathogenic variant was found, c.532delG (p.E178Nfs*15) (CancerNext-Expanded® +RNAinsight®Ambry Genetics).
Conclusion: Despite a growing population of gender aware patients, research is limited on genetic counseling for those individuals with a hereditary cancer predisposition syndrome. Clinical guidelines have been developed and adopted for this population in terms of management, but predictive testing for hereditary cancer syndromes is not the standard of care. Our recommendation is that individuals considering gender reassignment surgery and hormonal therapies consider multigene hereditary cancer panel testing to further stratify their risk for cancer prior to initiation.

P3-04-15: Dietary-Advanced Glycation End products and breast cancer risk: Evaluating MYC dependency within the context of AGE-RAGE signaling in cancer associated fibroblasts

Gowtami Aishwarya Panguluri, Bradley A Krisanits, Jackson Lane, David P Turner, and Victoria J Findlay

Breast cancer ranks as the second leading cause of cancer-related mortality among women. The importance of lifestyle factors in cancer prevention is gaining momentum. Advanced Glycation End-products (AGEs) are reactive metabolites formed as a result of a spontaneous non-enzymatic reaction between reactive carbonyl and amine groups, they are irreversible and they accumulate in our bodies as we grow older. Dietary habits including high fat, high sugar and ultra-processed foods, contribute to the increased consumption of AGEs, and our collaborative group have linked the increased consumption of AGEs to the increased incidence of breast cancer and worse outcomes associated with the disease. Unfortunately, the mechanisms linking AGE consumption and breast cancer progression remain inadequately understood. AGEs can bind to and activate the Receptor for AGE (RAGE), prompting inflammatory responses and oxidative stress, potentially contributing to cancer development. We have developed a unique dietary-AGE mouse model to mimic human AGE consumption. In recently published studies, we found that mice fed a high AGE diet during puberty had dysregulation of normal mammary gland development. We also found hyperproliferative lesions in the high AGE fed mice with increased stromal recruitment including fibroblasts and macrophages. Primary fibroblasts isolated from the mouse mammary glands displayed an activated phenotype, similar to that observed in cancer associated fibroblasts, and led to increased epithelial cell migration and invasion in co-culture assays when compared to fibroblasts isolated from regular fed mice. We were able

to recapitulate this phenotype with exogenous AGE treatment ex vivo, and found that the AGE-mediated effect on migration was dependent on RAGE expression in fibroblasts. In new unpublished studies, we found that dietary AGE consumption promotes aggressive breast tumor growth in vivo. However, we are unable to assess the dependency on stromal RAGE in vivo as we found that the Met1 breast tumor cells do not grow in RAGE null mice. To address this, we are performing 1) co-injections studies in RAGE null mice with breast tumor cells and primary fibroblasts isolated from wildtype mice to ask whether fibroblast RAGE is sufficient for tumor initiation and AGE-mediated breast tumor growth; and 2) co-injection studies in wildtype mice with breast tumor cells and primary fibroblasts isolated from RAGE null mice to ask whether fibroblast RAGE is necessary for AGE-mediated tumor growth. Future studies with RAGE flox mice are planned to assess the impact of dietary AGE on tumor growth in mice with fibroblast specific deletion of RAGE. Based on the importance of MYC in breast cancer and its role as a downstream effector of AGE-RAGE signaling, we hypothesized that MYC may be required for the AGE-mediated effects on epithelial cellular migration. Therefore, we inhibited MYC in fibroblasts through transfection of shMYC constructs, lentiviral infection of shMYC virus and pharmacologically with small molecule inhibitors. These fibroblasts were then used in the epithelial migration co-culture assays. We found that inhibition of MYC in fibroblasts negated the AGE-mediated effects on epithelial cell migration similar to that observed with fibroblasts lacking RAGE. We are now assessing whether MYC is required for AGE-mediated fibroblast activation. Our current data support the idea that AGE-RAGE signaling activates a MYC transcriptional program in fibroblasts to promote epithelial cell migration. Taken together, these data support the idea that dietary intervention in young women may reduce breast cancer risk.

P3-04-16: Tumor Tissue Slices from Breast Cancer Patient Derived Xenografts as an Ex Vivo Method for Treatment Response

John Landua, Ping Gong, Lacey Dobrolecki, Christina Sallas, Michael T. Lewis

Introduction: The generation of patient derived xenografts (PDX) by implanting human breast tumors in the epithelium-free mammary fat pad of immunodeficient mice has allowed researchers to overcome the difficulties in consistently obtaining cancer tissue and provides a more biologically accurate model compared to in vitro cell lines. PDXs also allow for the propagation of a cohort of human breast tumors in which to perform preclinical studies to investigate treatment response. However, due to the costs associated with immunodeficient mice, drugs, and labor, these studies can become cost prohibitive. Therefore, we developed a method to generate and maintain thin slices of PDX tumors to provide a cost-effective platform for studying drug interactions while preserving the cellular heterogeneity and microenvironment of the solid tumor. Method: PDXs were harvested from tumor bearing mice, cored, and then sliced with the Alabama R&D Tissue Slicer. Tumor slices were maintained in a high nutrient media in an oxygen permeable plate to support cellular functions and reduce stress throughout multiple cell layers. Results: Cultured slices were compared to the original tumor via immunohistochemical biomarkers

and transcriptional profiling. Using the tissue slice method, we were able to treat PDX models with chemotherapy, inhibitors, and other small molecules ex vivo to recapitulate results shown in vivo.Discussion: The PDX tumor slice platform can offer a cost effective alternative to investigate treatment response and molecular mechanisms before investing in expensive mouse studies.

P3-04-17: 16q is a breast cancer suppressor arm

Sean Egan, Idil Eda Temel, YeJi An, Katelyn Kozma, Amanda Loch, Wei Wang

The loss of chromosome 16q is the single most common genomic event in BC, occurring in over 50% of tumors. Despite this, the literature is focused almost exclusively on the importance of focal mutations that show a maximum frequency of ~35-40%. We have modeled 16q loss by deleted this region in mammary epithelium of genetically modified mice. This event was sufficient to induce mammary tumor formation. This finding establishes for the first time that 16q is a BC-suppressing chromosome arm. We have gone on to identify two genes on 16q that can promote tumor formation when one copy is lost (Cbfb and Ankrd11, each in cooperation with Pik3caHR). We have also identified mutations that cooperate with 16q syntenic sequence loss in spontaneous tumors from our 16q mouse model (c-Met gene amplification and p53 mutation) as well as in a Sleeping Beauty transposon-based screen (identifying mutations that enhance Ras and Rho signaling). Finally, we have used transcriptional profiling and proteomics to compare matching mammary tumors with/without 16q-syntenic sequence loss, which revealed that hemizygous deletion of this region suppressed keratinocyte-like differentiation in mammary tumors.

P3-04-18: An immunological mechanism of resistance to CDK4/6 inhibitors in breast cancer

Claudia Galassi, Giulia Petroni, Lorenzo Galluzzi

Background: Resistance to CDK4/6 inhibitors (CDK4/6is) underlies treatment failure in patients with HR+HER2- breast cancer (BC) (Pandey et al., 2019). Thus, strategies breaking resistance to CDK4/6i+ET in women with HR+ BC are urgently awaited. Recent findings from Dr. Galluzzi in various immunocompetent mouse models of HR+ BC demonstrate that the CDK4/6i palbociclib (P) can be successfully combined with radiotherapy (RT) when delivered according to a precise treatment schedule (Petroni et al., 2021). These data inspired the initiation of a prospective, randomized phase II clinical trial comparing standard-of-care CDK4/6is plus ET vs RT followed by (à) CDK4/6is plus ET in patients with oligometastatic HR+ BC (NCT04563507).
In this context, we set out to dissect the immunological mechanisms underlying sensitivity vs. resistance to treatment in HR+ BC exposed to P+ET vs. RTàP+ET.
Methods: To dissect the impact of these treatments on HR+ BC, we performed scRNAseq on CD45+ cells infiltrating medroxyprogesterone acetate /7,12-Dimethylbenz[a]anthracene

(MPA/DMBA)-driven carcinomas established in immunocompetent mice (a model of luminal B BC), bioinformatic analysis on public patient datasets, functional studies and efficacy studies.
Results: We identified a hypoxia-inhibitable CCL2-depedent pathway recruiting IL17A+ γδ T cells to mouse HR+HER2- BCs after CDK4/6is, which repolarized tumor-associated macrophages (TAMs) towards an immunosuppressive CX3CR1+ phenotype. IL17A or γδ T cell signatures, as well as intratumoral γδ T cell or CX3CR1+ TAM abundance, correlated with tumor grade and reduced survival in two cohorts of HR+HER2- BC patients. Consistent with mouse data, circulating γδ T cells and plasma CCL2 levels negatively correlated with progression-free survival (PFS) in two series of HR+HER2- BC patients receiving CDK4/6is. Moreover, intratumoral γδ T cells were increased in HR+HER2- BC biopsies upon relapse on CDK4/6is compared to paired baseline biopsies. CX3CR1+ TAMs had negative prognostic impact in patients with HR+HER2- BC treated with anti-PD-1 and RT.
Conclusions: Our observations suggest that γδ T cells and CX3CR1+ TAMs may favor resistance to CDK4/6is in patients with HR+HER2- BC, and hence constitute potential targets to delay disease progression.

P3-04-19: Metronomic cyclophosphamide/iodine is proposed as an effective treatment for breast cancer: comparing intraperitoneal and oral administration in a murine model.

Evangelina Delgado-González, Ericka de los Ríos-Arellano, Brenda Anguiano, Carmen Aceves

Metronomic chemotherapy, which consists of the oral administration of conventional drugs in a continuous schedule at minimal effective doses, has shown promising results in metastatic cancer clinical trials. This treatment shows antiangiogenic and immunomodulatory actions with moderated secondary effects. The use of cyclophosphamide in a metronomic manner (mCpp) combined with immunotherapy improves considerably the antineoplastic response, but it is still expensive. Studies from our group show that molecular iodine (I2) reduces the viability of cancer cells and, with chemotherapeutic agents, activates the antitumoral immune response and diminishes side effects. The present work evaluates the adjuvant of oral I2 with mCpp using a murine model of mammary cancer. Female Sprague Dawley rats with 7,12-dimethylbenzantracene-induced tumors received mCpp in two forms: intraperitoneal (50 and 70 mg/kg two times/week, iCpp50 and iCpp70) and oral (50 mg/kg daily, oCpp50). I2 (0.05%, 50 mg/100 mL) and oCpp50 were dispensed in the drinking water for three weeks, during which body weight and tumor growth were registered. iCpp70 was the most efficient antitumoral dose but generated severe body weight loss and inflammation in the bladder epithelium suggesting the installation of hemorrhagic cystitis (HC). I2 prevented body weight loss, exhibited adjuvant actions with all doses of mCpp decreasing tumor growth, and canceled HC. Mechanisms include decreases in vascular endothelial growth factor (VEGF) and Survivin protein expression. oCpp50+I2 diminished angiogenic signals (CD34, vessel-length,

and VEGF content) and proinflammatory cytokines (interleukin-10 and tumor necrosis factor-alpha) and increased cytotoxic (lymphocytic infiltration, CD8+ cells, Tbet and interferon-gamma) and antioxidant markers (nuclear erythroid factor-2 and glutathione peroxidase). In conclusion, I2 enhances the effectiveness of oCpp with minimal side effects, allowing us to propose this binomial as an excellent candidate to scale it to a clinical protocol.

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P3-04-20: Anti-cancer Efficacy of Natural Killer Cells in Breast Cancer Cell Lines and Patient-derived Organoids

Ilkyun Lee, Suk Jun Lee, Seungji Lee

Background: Currently, immunotherapy through Adoptive Cell Transfer (ACT) of immune cells, such as T cells or Natural killer cells (NK cell), is being proposed as a new treatment in the treatment of various cancers. NK cell-based therapeutic research has been conducted mainly in blood cancer until recently and is currently being tried in many solid cancers.PurposeThe purpose of the study is to examine the anti-cancer efficacy of natural NK cell-based ACT for breast cancer using breast cancer cell lines and patient-derived breast cancer organoids, which mighty be an alternative model to predict the outcome of developing therapeutics.
Methods: The isolated PBMCs from healthy donors were cultured and expanded following a previously published protocol. Characterization of NK cells was performed by flow cytometry and Enzyme-linked immunosorbent assay (ELISA). Expanded NK cells were evaluated for their anti-cancer efficacy in vitro (breast cancer cells MCF-7 and MDA-MB-453) and in vivo (xenograft breast cancer model using MCF-7 breast cancer cells). Breast cancer organoids using breast cancer cell lines (MCF-7, MDA-MB-231, and MDA-MB-453) and patient tissue were generated. Varying number of NK cells (1x104 – 1x105 cells) were applied to examine the cytotoxic effect of NK cells on the patient-derived organoids. The morphological and size changes of organoids were monitored for any sign of disintegration of the organoids for up to 2 weeks.
Results: In the 2 weeks expanded PBMCs, it was possible to secure a sufficient number of NK cells, and it was confirmed that NK cells had anti-cancer efficacy against breast cancer cell. In the xenograft animal model, the expanded NK cells tended to inhibit tumor growth, but they were not statistically significant.The organoids derived from breast cancer cell lines and breast cancer patients were successfully cultured. In the MCF-7 xenografted tumor mass and organoids made of MCF-7 showed co-localized same ER and PR expression

pattern.
However, in the case of breast cancer-derived organoids, the expression of receptors in the patient's original tumor and organoids was not completely consistent. In addition, no significant anticancer effects of NK cells have been confirmed in organoids derived from breast cancer patients.
Conclusion: NK cell-based treatment was judged to have value as an auxiliary treatment method rather than a main treatment method in the treatment of breast cancer. In the case of organoids derived from breast cancer patients, the expression patterns of receptors in the patient's original tumor and organoids did not completely match, which was thought to reflect the diversity and polymorphism of breast cancer cells. For more accurate characterization and evaluation of effectiveness as an alternative animal model, it will be necessary to conduct phenotypic analysis on a large number of organoids.

P3-04-21: Optimization of lentiviral transduction of a triple-negative breast cancer patient-derived xenograft organoid for modeling tumor cell quiescence and associated treatment resistance

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Triple-negative breast cancer (TNBC, lacking expression of estrogen and progesterone receptors and amplification of HER2) is an aggressive and drug-resistant subtype. Current standard-of-care for early-stage disease with neoadjuvant chemoimmunotherapy results in a pathologic complete response in only ~60% of patients. Proliferative heterogeneity naturally occurs in most tumors, where tumor cells exist in multiple different states with varying proliferative potential—states of active division, long- and short-term quiescence, pre-cell cycle entry, and stress. TNBC tumors exhibit a higher percentage of actively cycling, or Ki-67–positive cells, compared to other molecular subsets of breast cancer, which has been shown to predict higher pathologic complete response rates following neoadjuvant chemotherapy. Unfortunately, despite this, TNBC patients experience worse overall survival rates driven by higher rates of relapse. It has been proposed that a significant portion of noncycling, or Ki-67–negative, cells are in a G0-like, or quiescent, state and predicted therefore to be resistant to cytotoxic chemotherapies that rely on actively dividing cells. The presence of a tumor cell population in a quiescent/G0-like state has been increasingly recognized across many tumor types, including in breast cancer.
Patient-derived tumor organoids have emerged as an appealing model system that retains more cellular heterogeneity from primary tumors compared to widely used two-dimensional cell lines. Organoids show remarkable complexity by single-cell RNA sequencing, representing many more cell states compared to best available two-dimensional cell lines that most closely resemble the mutation, copy number variation, gene expression and protein expression profiles of patient tumors.
A mutant version of p27 containing mutations blocking binding to cyclin/cdk complexes, but retained proteolysis in S/G2/M, combined with an mVenus fluorescent protein (p27-mVenus) has been previously validated as a genetic reporter for steady-state G0 readouts.

We have optimized lentiviral transduction of a triple-negative breast cancer patient-derived xenograft organoid with the p27-mVenus reporter to label quiescent organoid subpopulations. Optimized parameters include method of lentivirus concentration, multiplicity of infection, duration of transduction, presence of protamine, presence of a Matrigel base layer, presence of RetroNectin and use of spinoculation. We will use this p27-mVenus TNBC organoid reporter line to study the response of quiescent cell populations to current standard of care treatments and how they contribute to therapeutic resistance.

P3-04-22: An organoid model derived from a patient with breast angiosarcoma

Xiaoling Liu, Meiyang Huang, Jicheng Li, Aishi Deng, Dong Chen

Background: Breast angiosarcoma (BA) is an extremely rare and highly aggressive breast malignancy, accounting for less than 1% of all breast malignancies and less than 5% of all soft tissue sarcomas. BA is associated with a poor prognosis due to a high risk of postoperative recurrence and future metastases, highlighting the importance of postoperative adjuvant therapy. However, the effectiveness of adjuvant chemotherapy for BA is still unclear, with relevant studies mainly based on case reports and small-scale studies. It is urgent to establish a reliable in vitro preclinical model to explore individualized treatment for BA.
Methods: We reported a case of primary BA in a 36-year-old premenopausal woman who underwent a right-sided mastectomy. In order to investigate the most suitable drugs for this patient, we used the postoperative tumor specimen for digestion and preparation of organoid models. And the BA specimens and organoids were fixed and embedded to make paraffin sections. Both of them were then characterized by H&E and immunofluorescence staining. Subsequently, organoid models were used for screening of sensitive chemotherapy and targeted drugs.
Results: The main clinical presentation of this case is a rapidly growing mass in the upper outer quadrant of the right breast, accompanied by slight purplish skin discoloration. The pathological diagnosis showed primary breast angiosarcoma, with immunohistochemical staining positive for CD34, CD31, ERG, and FLI-1, and a high Ki-67 index (60%). We successfully established an organoid model for BA, which could be passaged continuously and cryopreserved. The BA organoids closely recapitulated the histological features and captured the marker expression in the original tumor, including CD31, CD34, c-Myc, CD117, Ki-67, even after a long-term culture. The dose titration tests of 14 chemotherapeutic and targeted drugs displayed differential drug responses with diverse IC50 values. Among all the drugs, anthracycline, paclitaxel, and tyrosine kinase inhibitors such pazopanib showed excellent anti-tumor effects. Inversely, ifosfamide and endocrine drugs showed no obvious anti-tumor effects.
Conclusion: This study indicate that patient-derived BA organoids may be a novel preclinical model to investigate personalized therapy for patients with BA.

P3-04-23: Utilization of a novel breast microphysiological system to investigate the influence of tumor microenvironment on hormone receptor positive breast cancer

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Introduction: Hormone receptor positive (HR+) breast cancer accounts for nearly 60% of all breast cancer cases, with roughly 40% of patients experiencing resistance to endocrine treatment. There are known mechanisms of HR heterogeneity in breast cancer cells such as estrogen receptor-a gene (ER) gene mutations, co-activators, enhanced Ki-67 expression and growth factor activated pathways. Despite this, we do not have adequate predictive markers for response to therapy. Currently we have an incomplete understanding of how heterogeneity in the tumor microenvironment (TME) impacts the ER response in breast cancer. It is well established that the TME can modulate cellular proliferation, survival, and resistance to therapy in breast cancer. There is currently a gap in the ability of models to accurately mimic the TME in vitro, thus limiting our understanding of drug resistance and development of novel therapeutics. Here, we have modeled the pre-menopause, obese HR+ breast TME through the incorporation of six pre-menopause, obese human breast tissue (HBT) donors into a novel ex vivo breast tumor model to investigate the influence of the TME on HR+ breast cancer response to endocrine treatment.
Methods: Ex-vivo breast tumors (EVBTs) were created by sandwiching. and then anchoring, healthy human breast tissue seeded with breast cancer cells between two confluent monolayers of breast adipose-derived stromal cells (brASCs). Breast adipose tissue from six different young (<50 years of age), obese (BMI≥28) donors was used to construct the EVBTs which were then seeded with the same representative HR+ breast cancer cell line and treated with vehicle, E2 100ρM, or Fulvestrant 1μM for 48 hours in median environment depleted of exogenous hormones and growth factors. RNA sequencing was used to demonstrate that the breast cancer cell line signatures was retained in the EVBT system and demonstrated ER response through evaluation of ERα and the ER responsive genes (PGR). Additional analysis done with qRT-PCR demonstrated relative gene expression changes in the microenvironment in response to endocrine treatment. To determine the source of transcriptional changes, individual cell populations were evaluated for ER and ECM genes using qRT-PCR following treatment with endocrine therapy.
Results: Results demonstrated the retention of the breast cancer cell line transcriptome in the ex vivo breast tumor for up to 14 days in vitro. In addition, some donors displayed decreased PGR gene expression after treatment with ICI, elevated ERα gene expression, and increased COL6A6 expression. compared to breast tissue not seeded with HR+ breast cancer. Post-endocrine treatment qRT-PCR results showed variable ECM-gene expression changes between the breast tissue donors. To determine the source of the ECM changes, HR+ breast cancer cells and brASCs were evaluated post-endocrine treatment. Results demonstrated that expression of COL6A6 increased in breast ASCs cultured alone following treatment with Fulvestrant. No significant changes were noted in HR breast cancer cells

cultured alone after endocrine treatment.
Conclusion: Given the impact of the TME in breast cancer pathology and therapeutic resistance, it is critical for the contributions of the TME to be included in pre-clinical models of breast cancer. The findings from this study are ongoing, however, data suggest ECM remodeling occurs in response to endocrine therapy which is influenced by the TME. Further studies are needed to better define the interaction of ECM with HR+ breast cancer as well as the impact of endocrine therapies on ECM and TME remodeling.

P3-04-24: Targeting nucleosome synthesis as a therapeutic strategy for triple-negative breast cancer

Joshua Gruber, Harsh Goar, Shreenidhi Rajkumar, Danielle Dixon

The paradigm of modern cytotoxic chemotherapy is to impair DNA replication to prevent cell division. During this process, newly synthesized DNA is wrapped around histones produced de novo to create the fundamental unit of chromatin: nucleosomes. Yet, despite the 50+ approved oncology drugs that target DNA synthesis, there are currently no therapeutic strategies that target nucleosome production. This gap represents a large and untapped opportunity to develop novel, safer and more efficacious anti-cancer therapies. We have focused on histone acetyltransferase 1 (HAT1), an enzyme that acetylates and stabilizes newly synthesized histones in the cytosol to support nucleosome production. Our previously published data demonstrated that HAT1 controls histone production by also binding histone H4 promoters (Gruber et al. Mol. Cell 2019). In the absence of HAT1, cells cannot effectively synthesize nucleosomes and tumor growth is impaired. To further develop rationale for targeting HAT1 for breast cancer we bred the HAT1+/-heterozygous mouse to the MMTV-PyMT model of triple-negative breast cancer. Despite normal development, animals deficient for a single HAT1 allele in the MMTV-PyMT background evinced delayed mammary tumor onset, decreased tumor size and increased survival compared to littermate controls (median survival 172 v. 196 days for WT v. het; log-rank $p < 0.004$). Histologic examination of tumors revealed a strong correlation between Ki-67 and HAT1 protein expression ($R2 = 0.51$, $p < 0.01$). HAT1 expression in heterozygous animals was primarily diminished in normal mammary glandular structures compared to WT controls, but HAT1 levels were high in tumors, which mirrored high expression of HAT1 and related co-factors in a panel of human triple-negative breast cancers. Since HAT1 primarily binds and regulates histone H4 promoters to coordinate nucleosome production further experiments were performed to test the requirement for histone H4 genes for mammary tumorigenesis. Biallelic CRISPR/Cas9 editing of either promoter or coding regions of three separate histone H4 genes in triple-negative breast cancer cell lines demonstrated that all edits diminished tumor growth potential in vivo. In addition, tumor growth could be rescued by re-expressing a single copy of histone H4 into cells bearing H4 gene edits, demonstrating the specificity of these edits. These results establish that the HAT1-histone H4 axis is a critical dependency for triple-negative breast cancer growth. Our lab has recently published the first small molecule HAT1 enzymatic inhibitor, which demonstrated

anti-tumor activity and animal safety in vivo (Gaddameedi et al. J. Med. Chem. 2023). Thus, small molecule strategies to target the HAT1-H4 axis could have therapeutic relevance for triple-negative breast cancer with a goal of improving patient outcomes.

P3-04-25: Investigating Proteostasis in Endocrine Therapy-Resistant Luminal A Breast Cancer

Anthony Peidl, Shaymaa Bahnassy, Henry Vo, Xiaolian Gao, Tasneem Bawa-Khalfe

Proteostasis (or protein homeostasis) maintains a functional proteome through a network of proteins that regulate protein synthesis, folding, modification, trafficking, and degradation. Dysregulation of the proteostasis network drives multiple diseases including neuropathies and cancer. Various components of the proteostasis network, such as the heat-shock protein (HSP) family, are known oncogenes and attractive targets for therapies. Canonical studies establish HSPs, including small HSP HSP27, as chaperones that facilitate proper protein folding and prevent toxic protein aggregation. More contemporary reports highlight the enzymatic properties of HSP27 as a member of the ubiquitin superfamily of proteins. Specifically, HSP27 directs ubiquitin-tags on misfolded/unfolded proteins and targets to the proteasome for degradation. In addition, HSP27 serves as a ligase for ubiquitin-like SUMO modification of select substrates; our recent report highlights the androgen receptor as a novel substrate for HSP27-directed SUMOylation in breast cancer. Specifically, we observe a concurrent induction of SUMO and HSP27 in endocrine-therapy resistant (ET-R) hormone receptor positive (HR+) breast cancer. Yet it is unclear how induction of HSP27 impacts proteostasis and aggregate formation in this highly aggressive cancer type. New data now shows a change in HSP complex formation between endocrine-therapy sensitive (ET-S) and ET-R breast cancer. We show increased protein aggresome formation in ET-R breast cancer, with ongoing drug treatment studies targeting Hsp27 and the SUMOylation machinery being performed to assess changes in aggresome accumulation in these models. Emerging molecular docking studies elucidate a novel binding pocket for targeted drug therapies that could inhibit the SUMO/SUMO-interacting motif (SIM) interactions for Hsp27. Ongoing protein-protein interaction studies analyzing SUMOylation and ubiquitylation of Hsp27 and target substrates as well as computational studies using small molecule and peptide drug screening, molecular docking, and molecular dynamics will help determine how we can target Hsp27 in ET-R breast cancer to restore its chaperone activity.

P3-04-26: DCIS turns fatal: Thrombotic microangiopathy as the cause of death in undiagnosed recurrent metastatic breast cancer

Samantha El Warrak, Naga Vaishnavi Gadela, Jeffrey Nascimento

Introduction: Pulmonary tumor thrombotic microangiopathy (PTTM) is a fatal condition caused by microscopic tumor cells. Very rarely, this can be the only presentation in

recurrent or metastatic solid tumors. It is an underdiagnosed condition with only a few reported cases in the literature because of its challenging diagnosis premortem. We present a fatal case of PTTM in metastatic breast cancer in a patient considered to be in remission.
Case presentation: A 34-year-old female presented to the emergency department with progressively worsening shortness of breath for one week. Her medical history was notable for left hormone-receptor negative breast ductal carcinoma in situ treated with mastectomy 3 years prior to presentation. On presentation to the hospital, she was hypoxic with an oxygen saturation of 86% on room air requiring nasal cannula(NC). Her physical examination was unremarkable. Laboratory findings were significant for D-dimer 2954ng/ml, C-reactive protein 8.24mg/dL, lactate dehydrogenase 1059U/L, ProB-type natriuretic peptide 524pg/ml, and mild transaminitis. CT-angiography of the chest was negative for pulmonary embolism with normal appearing lung parenchyma. CT-abdomen showed multiple hypoechoic liver lesions and sclerotic bone lesions suggesting possible metastasis. Echocardiogram was significant for fibrinous pericarditis, moderate pericardial effusion, normal ejection fraction, no signs of pulmonary hypertension or intra-cardiac shunt. Her hypoxia rapidly progressed within a 72-hour timeframe from 2L NC, high-flow NC, continuous positive airway pressure(CPAP) to mechanical ventilation. She was started empirically on prophylactic anticoagulation, colchicine and ibuprofen for pericarditis, ceftriaxone and doxycycline along with high-dose steroids for possible community acquire pneumonia, tick-borne illnesses and acquired respiratory distress syndrome. Despite efforts to halt this unknown process, she went into cardiac arrest with pulseless electrical activity and died despite aggressive cardiopulmonary resuscitation. An autopsy showed metastatic adenocarcinoma compatible with metastatic breast cancer.
Discussion: PTTM, also called carcinomatosis endarteritis, is a challenging premortem diagnosis and a fatal complication of tumor emboli from metastatic breast cancer. Breast cancer is the second most common cancer associated with PTTM following gastric cancer. Although the pathogenesis is not fully understood, it is attributed to remodeling of vessel walls and interstitial fibrosis leading to pulmonary infarction, alveolar hemorrhage, pulmonary hypertension and subsequent cor pulmonale. It manifests as cancer cells spreading through the intrapulmonary arterial tree, causing occlusion and sudden death. Devastatingly, it can be the only manifestation of recurrent undiagnosed cancer, as seen in our patient. As there are no standard guidelines to diagnose PTTM, physicians should have a high clinical suspicion to appropriately manage this catastrophic condition.
Conclusion: In conclusion, PTTM is an ambiguous clinical manifestation of metastatic breast cancer, especially when the cancer was considered to be in remission. Our case highlights the importance of considering tumor emboli as a cause of acutely worsening unexplained hypoxemia in patients with a history of cancer, regardless of stage.

P3-04-27: Resistance to neratinib in HER2+ breast cancer: Mechanistic insights and treatment approaches

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Background: Neratinib (Nrb), a potent irreversible pan-HER tyrosine kinase inhibitor (TKI) is approved for use in patients with HER2-positive (+) breast cancer. However, resistance continues to be a key clinical challenge. We previously reported using HER2+ breast cancer cell models that resistance to Nrb is associated with the co-acquisition of HER2 and PIK3CA mutations, which could be overcome by simultaneous blockade of the HER2 and the PI3K pathway. Here, we sought to corroborate the mechanistic underpinnings of Nrb resistance and to extend our studies to the in vivo setting to confirm the efficacy of a promising and potentially less toxic drug combination.
Materials and Methods: Our previously developed HER2+ BT474/AZ cells with acquired resistance to Nrb (NR) or lapatinib (Lap, LR) and their treatment naïve parental (P) cells were used. The Naïve and LR (harboring endogenous HER2 L755S) cells were engineered to ectopically express mutant PIK3CA (mutPIK3CA) using the lentiviral expression system. Upon short-term treatment with small molecule inhibitors targeting HER2 or AKT, either alone or in combination, characterization of relevant cell models, at the signaling level, was performed by Western blot (WB). Changes in cell growth and migration were assessed by methylene blue and Incucyte wound healing assays, respectively. Nude mice bearing GFP/Luc-tagged NR xenografts were treated with vehicle, Nrb, the AKT inhibitor capivasertib (Capi), or Nrb+Capi and monitored for primary tumor growth and metastasis (by bioluminescence imaging). Tumors harvested at the end of experiment were assessed by WB and IHC.
Results: While we previously reported that NR is associated with the co-acquisition of HER2 L755S and a pathogenic PIK3CA mutation (SABCS 2021), the role of PIK3CA mutations, when occurring alone in resistance to Nrb, warranted further investigation. Our new results show that ectopic expression of mutPIK3CA confers resistance to Nrb only when expressed in LR cells that already harbor endogenous HER2 L755S. Interestingly, ectopic expression of mutPIK3CA alone in naïve P cells could confer resistance to tucatinib (Tuca) and Lap but not Nrb. Nrb and Capi only when combined together but not as single agents were effective in overcoming resistance conferred by HER2 and PIK3CA mutations in this ectopic expression system, which agrees with our observation from the acquired NR model. Further, WB analysis of the NR cells showed that Nrb+Capi eliminated the levels of pS6 and induced high levels of the apoptotic marker c-PARP, while single agent Lap, Tuca, and Capi failed to do so. Importantly, our BT474 NR cells not only grow effectively as xenografts in the presence of Nrb but also metastasize to the lung, confirming our in vitro finding that the NR cells are highly migratory compared to P cells. Interestingly, Capi, both alone and in combination with Nrb, was significantly effective in inhibiting the NR tumor growth along with a trend to also inhibit its lung metastatic ability. Evaluating the status of the PI3K/AKT pathway in the available tumors harvested at the end of this experiment, we observed considerably lower

pS6 levels in tumors treated with Nrb+Capi, consistent with our in vitro results. Conclusions: Our data suggest that, in HER2+ BC, the efficacy of Nrb may be reduced by mutations in HER2 together with co-occurring mutations in key downstream mediators such as PIK3CA, which may also confer cross-resistance to other TKIs, especially HER2-selective TKIs such as Tuca. Overcoming this resistance requires the use of small molecule inhibitors co-targeting the mutated HER2 and the downstream component, a strategy that warrants further clinical testing. Our findings thus suggest that the genomic landscape of a HER2+ tumor should guide the treatment decision making in order to ensure the optimal use and sequence of currently available HER2 TKIs.

P3-04-28: Inhibition of FASN and ACC1 as a Potential Treatment for Advanced Endocrine Therapy-Resistant Breast Cancer

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Introduction: Over the past decade, there have been substantial advancements in improving survival rates for estrogen receptor alpha (ERα) positive breast cancer. Treatments such as selective estrogen receptor down-regulators and modulators (SERMs) like fulvestrant and tamoxifen, mTOR inhibitors like everolimus, aromatase inhibitors (AIs), and cyclin-dependent kinase inhibitors such as palbociclib, ribociclib, and abemaciclib have all contributed to extending the overall survival of breast cancer patients. Unfortunately, resistance to endocrine therapy is a common occurrence and all patients will eventually succumb to their disease. Metabolic rewiring is a biological hallmark of all cancers, yet there are currently no therapies that specifically target cancer metabolism. Fatty acid synthase (FASN) is a key enzyme in lipid metabolism and is overexpressed in more aggressive and therapy-resistant tumors, including breast cancers. FASN inhibitor, TVB-2640, has been evaluated in multiple tumor cell lines and in a phase 1 clinical study, and showed partial responses in 5 patients and multiple patients with prolonged stable disease (≥16 weeks). Acetyl-CoA-carboxylase-1 (ACC1/ACACA) is an enzyme that functions upstream of FASN to provide malonyl-CoA, a rate-limiting step in de novo long-chain fatty acid synthesis and is overexpressed in cancer. Previously, we showed that FASN inhibition in tamoxifen-resistant cells leads to inhibition of proliferation and reduced tumor growth through induction of endoplasmic reticulum stress. We therefore assessed the effects of FASN and ACC1 inhibition in fulvestrant- and palbociclib-resistant breast cancer.

Methods: We generated fulvestrant-resistant MCF7 cells by long term exposure to fulvestrant (MCF7/FR cells), and palbociclib-resistant (MCF7/RB1Crispr and ZR75/RB1Crispr) cells were generated through CRISPR/Cas9 knockout of the retinoblastoma (RB) gene. We assessed the impact of FASN inhibitor, TVB-3166, (and analog of TVB-2640 with slightly lower molecular weight for in vitro use), and ACC1 inhibitor, PF05175157 on proliferation, viability, cell cycle, and apoptosis in these cells, and tumor growth in xenografts. RNA sequencing of fulvestrant-, and palbociclib-resistant cells was performed to investigate gene expression. Unfolded protein response signaling protein

levels were analyzed by western blotting after treatment with TVB and PF05175157. Results: Inhibition of FASN and ACC1 lead to a marked inhibition of proliferation in fulvestrant- and palbociclib-resistant cells compared to the parental cells. However, inhibiting both FASN and ACC1 did not appear to produce an additive effect in these cell lines. TVB increased cells in the G1 phase, whereas PF05175157 increased the number of cells in the S-phase in fulvestrant-resistant cells. Both TVB and PF05175157 increased the number of apoptotic cells in fulvestrant- and palbociclib-resistant cells. RNA sequencing of fulvestrant-resistant and palbociclib-resistant cells showed that treatment with TVB results in alteration in unfolded protein response (UPR) pathway. Additionally, TVB and PF05175157 significantly inhibited tumor growth in mice to untreated controls.

Conclusion: Our preclinical data provide evidence that FASN inhibition by TVB-3166 or ACC1 inhibition by PF057175157 present promising therapeutic strategies for treatment of endocrine-resistant breast cancer. Further clinical development of FASN and ACC1 inhibitors for endocrine resistant breast cancer should be considered.

P3-04-30: Single cell analysis enables tracking the evolution of resistance to CDK4/6 inhibitors in ER+ breast cancer

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The combination of CDK4/6 inhibitors (CDK4/6i) with endocrine therapy prolongs survival of patients with ER+/HER2- metastatic breast cancer. Nearly all patients with advanced disease treated with this combination eventually progress, highlighting a need for therapeutic interventions that prevent the emergence of drug resistance. Drug tolerant persister (DTP) cells represent a reservoir of surviving cells from which drug-resistant clones eventually emerge. We hypothesize that targeting the DTP state which emerges under selective pressure from CDK4/6i could prevent the emergence of drug resistance. In vitro treatment of MCF7 and T47D ER+/HER2- breast cancer cells with the CDK4/6i Palbociclib and estrogen deprivation for 4 weeks suppressed cell viability with 2-5% of cells surviving treatment and achieving a DTP state. Cell cycle analysis by PI staining revealed that 95% of the DTP cells were arrested in G0/G1 after 4 weeks of continuous therapy. Upon drug washout, the DTP population resumed proliferation within ~1 week and regained sensitivity to retreatment with CDK4/6i, suggesting epigenetic reprogramming is responsible for the DTP state. Whole exome sequencing did not show enrichment of known genomic alterations associated with CDK4/6i resistance (e.g., RB1, FAT1, PTEN). Bulk RNA sequencing of MCF7 DTP cells revealed transcriptome reprogramming compared to untreated controls, which reverted upon drug washout to that of the treatment naïve state. While cell cycle-related gene signatures such as E2F targets and G2M checkpoint were downregulated, interferon response signatures were upregulated in MCF7 DTP cells relative to untreated controls. MCF7 persisters were characterized by enrichment in senescence and chemotherapy-induced stressed gene signatures, while diapause and MYC

target signatures were downregulated. These same signatures were similarly enriched in ER+/HER2- tumors from patients in the NeoPalAna study treated with neoadjuvant palbociclib and the aromatase inhibitor anastrozole compared to baseline biopsies.
To study evolution of drug resistance, we transduced MCF7 and T47D cells with the LARRY (Lineage and RNA recovery) barcode library to uniquely label and track each persister cells long-term, and identify programs that are causal to the emergence of drug resistant clones. We performed single cell RNA-seq on barcoded cells treated with palbociclib plus estrogen deprivation for 0 h, 72 h, and 4 weeks. Several clones that arrested in G0/G1 initially eventually expanded at 4 weeks, while other clones were eliminated. We are currently investigating which genes/pathways are upregulated in the barcoded clones enriched at the 4-week time point. We will then validate results from these single cell approach in cells surviving neoadjuvant treatment in the NeoPalAna trial.
In summary, a small subset of ER+/HER2- breast cancer cells survive long-term treatment with CDK4/6i plus estrogen deprivation and exhibits reversible transcriptomic features of DTPs. Our ongoing single cell-seq analyses may reveal transcriptional programs that enable the DTP state which, in turn, could be targeted to reduce survival of drug-tolerant cells and prevent the emergence of drug resistance.

P3-05-01: Social Determinants of Health in Patients with Metastatic Breast Cancer in Mexico

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Background: Breast cancer is the most common cancer in Mexican women, with a significant proportion diagnosed with metastatic breast cancer (mBC) at initial presentation due to access barriers and social disparities. Despite this, the impact of social determinants of health (SDH) on Mexican women with mBC remains understudied. This study aimed to describe SDH among women with mBC in Mexico and their influence on health outcomes.
Methods: A prospective cohort study was conducted across three tertiary public hospitals in Mexico City, enrolling adult patients with mBC undergoing first- or second-line treatment. At baseline, patients completed a standardized SDH screening tool (the American Association of Family Physicians' Social Needs Screening Tool) and questionnaires assessing medication self-efficacy (SEAMS, scale 13-39), quality of life (FACT-G, scale 0-108), and pain control (BPI, scale 0-10). Clinical data were collected from medical records. Personalized recommendations were provided by a patient navigator based on the patients' identified needs across five SDH domains: access to healthcare (transportation), education, economic stability (employment and finances), neighborhood and built environment (housing, food, and utilities), and social and community context (childcare and personal safety). Associations between SDH domains and health outcomes were analyzed.

Results: 329 patients (99.1% female) with a mean age of 58.2±12.7 years (range 24-91) were included. 168 (51.1%) had recurrent disease and 71.4% were under first-line therapy.
284 (86.3%) resided in urban areas. 203 (61.7%) reported a travel time to the hospital of <2 hours and 66.9% used public transportation.
177 (53.8%) had an educational level of less than high school. 73 (22.2%) were employed.
139 (42.2%) had a monthly household income of <415 USD. 137 (41.6%) had social security insurance.
154 (46.8%) were married or had a common-law partner. 269 (81.8%) had at least one child.
At baseline, the mean SEAMS total score was 32.8±6.2, and the mean FACT-G score was 80.8±10.6. 132 (40.1%) reported having pain; pain severity average score was 4.3±2.2, with a mean pain interference score of 4.7±2.9.
314 (95.4%) patients reported at least one SDH need in the following domains: 287 (87.2%) economic stability, 230 (69.9%) neighborhood and built environment, 123 (37.4%) education, 55 (16.7%) access to healthcare, and 46 (13.9%) social and community context. Overall quality of life was significantly different according to the number of SDH needs (p=0.018). The mean FACT-G score was lower among patients with an education need compared to those without it (78.9±10.8 vs 82.0±10.4, p=0.010). Age was significantly negatively correlated with the FACT-G score (r=-0.245, p<0.001). Pain severity and medication self-efficacy scores (p=0.63) were not associated with SDH needs, except for the patients with social and support needs (31.1±6.8 vs 33.1±6.1, p=0.049). Medication self-efficacy scores were also lower among those in second line therapy (31.7±6.6 vs 33.3±6.1, p=0.036).
Conclusion: A great proportion of Mexican patients with mBC exhibit diverse SDH-related needs that can affect treatment adherence and quality of life. Addressing these needs through interventions like patient navigation could potentially improve breast cancer related outcomes, and merits further study.

P3-05-02: The impact of ethnicity on benefit from novel drugs approved for breast cancer treatment: a systematic review and meta-analysis of randomized phase 3 trials of the last decade.

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Introduction: Asian patients, previously underrepresented in randomized clinical trials (RCTs), lately begun to be more equally enrolled. However, it is still unclear if they derive different benefit from breast cancer (BC) novel anticancer drugs. Thus, we did a systematic review and meta-analysis to evaluate the heterogeneity of treatment efficacy for Asian vs. non-Asian patients enrolled in the major phase III RCTs of the last decade, both in the advanced (aBC) and the early (eBC) setting. Moreover, since the underrepresentation of other ethnicities, especially Black, still limits their evaluation as individual subgroups, we

examined the outcomes of White vs. Non-White patients.
Methods: Novel anticancer therapies approved by FDA between January 2013 and December 2023 for BC treatment were identified. We systematically searched for the eligible RCTs as follows: (a) phase III RCTs with full-text article; (b) enrolling patients with aBC or eBC; (c) comparing the experimental vs. standard drug; (d) available progression free survival (PFS) in the aBC or event free survival (EFS), invasive disease free survival (iDFS) or disease free survival (DFS) in the eBC; (e) reporting the hazard ratio (HR) and 95% confidence intervals (95%CI) for outcomes in Asian and/or non-Asian subgroups or White and/or non-White subgroups. We calculated the pooled HR and 95%CI in ethnic subgroups using a random-effects model, and assessed the heterogeneity between the estimates using an interaction test.
Results: 23 phase III RCTs were identified in the aBC setting, with 1547 (11.1%) patients of Asian ethnicity. Experimental drugs tested included CDK4/6i (palbociclib, ribociclib, abemaciclib), SERD (elacestrant), PI3Ki (alpelisib), PARPi (olaparib, talazoparib), broad variety of anti-HER2 drugs (tucatinib, trastuzumab deruxtecan, pertuzumab, T-DM1, neratinib, margetuximab), anti-PD-1 and anti-PD-L1 drugs (pembrolizumab, atezolizumab) and anti-TROP2 drug (sacituzumab govitecan). 16 RCTs provided HR (95%CI) for PFS in the subgroup of Asians and 17 RCTs for Non-Asians. Overall, the pooled HR for PFS of the experimental arms vs. control arms was 0.58 (95%CI 0.49-0.70) for Asians and 0.61 (95%CI 0.56-0.67) for Non-Asians. 14 RCTs presented the results for the subgroup of White patients and 17 RCTs for non-White patients. The pooled HR for the experimental treatment arms vs. control arms for Whites was 0.64 (95%CI 0.57-0.72), and HR of 0.57 for Non-Whites (95%CI 0.49-0.65). The difference in efficacy in terms of PFS between Asians and non-Asians was not significant (p=0.478), nor between Asians and Whites (p=0.321) nor Whites and non-Whites (p=0.214). For the eBC analysis, we identified 8 phase III RCTs, with a total of 1630 Asian patients (16.4%), but only 4 (experimental drugs abemaciclib, neratinib, T-DM1 and olaparib) reported the outcomes in Asian and non-Asian subgroups. The pooled analysis showed an IDFS advantage for the experimental arms vs. control arms with HR of 0.66 (95%CI 0.50-0.87) for Asians and HR of 0.63 (95%CI 0.53-0.76) for non-Asians, with not significantly different IDFS benefit between Asians and non-Asians (p = 0.808). Pooled HR for IDFS was 0.63 (95%CI 0.52-0.76) for White patients and 0.66 (95%CI 0.52-0.86) for non-White patients. Again, there was no significant difference in the efficacy of experimental treatment arms neither between White and non-White ethnicity (p=0.729), nor between Asians and Whites (p=0.777).
Discussion: The magnitude of benefit from novel anticancer drugs approved for BC treatment in the last decade was not ethnicity dependent. Intrinsic genetic, pharmacogenomic and environmental variability between populations might not be so relevant to determine a different sensitivity to novel anticancer drugs including CDK4/6 inhibitors, ADCs and immune checkpoint inhibitors. This finding is reassuring for the broad applicability of future results of trials conducted with ethnic imbalance.

P3-05-03: Using large-scale, population- or hospital-based data, to identify disparities by race in the use of Oncotype DX: a systematic review

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Background: In the last decade, genomic tests have been implemented in clinical pratice to gauge the added benefit of adjuvant chemotherapy in reducing the risk of relapse for patients diagnosed with hormone-receptor positive, HER2-negative early breast cancer, eligible for genomic testing based on ASCO guidelines. Several tests are available, but the most widely used is Oncotype DX (ODX). There is evidence for potential disparities in the uptake of ODX by race or other social determinants of health. However, most results derive from clinical trials or studies conducted in academic settings. Data with optimal population coverage are important for unbiased identification of potential barriers in access to care. We aimed to review systematically these large-scale results.
Methods: We considered longitudinal, observational studies of women eligible for ODX testing. Eligible studies had to be population-based or hospital-based, regional or nation-wide, and to include information on the uptake of ODX or treatment decision patterns, by race or ethnicity. The outcomes of interest were the odds of being offered ODX for women who were not non-Hispanic White compared to women who were non-Hispanic White. We searched Embase, PubMed and MEDLINE, using complex search strategies. We selected publications based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.
Results: We retrieved 1,854 publications. After selection by title, abstract and full text, six publications were deemed eligible for inclusion in the systematic review, but only five were unique in terms of calendar time, study population or inclusion criteria. Three studies were based on the National Cancer Data Base (NCDB), a hospital-based dataset covering 70% of the US population, while two were based on the Surveillance, Epidemiology and End Results Program (SEER), a population-based dataset that covers approximately 48% of the US population. NCDB covered breast cancer diagnoses for 2010-2014, while SEER covered a longer calendar period, from 2004 to 2014. Three studies included women with node-negative and node-positive disease, while two were restricted to women with node-negative disease. In SEER, around 70% of the patients receiving ODX were non-Hispanic Whites (NHW), while only 10% or less were non-Hispanic Blacks (NHB) or Hispanics. In 2004, NHB and Hispanics were up to 50% less likely to receive ODX than NHW (odd ratios (OR) around 0.50). By 2014, these disparities had diminished to some extent, but they were still remarkable (OR around 0.70) for patients with node-positive disease. In the SEER data, the odds of receiving chemotherapy did not differ by race, but in the high-recurrence score group, NHB were less likely to receive chemotherapy than NHW (OR 0.76). In NCDB, the proportion of NHW among tested women was even larger than in SEER (around 90%), and racial/ethnic minorities were up to 25% less likely to be tested than NHW, during 2010-2014 (OR 0.76). In NCDB, NHB or Hispanics were more likely to receive chemotherapy than NHW (OR around 1.20), but within a given RS group, chemotherapy was equally used between racial/ethnic groups.

Discussion: This review provides robust evidence for racial disparities in access to ODX. The evidence for disparities in chemotherapy use after testing is less clear. This pattern suggests that barriers to optimal care may arise in referral pathways or in awareness of the relevance of testing among women and oncologists. The disparities slightly subsided over time, but they remain remarkable. Data were only available for women diagnosed up to 2014; further studies are warranted to track progress in reducing such inequities. All the eligible studies were conducted in the US, making it urgent to collect data from other countries where the ethnic makeup is equally diverse.

P3-05-04: Understanding access to HER2-targeted therapy among breast cancer patients treated in a resource-constrained setting: Cross-sectional observational cohort analyses

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Introduction: Improving access to healthcare in low and middle-income countries is a complex multidimensional challenge. Bio-similar and generic medicines positively impacted access to oncology medications by injecting price competition and helping negotiate reduced prices. Before the availability of biosimilars in India, access to HER-2 targeted therapy was reported for less than 10% of eligible patients. In this retrospective study we aim to understand overall patterns of access to HER-2 targeted therapy in women treated for breast cancer (BC).
Methods: A consecutive series of breast cancer patients diagnosed between, Jan 2023 and Dec 2023 were included in the analyses. The study's primary endpoint was to identify the proportion of patients with access to HER-2 targeted therapy (at least 1 session of targeted therapy) among eligible patients. The secondary endpoint was to describe the source of access and identify the proportion among those with access compliant with total planned targeted therapy (as decided by the treating physician).
Results: In the specified period, 1147 new BC patients were diagnosed, of whom 203 (17.6%) were HER-2 positive. Eleven patients had no further diagnostic workup at our center so were excluded from further analyses. The majority were non-metastatic (NMBC) at diagnosis (76% - 146/192). De novo metastatic BCs in 24% (46/192) and nine patients had recurrent disease. The median age was 52 years (SD 11.0, range 28 - 87). Overall access to HER-2 targeted therapy was 70.3% (135/192) and was similar in MBC (30/46 – 65%) and NMBC cohorts (105/146 – 71.9%). The majority of the patients with access used biosimilar formulations (overall – 87.3%; trastuzumab - 89.7%; ado-trastuzumab-emtansine - 99.4% & no biosimilar available for pertuzumab). In the NMBC cohort, the sources for access to targeted therapy were government-sponsored (37/105 - 37.1%); non-governmental-organization partnership (26/105 - 24.7%); self-pay or out-of-pocket (26/105 - 24.7%); and private medical insurance (14/105 - 13.3%). In the NMBC cohort compliance to planned targeted therapy was for 88.5% of patients (93/105, 20 completed &

73 ongoing therapy). The mean number of targeted therapy cycles in NMBC was 9.8 (SD 4.3, range 2 – 17). The major reason for incomplete therapy among those with access remains non-compliance related to social concerns (travel, leave for the caretaker, etc.) rather than resource limitations.
Conclusions: Availability of bio-similar drugs improved access to HER2-targeted therapy. Fostering partnerships between public and private sectors, including pharmaceutical companies, to support cancer therapy and better patient education can improve access further to HER2-targeted therapy in BC.

P3-05-05: A Comprehensive Study of Breast Cancer Mortality and DALY Trends considering Smoking as a Risk Factor from 1991-2021 in the USA and exploring the State-level disparities

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Introduction: Breast cancer is the second leading cause of cancer death in women. Smoking has been considered an important risk factor. Over the past 25 years, intensive screening strategies have been implemented to detect breast cancer early. Numerous legislative acts have also been passed to prohibit smoking and public tobacco use. In this study we highlight the trends in breast cancer mortality and disability adjusted life years (DALY) using tobacco as a risk factor with a focus on sex and state wise disparities.

Method: Data on Age-standardized death rates and Disability-Adjusted Life Years (DALY) for breast cancer with smoking as a risk factor were extracted from Global Burden of Disease database. Information about males and females across all U.S. states from 1991 to 2021 were studied. To evaluate performance, the percentage decline in mortality and DALY for every state was computed from 1991 to 2021 and compared to the overall trend in the US. Joinpoint regression analysis was used to examine gender and temporal trends in the US.

Results: Mortality rates in the US decreased by 55.1% from 1991 to 2021. Within the States, the largest reduction was seen in the state of Massachusetts (71.05%) and least decrease was observed in Mississippi (31.89%). On sub-group analysis based on sex, mortality decreased by 51.4% for females and 46.2% for males in the US. Within the States, Massachusetts had the most significant decrease in breast cancer mortality associated with smoking tobacco in females (70.7%) Mississippi had the lowest reduction for both at 30.25% for females and 22.3% in males.
In the US, DALY rates decreased by 57.42% overall, with a more significant decrease in females (56.79%) than in males (46.03%). On state-specific trends, some of the notable decrease were seen in Massachusetts (73.61%), California (65.06%) , New York (66.7%), Maryland (62.06%) and Connecticut (64.96%) from 1991 to 2021. In terms of sex specific data, Massachusetts has shown the greatest reduction in DALY among females (73.4%) and

males (61.45%). Lowest reduction in DALY was seen in West Virginia (30.8%) in females and Mississippi (19.53%) in males.
Conclusions: The results indicate a notable decrease in mortality and DALY related to tobacco-associated breast cancer. This is likely due to improved screening strategies and treatment options. These findings also underscore the advances in the breast cancer landscape. However, substantial state-level disparities remain, emphasizing the need for further epidemiological studies to identify and address the underlying factors.

P3-05-06: The Development of a Hispanic/Latinx Breast Cancer Clinic

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Background: Breast cancer is the leading cause of death for Hispanic women. Even though Hispanic women are 30% less likely to develop breast cancer than non-Hispanic whites, studies have reported a higher risk of breast cancer mortality for Hispanic women compared to non-Hispanic white women. Many factors influence the disparate diagnosis and mortality, including genetic, environmental (estrogen -related,) and socio-economic. Important social factors include the lower rates of mammography screening, longer times to diagnosis after the abnormal test or palpable abnormality. Increased mortality may also be related to tumor specific factors, including increased incidence of triple negative as well as HER2 -positive breast cancer, both subtypes associated with a worse prognosis. Stage at diagnosis is another important factor as Hispanic patients are less likely to have localized stages I and II and higher likelihood to have stages III or IV cancer, which are associated with a much worse prognosis.Once diagnosed with cancer, Hispanic patients are less likely to receive evidence -based treatments, including surgery, chemotherapy, radiation, endocrine and targeted therapy treatments. Similarly, adherence and compliance to adjuvant endocrine therapy is frequently not optimal. Finally, Hispanic patients represent a very small percentage of patients participating in breast cancer clinical trials.

Methods: To address some of the factors related to disparate health outcomes, Northwestern Medicine established The Hispanic Breast Cancer Clinic in September 2023. The clinic's aims are to elevate the care being offered to Hispanic breast cancer patients in their native language. The clinic is doing so by connecting the patient to resources and addressing social determinant of health that could prevent prompt initiation of therapy. By offering evidence-based guideline concordant recommendations for surgery, radiation, and systemic therapy and for appropriate patients, connecting them to clinical trials. The clinic consists of a Spanish-speaking breast cancer oncologist, nurse, medical assistant, administrative assistant, research coordinator and social worker. ResultsThe clinic has served 110 patients, enrolled 7 patients in therapeutic clinical trials, initiated four collaborative research projects, including a study evaluating meditation (29 pts), a phone app to provide breast cancer education in Spanish (60 pts), a questionnaire to determine social determinants of care factors (15 patients) and a program helping patients to cope with anxiety (22 patients).Two additional projects are in development including a survey

regarding clinical trial barriers, as well as a second questionnaire regarding patient reported outcomes related to the Hispanic clinic. We have a submitted grant applications and received a recent grant to fund a collaboration between Northwestern Medicine and the local county hospital (Stroger)to transfer 2 to 4 patients per week for education, consultation, and discussion regarding diagnosis, prognosis, treatment, and clinical trial options.All patients are evaluated by our social worker and are connected to a variety of resources for additional support.

Conclusion: There are many factors that have resulted in worse mortality for Hispanic patients with breast cancer. The factors may be social, genetic or tumor-related. The aim of the Hispanic clinic is to elevate the care and ultimate the outcome of Hispanic patients, once diangosed with Breast cancer by addressing their specific needs. The clinic serves as both a vital resource for patients and a research hub, fostering collaboration with faculty to address pressing questions about breast cancer care, and trust among Hispanic women. This initiative marks an important step towards achieving health equity and advancing tailored healthcare solutions. We will update the numbers of patients participating in the clinic as well as the collaborative projects that are currently ongoing.

P3-05-07: Early Pilot Results From the Navigator-Assisted Hypofractionation (NAVAH) Program to Aid African-American Breast Cancer Patients

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Purpose/Objective(s): In the United States, African-Americans have the highest overall cancer death rate and shortest survival time of any racial or ethnic group. By far the most commonly diagnosed cancer in African-American women is breast cancer. Consequently, breast cancer provides an excellent opportunity to address barriers impeding equal access to optimal treatment, for which radiation therapy (RT) has been established as essential by Level 1 evidence. Disparities in access to RT, mortality rates, and treatment outcomes among African-American breast cancer patients compared to other populations highlight the urgent need for targeted interventions. The Navigator-Assisted Hypofractionation (NAVAH) program, with its innovative patient navigation approach and culturally sensitive survey, aims to better identify the specific barriers faced by this population. This study is an early report of our experience piloting a culturally sensitive survey in an ongoing NAVAH program clinical trial involving African-American breast cancer patients (ClinicalTrials.gov# NCT05978232).

Materials/Methods: Following IRB approval, African-American breast cancer patients having been referred for RT in multidisciplinary tumor board, seen by Radiation Oncology, and consented to receive RT were approached to participate in trial participation. Surveys were conducted in person or by telephone prior to RT onset. Survey information was

assessed by topic category and survey responses were amalgamated into a representative score for each category (outstanding, excellent, good, average, below average). Survey categories involved availability (access to a medical center, coordinating care, and overall quality of care), accessibility (transportation, distance to care, and healthcare literacy), affordability (financial considerations, employment, and level of education), accommodation (access to internet, navigating transportation), and acceptability (comfort and prejudice among interactions with the system).

Results: Trial enrollment rate thus far has been 67%, with a total of 14 patients having completed the survey; 6 in person and 8 by telephone. Median survey completion time was 27.5 minutes (range=10-45 minutes). Median distance from RT treatment was 5.3 miles (range=3.7-11.4 miles). The mean response noted outstanding availability, excellent accessibility, good affordability, excellent accommodation, and good acceptability. Although satisfaction and trust in interactions with doctors and nurses scored well, responses in the acceptability category highlighted a high perception of disparities in the medical system, including high prevalence of racial prejudice, and high prevalence of treatment differences between high-income and low-income patients in clinical settings. Less than 10% of patients reported using alternative/complementary medicine for their cancer prior to being evaluated for treatment.

Conclusion: Our early NAVAH program findings indicate that this survey design is feasible in the African-American breast cancer population, with early findings revealing optimal availability of care and excellent accessibility to and accommodation for care, with almost no patient using alternative/complementary medicine for their cancer. These findings are tempered by concerns regarding prejudice by patient race and socioeconomic status. Further implementation as this trial accrues will provide more definitive and comprehensive answers regarding these and additional other categories in the survey, including cancer screening, financial toxicity, and perception of treatment during cancer care. Additional investigation involving patients actively receiving breast cancer RT remains ongoing.

P3-05-08: Examining Racial Disparities in the Association between Food Swamps, Liquor Store Density, and Postmenopausal Breast Cancer Mortality among Georgia Cancer Center Patients

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Purpose: Breast cancer (BRCA) is the 4th leading cause of cancer death in the United States (U.S.) and is one of 13 obesity-related cancers. Healthy food consumption (i.e. eating fresh fruits and vegetables, whole grains, less processed foods) is a protective factor shown to decrease obesity risk and postmenopausal BRCA death, but racial and ethnic disparities regarding postmenopausal BRCA continues to persist. Also, residing in geographical areas

with no access to healthy food options and more unhealthy food options (i.e. food swamps) reduces access to healthy foods and has been severely understudied. The influx of liquor stores in the South, or businesses that advertise and sell unhealthy food products including processed foods and alcohol, may contribute to elevated postmenopausal BRCA death. We assessed the relationship between residing in food swamps and liquor store density with risk of postmenopausal BRCA death among a subsample of Georgia Cancer Center patients.
Methods: We conducted a retrospective cohort analysis utilizing 2012, 2014, 2015, 2017, and 2020 data from the U.S. Department of Agriculture Food Environment Atlas and linked them via county FIPS codes with 2016 – 2022 postmenopausal BRCA death (restricted to 45+ years old) patient data from the Georgia Cancer Center's electronic medical record database. Food swamp score (FS) was calculated as the ratio of fast-food and convenience stores to grocery stores and farmer's markets. Liquor store density was calculated as the proportion of beer, wine, and liquor stores to Georgia county populations per 100,000. We categorized FS and liquor store density to low versus high; higher scores indicated patients residing in counties with poorer healthy food resources or greater access to liquor stores. Multilevel Cox proportional hazard models were used to estimate the association between FS and postmenopausal BRCA death.
Results: Out of 282 Georgia Cancer Center patients, the majority of NH-Black and Other patients and married patients resided in high FS and liquor store dense scored counties, respectively (p – value < 0.0001). Overall, patients at the high level of FS counties had an increased risk of postmenopausal BRCA death (adjusted hazard ratio (aHR) = 4.00, 95% CI = 1.06 – 15.1). In our fully adjusted models, NH-Black and Other patients residing in high FS scored counties had a non-significantly higher risk of postmenopausal BRCA death (adjusted hazard ratio (aHR) = 2.27, 95% CI = 0.37 – 14.0) compared to NH-Whites living in low FS scored counties.
Conclusions: Our subsample of cancer patients living in the worst food environments had increased risk of postmenopausal BRCA death. Local and state policymakers should make a concerted effort to partner with community stakeholders and farmers to employ sustainable approaches at combating obesity and BRCA by increasing healthier accessible food sources (e.g. community gardens, indoor hydroponic vertical gardens).

P3-05-09: Invasive Lobular Carcinoma Has Aggressive Molecular Subtypes in African American Women

Genevra Magliocco, Roy Khalife, Anthony Magliocco

Introduction: Breast cancer is a heterogeneous disease with many biological subtypes. Invasive lobular carcinoma (ILC) is a rarer subtype of increasing clinical importance, with distinct clinical pathological and molecular features that are not fully understood. The incidence of ILC is increasing compared to ductal carcinoma in the US. While the incidence of breast cancer is lower in African American/Black (AA/B) women compared to White women (W), mortality rates are disproportionately higher. The reasons for this disparity are unclear. The aim of this study is to better characterize the molecular features of lobular breast cancer and determine if differences exist between AA/B women compared to W women.

Methods: The Cancer Genome Atlas firehose data set was analyzed in cBioPortal, comparing AA/B (n=16) and W (n=166) women with lobular breast cancer. Statistical tests including linear cox regression, Fisher's exact test, and chi square analysis were performed using R (version 4.4.1, 2024). Variables included race, stage, age, and hormone receptor status. Analysis compared differences in overall and disease-free survival and interpreted molecular differences between the two groups. ClinVar was used to determine frequency of germline mutations in the general population.

Results: There were no differences for distribution of age or stage between racial subgroups. Proportions for stage were 18.7% AA and 15.7% W for stage 1, 62.5% AA and 50.3% W for stage 2, and 18.8% AA and 33.9% W for stage 3. The median age was 59.3 for AA/B and 62.0 for W.

Overall survival at 36 months was worse (76.7%) in AA/B compared to (94.6%) in W women with ILC (p=0.056). In this cohort, age and stage were not associated with differences in overall survival.

Estrogen receptor (ER) negative status was more prevalent in AA/B subgroup with ILC (22%) compared to W women with ILC (2%). Overall survival at 36 months for patients with ER negative ILC was shortened compared to ER positive ILC (50% vs 98% respectively, p=0.0001). However, when separated by racial subgroup, ER status was not associated with differences in overall survival for AA/B vs. W. Patients with ER negative ILC were 14.29 times more likely to have a TP53 alteration compared to ER positive ILC (p= 0.002).

TP53 mutation was more prevalent in AA/B (37.6%, n=6) compared to W (3.6%, n=6) in lobular cancer. Within the AA subgroup, disease free survival at 36 months for patients with TP53 alterations was 25% (n=5) compared to 100% for patients with unaltered TP53 (n=11) (p=0.0004). Within the W subgroup, patients with TP53 alterations were less common and associated with longer disease-free survival at 3 months (100%, n=6 for altered) compared to unaltered TP53 (89.5%, n=160) (p=0.50). Among the 6 AA/B patients with TP53 alterations, four had allele frequencies of 0.4-0.7, possibly suggestive of germline mutation. A search of ClinVar revealed two out of the four TP53 mutations were previously seen in Li Fraumeni syndrome (R248W, L255 del).

Conclusion: Overall survival for ILC was worse in AA/B women compared to their W

counterparts, consistent with existing literature. Differences in hormone status and inherited and somatic gene mutations were identified between racial subgroups. Among ILC there is an aggressive subtype characterized by ER negativity and TP53 mutations. This subtype may be more common in AA/B. These results suggest that some TP53 mutations in AA/B may be inherited; this could have implications for future targeted TP53 therapy in AA/B. However, analysis is limited by small sample size for AA/B women with ILC, and absence of detailed sociodemographic, treatment and outcome data. Future studies should include a larger size of AA/B, and control for sociodemographic and treatment variables.

P3-05-10: Racial Disparities in Clinical Trials of PARP Inhibitors for Breast Cancer - A Comprehensive Review

Elizabeth John, Simran Sekhon, Parnian Kheirkhah Rahimabad, Noufil Adnan, Arindham Bagchi

Breast cancer is the most common cancer worldwide. When it comes to African American women (AAW), their mortality rate is 40% higher compared to other ethnic groups in the US. AA patients are younger in age at diagnosis, have a higher incidence of triple-negative breast cancer (TNBC), and AA males have higher incidence of breast cancer. These suggest that genetic predisposition plays an important role in the disparate outcomes based on race. An unbiased analysis of TNBC cases has shown that the prevalence of pathogenic germline BRCA1 and 2 mutations is approximately twice as high as in breast cancer overall. Additionally, there is limited data on the enrollment of racial and ethnic minorities in the pivotal trials for PARP inhibitors, which have shown effectiveness in treating metastatic breast cancer associated with germline BRCA1 or BRCA2 mutations.
We conducted a systematic review of phase II/III clinical trials of PARP inhibitors in BRCA 1 and 2 mutated breast cancer. Our review examined the demographic data reported in these trials from 2016 to 2023. To highlight the disparities in the enrollment of minority groups, we calculated the enrollment fractions of various racial groups in these trials. We gathered studies from PubMed, Embase, Scopus, CENTRAL, Clinicaltrials.gov, and Cochrane.
Out of 56 full-text studies identified, 14 met the inclusion criteria, totaling 5,067 patients. Five trials were excluded due to missing racial data. Our review included 2845 White pts, 136 African American (AA) pts, 83 Hispanic pts, 662 Asian pts, and 247 pts from other racial backgrounds. Among the enrollees, 71.6% were White, 16.7% were Asian, 6.2% were in the "other" category, 3.4% were AA, and 2.1% were Hispanic.
We compared the racial demographics of trial participants to breast cancer incidence rates using SEER*Stat software. We applied time series analysis (ARIMA models) to estimate the incidence rates for 2021 to 2023. White, African American, Asian, and Hispanic racial groups were consistently represented in the clinical trials and the SEER dataset. We calculated the Enrollment Fraction (EF) by comparing the number of trial enrollees to the estimated number of cancer cases for each racial subgroup and logistic regression to determine the odds ratio (OR) of clinical trial enrollment across racial groups, considering a P value of < 0.05 as statistically significant.

Compared to the White pts as the reference group, the enrollment OR was 0.28 for AA, 0.14 for Hispanic, and 2.13 for Asian. All these differences were statistically significant (P < .0001). The EF was highest for Asians (0.95%), then White (0.45%), AA (0.13%), and Hispanic (0.06%). Although Asians had higher EF and OR compared to the White group, this estimate does not reflect the enrollment of this population from the US. The Asian trial enrollees were primarily from Asian countries rather than from the Asian population in the US. Therefore, comparing Asian trial enrollees to the Asian cancer incidence cases in the SEER dataset may lead to invalid estimations due to the differing populations. However, other racial minorities were primarily recruited from within the US. To address this issue, we ran an analysis excluding Asians to assess their influence on the estimates of the remaining racial groups. When excluding Asians, the proportion of trial enrollment was even higher for Whites (92.9%), followed by AA (4.4%) and Hispanics (2.7%). Excluding Asians, the EF of other categories remained the same. The OR of enrollment for AA and Hispanics remained the same and statistically significant after excluding Asians.
Racial minorities are consistently underrepresented in pivotal PARP inhibitor trials, contributing to the race-based disparities in breast cancer. These disparities result in a lack of representative data, limiting the generalizability and efficacy of PARP inhibitors.

P3-05-11: Patient Experience and Perceptions Related to Breast Health, Mammography and Artificial Intelligence in Healthcare

Nancy Brinker

The Promise Fund and Hologic Inc. have partnered to expand access to AI-supported breast cancer screening exams in a medically underserved population. As part of this initiative, patient focus groups and patient navigator interviews were conducted to explore patient experiences and perceptions related to breast health, mammography, and artificial intelligence (AI) in healthcare. These interviews were held, virtually, between February and March 2024, and focus groups were held, in-person, on April 9-10, 2024, with participants at FoundCare and the Community Health Center. FoundCare, a federally qualified health center (FQHC), and Community Health Center, a free clinic, both collaborate with the Promise Fund in promoting access to women's breast and cervical health screenings, diagnostic follow up and cancer care services.
The in-person sessions, attended by twenty patients, and the virtual interviews, attended by six patient navigators, were designed to represent diverse linguistic, racial, and cultural backgrounds, including multiple Spanish-speaking communities from Mexico, Dominican Republic, Guatemala, Chile, and Venezuela. All patient navigators were adult women from various free clinics and FQHCs. Patient participants were adult females, all at or below 200% of the Federal Poverty Guidelines.
Key themes from the discussions included:
1. Health Information Sources: Patients primarily relied on the internet, community health centers, medical visits, family, and cultural community communications for health information. Some patients expressed difficulty in keeping up with health information and

emphasized the importance of direct medical consultations with providers and preventive care. Patient navigators were a critical source of information once the patient could access and be connected to that resource. Clinical health and more upstream social services, such as transportation to health appointments and family food security, were also critical needs that patient navigators were key in facilitating.

2. Mammogram Experiences: Experiences with mammograms varied, with some patients reporting pain and discomfort, particularly those with implants. Regular screenings, family history of breast cancer, and personal vigilance were common motivators for mammograms. Financial barriers, insurance issues, and the need for patient advocacy were frequently highlighted.

3. AI in Healthcare: Patients had mixed levels of awareness and understanding of AI. Many associated AI with advanced diagnostics and potential improvements in early disease detection and surgical precision. Concerns included the potential loss of personal interactions with healthcare providers, privacy issues, and the fear of job displacement. However, patients had key opinions on AI that were more favorable than anticipated. There was optimism about AI's ability to enhance diagnostic accuracy, patient experience, provider trustworthiness, and treatment outcomes. Additionally, both patients and patient navigators preferred that physicians and AI work together. Patient navigators were even more familiar with AI. Given the critical role patient navigators hold, their knowledge may play an integral role in improving patient education and access to new technologies in the future.

4. Whole Health Experience: Patients expressed a desire for a more comfortable and supportive mammography process, greater access to care, enhanced trust between patients and providers, and comprehensive information sharing across healthcare providers. Education on breast cancer, early detection, and AI in healthcare was identified as a critical need.

Conclusion

The findings underscore the importance of trustworthy, patient-centered approaches in healthcare, particularly in the integration of AI technologies. Enhancing patient education and provider transparency, improving access to preventive services, and maintaining the human element in healthcare are essential for optimizing patient outcomes and satisfaction.

P3-05-12: The Prognostic Impact of Stress, High Levels of Tumor Infiltrating Lymphocytes and Race in Women Diagnosed with Triple Negative Breast Cancer

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Introduction: Tumor infiltrating lymphocytes (TILS) are the all-encompassing category of lymphocytes within the tumor microenvironment. High levels of TILS in triple negative breast cancer (TNBC) are associated with better response to chemoimmunotherapy and serve as a valuable prognostic marker. In particular, CD8+ T cells have been shown to mediate neoplastic progression. Many of these same immune players, however, are also

recruited during stress. The CD8+ exhausted phenotype has been documented as a downstream effect of stress, ultimately compromising cancer immunosurveillance. Chronic stress has been noted to directly inhibit the antitumor function of TILS. Furthermore, black women have been observed to have more robust immune systems with overall poorer outcomes. In this pilot study, we looked at the interplay of psychosocial distress scores, high levels of TILS, and race.
Methods: This is a historical cohort study of patients with TNBC from two metro Detroit community hospitals. We studied patients collected from December 31st, 2015 through January 1st, 2019. We assessed progression free survival at 3 years (PFS3), 5 years (PFS5) and overall survival at 5 years (OS5). We obtained stress scores via the National Comprehensive Cancer Network (NCCN) distress thermometer. We collaborated with the pathology department to delineate specimens with high TILS, defined as >50%.
Results: We included 27 patients in total. Twenty identified as white, six identified as black, and one did not disclose race. The mean age was 55.7 ± 13.8 (sd) years. The mean distress score was 5.0 ± 3.5 out of 10. High TILS was found in 32% of the group. Though not statistically significant, our data showed the following trends:

Patients with high TILS had higher distress scores (mean distress score 6.4 ± 2.8 vs 3.7± 3.2, p=0.05)
Patients with higher distress scores had worse outcomes. Mean distress scores for patients that did not reach PFS3, PFS5 and/or OS5 were higher. (PFS3 6.6 ± 3.6 vs 3.7 ± 3.1, p=0.6; PFS5 5.4 ± 3.9 vs 3.9 ± 2.8, p=0.3; OS5 5.8 ± 3.8 vs 3.6 ± 2.8, p=0.1)
Patients with high TILS were less likely to achieve PFS3 (28.6% vs 70.6%, p=0.06), PFS5 (28.6% vs 57.1%, p=0.2), and were more likely to die by five years (71.4% vs 35.7%, p=0.1).
Black women were less likely to achieve PFS3 than white women (50.0% vs 57.9%, p=0.7), PFS5 (25.0% vs 47.1%, p=0.4) and were more likely to die by five years (75% vs 47.1%, p=0.3).

Conclusion/Future Directions: Consistent with existing literature, we demonstrated that black women had poorer outcomes overall. Higher distress scores were also associated with poorer outcomes. Interestingly, we showed that patients with high TILS had worse PFS and OS, which is in opposition to current literature. Multivariable analysis was not indicated as only one variable was statistically significant. We postulate that with a larger population, we may see meaningful and statistically significant stratification among distress scores, race and high TILS.

P3-05-13: Determinants for concordance of ASCO quality of care measures in women with breast cancer in a Brazilian cohort

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Introduction: Breast cancer is the main cause of cancer death among women in Brazil, accounting for 1 in every 6 deaths. ASCO has developed quality measures on the administration of adjuvant endocrine therapy, radiotherapy and chemotherapy to improve the quality and accessibility of cancer care. We applied these measures to describe the quality of care offered to women with breast cancer in a public hospital in order to identify its determining factors.
Methods: Retrospective cohort study of patients diagnosed with breast cancer at the National Cancer Institute (HC III/INCA). Patients treated from April, 2016 to October, 2018, with stages I to III were included. Statistical analyzes were carried out in the Software Statistical Package for the Social Sciences (SPSS®) environment. The association between independent variables and outcome was performed using the crude odds ratio (OR). The variables that presented $p<20$ in the univariate analysis were included in the multiple logistic regression model using the forward stepwise method, and those with $p<0.05$ remained in the final model. This study and the respective amendment were approved by the Research Ethics Committee (CEP) of the National Cancer Institute (n°. 1,400,320 and 6,224,883), in accordance with the ethical principles established by the National Health Council.
Results: Of the 1556 eligible patients, 590 were excluded. 322 (44.8%) initiated endocrine therapy within 365 days of diagnosis with a median time to initiation of 379 (IQR 278.8-445.5) days. 78 (35.3%) began radiotherapy after breast conserving surgery within 365 days of diagnosis with time mean to start this treatment of 430 (SD ±140.4) days. Regarding women with hormone receptor-negative breast cancer, 9 (100%) received chemotherapy after 120 days from diagnosis. After adjustment, age >60 (OR 1.49; IC95% 1.6-2.9; $p<0.001$ and OR 2.74; IC95% 1.42 - 5.28; $p<0.003$) and locally advanced staging (OR 4.03; IC95% 2.89 - 5.62; $p<0.001$ and OR 13.47; IC95% 3.05 - 59.44; $p<0.001$) were predictors non-concordance with endocrine therapy and radiotherapy, respectively. On the other hand, Luminal A-like tumors had a greater chance of agreement with the start of hormone therapy and radiotherapy before 365 days after diagnosis (OR 0.40; IC95% 0.27-058; $p<0.021$ and OR 0.22; IC95% 0.11-0.46; $p<0.03$). Patients who underwent adjuvant radiotherapy had a greater chance of not starting treatment with hormone therapy within 365 days. (OR 1.69; IC95% 1.14-2.50; $p<0.009$).
Conclusion: We described breast cancer care in a major Brazilian institution underperforming the quality measurements suggested by ASCO. Age over 60, advanced staging and adjuvant radiotherapy were predictors of non-agreement while Luminal A-like tumors increased the chance of measurement agreement. The measures were informative about the quality of public care, making it possible to identify determining factors that interfere with the provision of high-quality care.

P3-05-14: Male Breast Cancer Disparities & Demographics: A National Inpatient Sample Database Analysis & Literature Review

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Background: Male breast cancer (MBC) disparities exist due to variations in MBC diagnosis and management. We aim to study the literature on MBC disparities and understand the demographic characteristics and utilization patterns of healthcare among MBC patients using a large-scale population-based sample.
Methods: We conducted a staged literature search on databases using relevant MeSH keywords following PRISMA guidelines. After full-text analysis, only relevant articles on MBC disparities were included. For the database study, we used inpatient hospitalization data from the National Inpatient Sample (NIS) between January 1, 2016, and December 31, 2019. We obtained data of male patients with primary or secondary discharge diagnoses of primary breast cancer (ICD10-CM codes C50 & D05) to analyze demographics, inpatient cost, and length of stay.
Results: Nine studies were gathered using the MeSH framework, with six deemed relevant. The American Cancer Society in 2022 reported the highest incidence of MBC in white men (2,650 cases), followed by black/African American men (510 cases). MBC exhibited a slightly higher mortality (530 deaths) compared to female breast cancer, attributed to delayed diagnosis and later-stage detection.
Our NIS database analysis of 1803 hospitalizations supported the demographic findings above, with predominantly White (70.83%), followed by Black (17.72%) and Hispanic (6.61%) patients. The mean age was 67.8 years, with Medicare as the primary insurance coverage (65.02%). Most cases exhibited Charlson Comorbidity index scores of 3 or more (84.80%), with mainly non-elective admissions (77.75%). Hospitalizations were widespread across census divisions, predominantly in urban teaching hospitals (72.77%) and large facilities (48.75%). The mortality rate among hospitalizations for primary MBC was 4.9% (88/1803), with a mean length of stay of 5.2 days & mean hospital charges of $58,279.
The literature shows that non-modifiable and modifiable risk factors contribute to these racial disparities. Men diagnosed with breast cancer often face unique challenges, including delayed diagnosis, limited awareness, and a lack of tailored treatment options. Addressing MBC disparities requires multifaceted approaches involving raising awareness, increasing education, expanding research efforts, and providing comprehensive support services. Collaboration among healthcare professionals, advocacy organizations, researchers, and policymakers is crucial for reducing disparities and improving outcomes.
Conclusions: Our database analysis indicates primary MBC predominates among the elderly, whites, and Medicare beneficiaries, and we need a multifaceted approach to address these disparities. Males should also be educated to recognize breast cancer signs and seek timely medical care, as early detection enhances outcomes significantly.

P3-05-15: Race, Stress, and Breast Cancer: A Scoping Review

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Background: Various physiological and biological pathways triggered by toxic stress may influence breast cancer risk and progression, and these impacts may be differential based on socially constructed racial categories, but the underlying mechanisms are complex and poorly understood. To explore this relationship, we systematically reviewed the literature on the relationships among race, stress (psychological and biological), and breast cancer (BC).
Methods: We searched PubMed (prior to August 2023), Embase, Web of Science, PsycINFO, and Scopus (prior November 2023) to identify relevant peer-reviewed studies. We included studies investigating various measures of stress in women with breast cancer across different racial/ethnic groups. We excluded abstracts, posters, gray literature, non-US studies, non-English publications, studies on other cancers, studies on breast cancer risk without diagnosis, and studies focused on quality of life, physical concerns, symptoms, distress, or burden. Two independent reviewers screened titles and abstracts, and subsequently, independently reviewed full text with disagreements resolved by discussion or a third reviewer. Data extraction using a standardized form and thematic synthesis were also conducted by two independent reviewers with discrepancies resolved through consensus discussions.
Results: Our review identified 30 articles examining the association between race, stress, and breast cancer; 21 were original observational cross-sectional (n=12) or longitudinal studies (n=9) that utilized data from population-based registries, clinical registries, clinic settings, and cancer registries. Nine other articles identified were narrative or systematic reviews. Primary studies explored biological markers including measures of allostatic load (AL), epigenetic changes, and social determinants of health. Studies suggested Black women with breast cancer exhibited higher AL and shorter telomeres compared to White women, suggesting that racial differences exist in objective measures of physiologic stress among patients with breast cancer. Epigenetic modifications were also noted to be associated with social determinants of health, including socioeconomic status potentially influencing breast cancer risk and aggressiveness. Studies highlighted the significant association between neighborhood disadvantage, social support, and psychosocial stress among women of color with breast cancer. These findings emphasize the complex interplay between individual, neighborhood, and societal factors and suggest disparate social conditions may be a driver of higher stress among breast cancer patients of color.
Conclusion: This review provides a landscape view of the current limited evidence on the complex interplay between social stressors, biological responses, and racial and ethnic disparities in breast cancer outcomes. The findings highlight the significant, yet often overlooked, role of chronic psychosocial stress in the disproportionate burden of breast cancer faced by marginalized racial and ethnic groups. This study highlights the need for further mechanistic exploration and evaluation of multilevel, targeted interventions including individual-level stress management techniques, neighborhood-level

improvements to resources, and societal-level policies addressing discrimination and limited access to healthcare. Additionally, identification of biomarkers and pathways that capture the intricate interplay between social stressors and biological responses are crucial for informing more effective and equitable approaches to breast cancer prevention, early detection, and management.

P3-05-16: Extreme ESR1 polyclonality, mutational dynamics and effects on treatment outcomes in patients with ER+ metastatic breast cancer

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Background: Mutations in ESR1 are commonly acquired on endocrine therapy (ET) and confer variable resistance to estrogen-directed treatments. The impact of polyclonality, including the dynamics of ESR1-mutant clones, interactions with co-occurring mutations, and associations with treatment outcomes have not been fully explored. In this case series, we investigate the course of 6 patients (pts) treated for ER+ metastatic breast cancer (MBC) who demonstrate extreme ESR1 polyclonality.
Methods: We conducted a retrospective case review by querying clinical databases across 3 academic cancer centers. We selected all pts with >= 10 concurrent ESR1 variants on any circulating tumor DNA (ctDNA) Guardant 360 test in MBC, defined as extreme ESR1 polyclonality. We explored prior lines of therapy, sites of metastases, frequency of ESR1 variants, commonly occurring co-mutations, and clonal changes in ESR1 in response to treatments.
Results: In our preliminary cohort (n=6), 5/6 pts were post-menopausal at primary diagnosis (median age 53.3y; 59.5y at first metastatic recurrence). 5/6 pts were ER+/PR+/HER2-; 1pt was ER+/PR-/HER2-. 4/6 pts received adjuvant chemotherapy (3/4 ddACT, 1/4 TC); 4/6 received adjuvant AI for >= 4 years (1 pt declined therapy; 1 pt found to have de novo metastasis). All 6 pts had bone metastasis at first metastatic diagnosis; 2/6 pts also had lymph node and adrenal involvement.
Pts had serial ctDNA testing (range 1-4). On initial ctDNA test, there was a range of 0-31 ESR1 clones with a median of 5.5. Pts received a median of 1 line of ET in the metastatic setting prior to first ctDNA test. The most frequent ESR1 clone was Y537N, followed by Y537S, L327R. Capecitabine use was associated with a decline in polyclonality from 10, 16 and 20 variants to 4, 8 and 9, respectively. In 1 pt, eribulin and abraxane did not have the same effect; ESR1 polyclonality was maintained. One pt with 1 ESR1 clone after 2.5 years of fulvestrant+palbociclib developed 15 additional clones after 1.5 years of an experimental SERD+everolimus. Another pt with 1 ESR1 clone after anastrozole+palbociclib developed 9 additional ESR1 clones after 10 months of AKT inhibition+anastrozole. This same pt had a reduction to 4 ESR1 clones on capecitabine with an expansion to 8 clones on a subsequent experimental ADC.
The most frequent co-alterations were in FGFR1 and CCND1, both occurring in 3/6 pts. In 1

pt with 12 ESR1 variants, only TP53 was co-mutated; another pt with 16 ESR1 variants had only AKT1 co-mutated. 1/6 pts carried PIK3CA mutations; this pt had 31 ESR1 variants on initial ctDNA test with 3 PIK3CA clones. Tumor mutational burden will be reported at the conference.
Conclusions: This is the first investigation of dynamic changes in
extreme ESR1 polyclonality in association with treatment outcomes in pts with ER+ MBC. Capecitabine was associated with a decrease in detectable ESR1-mutant clones, while increased polyclonality was found at progression on SERD+everolimus, AKT-inhibitor+AI, and an ADC. Although extreme ESR1 polyclonality is rare, understanding mutational dynamics will improve our understanding of polyclonality more broadly and its impact on treatment resistance. Additional analyses of these and additional pts will be presented at the conference.

P3-05-17: Identifying immune-related predictive factors for post-eribulin therapy in patients with HER2-negative advanced breast cancer - A multicenter retrospective study

Junichiro Watanabe, Yoshiya Horimoto, Yasuo Miyoshi

Background: In the EMBRACE study, eribulin (ERI) monotherapy improved the overall survival (OS) of patients with HER2-negative advanced breast cancer (HER2-ABC) [Cortes, 2011, Lancet Oncol]. A post hoc analysis of the EMBRACE study identified the baseline absolute lymphocyte count (ALC) as a predictive marker in the ERI arm [Miyoshi, 2017, Breast Cancer]. However, there is no report regarding predictive factors of post-ERI OS so far.
Patients and methods: We retrospectively reviewed the medical records of HER2-ABC patients who received ERI therapy from July 2011 to March 2018 in two institutions. Clinical data were extracted from medical records, including ALC, neutrophil-to-lymphocyte rate (NLR), and other clinically significant factors (age, visceral lesion, estrogen receptor, nuclear grade, anthracycline history, taxane history). Statistical analyses were performed using the Kaplan-Meier method, log-rank test, Wilcoxon's test, and Cox hazard model. OS was defined as follows; OS1, survival from the initiation of ERI to death of any cause; OS2, survival from the initiation of post-ERI therapy and OS3, survival from the termination of post-ERI therapy, respectively.
Results: We identified 218 HER2-negative ABC patients who underwent ERI therapy, and 210 out of 218 patients were eligible for analyses. Of 210 patients, 153 patients (72.9%) had recurrent disease, 164 (78.1%) patients revealed hormone-receptor-positive, 180 (87.6%) patients had a history of anthracycline and/or taxane treatment, and 126 (60.0%) had the visceral disease at the initiation of ERI therapy. The median treatment line for ABC of ERI was 2 (ranged 1–9). The median OS (range) was; OS1, 540.0 (41-2700) days; OS2, 337.5 (0-2267) days, OS3, 175.5 (0-1974) days, respectively. According to the multivariate analyses, factors significantly associated with OSs were for; OS1, Time to discontinuation (TTD) of ERI therapy as ≥120 days (hazard ratio [HR], 0.286; 95% confidence interval

[95%CI], 0.193-0.426; P <0.0001), ALC ≥1,500 at the initiation of ERI therapy (HR, 0.610; 95%CI, 0.374 -0.995; P = 0.0479); OS2, ALC ≥1,000 at the initiation of post-ERI therapy (HR, 0.610; 95%CI, 0.416-0.895; P = 0.0115), TTD of ERI therapy (HR, 0.710; 95%CI, 0.525-0.960; P = 0.0262); OS3, TTD of ERI therapy (HR, 0.682; 95%CI, 0.501-0.928; P = 0.0150). Meanwhile, there was a correlation between the TTD of ERI therapy and ALC at the initiation of post-ERI therapy (HR, 0.741; 95%CI, 0.551-0.997; P = 0.0480). Looking at factors affecting the TTD of ERI therapy were anthracycline-based regimen (HR, 1.788; 95%CI, 1.175-2.720; P = 0.0067) and high nuclear grade (HR, 1.526; 95%CI, 1.034-2.254; P = 0.0335), however, no independent factor related to TTD of post-ERI therapy was identified. Conclusion: According to our real-world study we found that 1) a maintained ALC at the initiation of ERI therapy was significantly associated with better outcomes, i.e., both OS1 and OS2 of HER2-ABC patients, 2) ERI therapy responders, i.e., TTD of ERI therapy ≥120days, had been maintained their ALC subsequently, and 3) patients with longer TTD of ERI therapy had been maintained both OS2 and OS3. Those findings support the significance of maintained ALC and patent survival in late-phase ABC [Jimbo, 2022, Breast Cancer Res Treat; Horimoto, 2024, BMJ Support Palliat Care], furthermore, those prompt the use of ERI in HER2-ABC patients who have a maintained ALC. Funding: not applicable.

P3-05-18: Prognostic value of ctDNA dynamics and protein biomarkers in patients treated with alpelisib (ALP) and endocrine therapy (ET) for HR+/HER2- PIK3CA-mutated advanced breast cancer (ABC) in all 3 cohorts of the phase II BYLieve study

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Background: The BYLieve study demonstrated efficacy and safety of ALP (PI3K-α inhibitor and degrader) + ET in patients (pts) with PIK3CA-mutated HR+/HER2− ABC post-progression of treatments including cyclin-dependent kinase 4/6 inhibitor (CDK4/6i). We assessed the prognostic role of ctDNA dynamics and protein biomarkers in pts from this study.
Methods: Pts on prior immediate treatment of CDK4/6i + aromatase inhibitor (AI, Cohort A, n=101), and AI followed by chemotherapy/ET (Cohort C, n=98) receiving ALP + fulvestrant and prior immediate treatment of CDK4/6i + fulvestrant receiving ALP + letrozole (Cohort B, n=97) were included for biomarker analysis. ctDNA fraction levels for samples collected at baseline (BL; Cycle 1, Day 1 [C1D1]) and at end-of-treatment (EOT) were analyzed using cfDNA 2.0 assay for all 3 Cohorts and samples collected at C2D1 (only for Cohort A & B) were analyzed using cfDNA 2.1 assay, both utilizing PanCancer analysis panel that covered coding exons of 579 genes. Genomic landscape alterations in Pi3K/AKT pathway and potential CDK4/6i resistance markers were assessed across those time points. Circulating proteins from a selection of matched patient/timepoint samples (Cohorts A, B and C, n=578) were assessed via SomaScan, an exploratory proteomics platform profiling 7,596

proteins from a single plasma blood sample. The association between ctDNA fraction and protein markers (SOMAmers) was assessed for all matched samples and the prognostic value of identified enriched SOMAmers was investigated at BL (all cohorts, n=292). Results: Among pts who had matched samples at C1D1 and C2D1, 15 of 82 in Cohort A and 11 of 80 in Cohort B had ctDNA fraction=0 at BL. In pts with ctDNA fraction >0 at C1D1, ALP treatment led to a rapid decrease in median ctDNA fraction at C2D1 in Cohort A (C1D1, 0.1; C2D1, 0.055; P=0.023) and Cohort B (C1D1, 0.126; C2D1, 0.079; P=0.044). No significant differences were observed in gene mutation frequencies (including ESR1 and TP53 mutations) across timepoints, in both cohorts, except the Pi3K/AKT pathway-related genes (AKT1,2,3, PIK3R1, PTEN) that revealed an increased number of alterations at EOT. The alterations in genes implicated in CDK4/6i resistance (CDKN2A, CDKN2B, RB1, PIK3R1, ERBB2, FAT1, NF1, CHD4) were consistent in both pt cohorts, but caution needed to interpret this due to limited sample size. Pts in Cohort A, with ctDNA fraction >0 at C1D1 and C2D1 had shorter PFS vs pts with undetectable ctDNA at C2D1 (P<0.0001). Correlation analysis between circulating proteins and ctDNA fraction at BL from matched samples revealed significant enrichment for SOMAmers positively (n=858) and negatively (n=316) correlating with ctDNA fraction. The top enriched SOMAmers for positive correlation with ctDNA fraction exhibited significant predictive value for poor PFS (P<0.0001), while the top enriched SOMAmers for negative correlation showed significant predictive value for favorable PFS (P<0.0001); both with comparable predictive value to ctDNA fraction. The combination of ctDNA fraction and protein signal at BL provided better prediction of PFS compared to either measurement independently. Identified SOMAmers also show evidence of further discrimination of PFS response in pts with ctDNA fraction=0. Conclusions: ctDNA fraction reduction at early timepoints demonstrates rapid and early response of ALP treatment in PIK3CA-mutated HR+/HER2– ABC. With the exception of Pi3K/AKT pathway-related genes, we observed consistency in alterations, especially in genes implicated in CDK4/6i resistance across timepoints suggesting that ALP does not alter overall tumor biology. Notably, an increase in alterations within Pi3K/AKT pathway genes at EOT may indicate a potential resistance mechanism and provide guidance on next lines of therapy The SomaScan analysis highlights independent value of circulating protein markers and their complementarity with ctDNA fraction, suggesting their prognostic value.

P3-05-19: High Prevalence of HER2-Low and Increased TIL Levels in ILC Patients with Residual Disease Following Neoadjuvant Therapy Provides a Rational for use of HER2-Antibody-Drug Conjugates or Immunotherapy Approaches

Melinda Sanders, Guadalupe Garcia, Paula I. Gonzalez-Ericsson, Laura Kennedy, Tina Chai

Background: Invasive lobular carcinoma (ILC) accounts for 5-15% of breast cancer (BC). Most ILCs are hormone receptor positive (HR+), HER-negative (HER2-) and lack associated tumor infiltrating lymphocytes (TILs), a state referred to as “immune desert”. The benefit of chemotherapy in reducing ILC recurrence is unclear, while endocrine therapy (ET) plays a

key role for patients with ILC. For patients with locally advanced disease, neoadjuvant ET or chemotherapy may be warranted to downstage ILC and permit breast conservation. However, pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) or neoadjuvant endocrine therapy (NET) in patients with ILC is significantly lower than that observed in no special type (ductal) carcinomas. Although tumor-infiltrating lymphocytes (TILs) predict response to neoadjuvant therapies in HER2-positive BC, their role in HER2-Low ILC is unknown. However, TIL counts > 5% in ILC have been previously correlated with decreased survival. HER2-Low BC are defined by an immunohistochemical score of 1+ or 2+ with a negative HER2 FISH test. The prevalence and implications of HER2-Low in ILC are currently poorly characterized.
Methods: Patients with ILC treated from 2018 to 2022 at our institution were identified from a breast cancer database. HER2-Low status was correlated with clinicopathologic features including TILs and treatment response among patients with HR+/HER2- ILC treated with neoadjuvant therapy (NAT) at our institution. Stromal TILs were quantified as < 5%, 5 to 10%, >10%.
Results: Between 2018 and 2022, a total of 196 women with ILC were treated at our institution. Median age at diagnosis was 63 years old (range 25–90 years). The majority of ILC tumors were HR positive (98%), intermediate to low grade combined histologic grade (92%) and 52% were HER-low. Of these, 37 women with ILC received NAT. Thirteen received neoadjuvant chemotherapy (NAC) and 24 received neoadjuvant endocrine therapy (NET). Median age at diagnosis was 59 years (25–82). Twenty-three (62%) were HER2-Low. None were ER-low positive. The majority (81%) were intermediate or high combined histologic grade and 7 (19%) had an intermediate or high proliferative rate. Collectively, most were cT2/cT3 (84%), multifocal (76%) and lymph node positive (76%). 54% had > 5% stromal TILs, 35% had associated tertiary lymphoid structures (TLS), and 24 (65%) had both. HER2-Low tumors were associated with higher TILs and presence of tertiary lymphoid structures (TLS) (65% vs 35% showed > 5% stromal TILs; 78% vs 24% had > 5% TILs and/or TLS, Fisher's test p= 0.0395). No other differences among clinicopathological characteristics were observed between HER2-Low and HER2-0 tumors. No patients achieved pCR and 95% were RCB II/III. Among patients with RCB II/III, 60% of those who received NAT and 77% of those who received NET were HER2-Low.
Conclusion: HER2-low status is common in ILC patients who receive NAT and is associated with higher TIL levels than HER2-0 tumors. The high prevalence of residual disease after NAT in HER2-low ILC suggests treatment resistance, and alternative treatment approaches are warranted. These could include antibody drug conjugates (ADCs) and/or immunotherapy-based approaches.

P3-05-20: Novel liquid biopsy technology reveals cancer-specific isoforms for breast cancer diagnosis and therapy

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Background: Developing new technologies for breast cancer diagnosis and therapy is crucial to improving patient outcomes and survival rates. Advanced molecular profiling enables earlier and more accurate detection of breast cancer, and can identify new targets for targeted therapies and personalized medicine. The most promising new technologies include detecting circulating tumor DNA (ctDNA) and RNA (ctRNA). Although ctDNA methods have shown promising diagnostic potential, in breast cancer ctDNA concentrations are extremely low. Current RNA approaches have focused on shorter RNAs, but these lack the resolution and complexity to provide more clinically relevant information for cancer detection and treatment.

Methods: Wobble Genomics have developed a novel liquid biopsy assay that utilizes full-length RNA sequencing from whole blood to create comprehensive transcriptome profiles. Combining Level-Up, our biochemical cDNA normalization technology, and TAMA, our full-length RNA annotation software, our platform maximizes efficiency of detection for ctRNA and enables accurate characterization of cancer-specific isoforms.

Patients: 200 patients with early breast cancer and matching controls with benign disease or no cancer on imaging have had 7.5 mL of blood collected. 2.5 mL of the blood is analyzed for transcriptome profiling. Sequential samples pre and post surgery are also being collected.

Results: From the first 130 cancers and controls analyzed, we have built an extensive breast cancer RNA database comprising 569K breast cancer specific transcripts with an average length of 1376 ± 793 bp. Of these, 11K, 146K, and 15K are unique to DCIS, stage 1, and stage 2 breast cancer, respectively, providing biomarker candidates with the potential of high diagnostic precision and higher informative value. While 36K of these cancer-specific RNA signals originate from protein-coding genes, the majority are characterized as novel long non-coding RNAs, which are not detectable by other platforms. RNA splicing patterns unique to cancer patients have been identified, uncovering previously overlooked identifiers for breast cancer.

Furthermore, based on the cancer-specific RNA discovered, we have constructed a therapeutically relevant protein-coding transcript database consisting of 2,184 isoforms that contain putative protein regions found only in cancer patients. Among 1,292 genes encoding these isoforms, 132 are established cancer genes, 30 of which are known oncogenes and 77 are tumor suppressor genes. These predicted cancer-associated protein sequences are primarily resulted from novel RNA splicing sites or new combinations of known splicing sites, and therefore constitute more pronounced amino acid changes from their normal protein counterparts that differ from single point mutations. This database provides a source of potential new targets for developing new cancer treatments, including mRNA vaccines and antibody-drug conjugates.

Conclusions: This completely novel platform can detect and characterize full-length breast

cancer specific RNA transcripts. This could allow earlier cancer detection and provide new insights into cancer behavior, biomarker discovery, and identification of new targets for drug development.

P3-05-21:Multicenter retrospective cohort study of the sequential use of antibody drug conjugates trastuzumab deruxtecan & sacituzumab govitecan in patients with HER2 low metastatic breast cancer: a subgroup analysis of next generation sequencing results.

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Background: Trastuzumab deruxtecan (T-DXd) and sacituzumab govitecan (SG) are both approved antibody-drug conjugates (ADCs) for patients (pts) with HER2 low metastatic breast cancer (MBC). Biomarkers that predict response and/or resistance to sequential use of these therapies are needed.
Methods: In this cohort of patients (pts) with HER2 low MBC treated with both ADCs (in either sequence), we previously reported real-world efficacy and subgroup analyses by age, sites of disease, and use of intervening therapies. Here, we provide a descriptive analysis of next generation sequencing (NGS) results in a subset of pts (N=66) who underwent liquid and/or tissue NGS testing at any of the following time points: prior to ADC1, prior to ADC2, or after receipt of both ADCs. Genomic information captured included specific genomic mutations, microsatellite instability status (MSI), and tumor mutation burden (TMB). Commercial testing per routine clinical practice was performed by Guardant Health, Foundation Medicine, Tempus, Caris, and/or UCSF CLIA-approved testing. We performed Cox proportional hazard analysis to evaluate the relationship between patient and treatment characteristics and genomic expression with real-world overall survival (rwOS) from the start of ADC1.
Results: The study cohort included 74 pts from 4 sites, with 66/74 (89%) with available NGS data from at least one-time point [47 (71%) HR+/HER2 low and 19 (29%) HR-/HER2 low]. The median number of tissue NGS was 1 for both the HR+ (range: 0-4) and HR - (range: 0-3) cohorts. The median number of liquid NGS was 1 (range 0-3) and 0 (range 0-4) for the HR+ and HR- cohorts, respectively. The median total number of NGS (any combination of liquid/tissue) was 2 (range 1-5) and 1 (range 1-5) for the HR+ and HR- cohorts, respectively. Types of NGS testing and timepoints were as follows: Prior to ADC1 liquid 9, tissue 31, both 10, no NGS16; Prior to ADC2 liquid 9, tissue 13, both 1, no NGS 43; after both ADCs liquid 16, tissue 4, both 1, no NGS 45.
In the entire cohort, 39 pts (59.1%) had a TP53 mutation; 29 pts (43.9%) had a PIK3CA mutation, and 13 pts (19.7%) had an ESR1 mutation. In the HR+ cohort, the most frequently detected mutations were PIK3CA in 26 (55.3%), TP53 in 24 (51.1%), and ESR1 in 13 (27.7%). In the HR- cohort, the most frequently detected mutations were TP53 in 15

(78.9%), PIK3CA and NF1 in 3 (15.8%). MSI testing demonstrated microsatellite stability (MSS) in all 66 patients. Median TMB was 4.70 (range: 1-14) for HR+ pts and 3.85 (range: 0.78-17.37) in HR- pts.
In multivariate analysis evaluating the impact of HR status, age, time since MBC diagnosis, ADC order, de novo MBC, visceral disease, CNS disease, PIK3CA status, and TP53 status on rwOS from the start of ADC1, shorter time since MBC diagnosis [HR 0.96 (0.96-1.0, p=0.010] was the only factor associated with longer rwOS in a Cox proportional hazard analysis. Of note, the presence of TP53 mutations or PIK3CA mutations did not impact rwOS [HR 0.70 (0.36-1.36), p=0.293 and HR 1.74 (0.81-3.74), p=0.156, respectively].
Conclusion: In this cohort of pts with HER2low MBC who received sequential ADCs, most pts underwent NGS testing, and the genomic landscape of mutations was consistent with incidence in the reported literature. The presence of TP53 or PIK3CA mutations did not impact rwOS from the start of ADC1. Given the heterogeneity in the type of NGS testing and the timing related to ADC administration, correlations with outcomes are limited. This study highlights the need for prospective evaluation of NGS information to clarify mechanisms of response and resistance to ADCs.

P3-05-22: Prognostic Significance of Tumor-informed Personalised Circulating Tumor DNA Monitoring in Breast Cancer Patients Undergoing Neoadjuvant Therapy

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Background: Minimal residual disease (MRD) in the peripheral blood of breast cancer (BC) patients undergoing neoadjuvant therapy (NAT) and surgery is associated with an increased risk of recurrence. Circulating tumour DNA (ctDNA) and its kinetics are often used to assess the presence of MRD in operable BC. There is currently no gold standard for evaluating MRD in BC patients treated with NAT. Therefore, we applied a sensitive tumour-informed personalised assay called OriMIRACLE™S-MRD explore associations between detectable ctDNA and prognostic value.
Methods: Clonal mutations in tumour tissue were identified by whole-exome sequencing. Then a personalised panel was designed to monitor ctDNA changes in patients' plasma at baseline of NAT, during NAT, after NAT and after surgery. CtDNA assay was performed using OriMIRACLE™S-MRD by Origimed (Shanghai, China).
Results: A total of 28 female patients with stage II-III breast cancer were enrolled, including 7 with triple-negative breast cancer and 21 with HER2-positive disease. The median age of the patients was 52 years (range: 28-64). Whole-exome sequencing analysis identified 93% (9096/9747) variants of uncertain significance (VUS), and approximately 63% of variants were copy number variants and fusions/arrangements, which are challenging to detect via liquid biopsy. Among all the genes involved in single nucleotide variants, 91% of the genes were detected in a single sample only. A personalised panel was successfully designed for each patient. Twenty-six individuals underwent single-sample testing at baseline. Twenty-

seven individuals collected 1-7 samples during NAT. Twenty-three individuals collected 1-2 samples after NAT, and 9 individuals collected 10 post-operative samples. In cases where more than one sample was collected during a given phase, the ctDNA status of that phase was defined as positive if any one of the samples tested positive. A given stage is defined as negative for ctDNA only if all samples within that stage are negative. The positivity rate of ctDNA at baseline was 92% (24/26). CtDNA concentration at baseline was found to be proportional to the postoperative grey and white matter hard zone volume ($R^2 = 0.64$). The ctDNA positivity rate decreased to 22% (6/27), 17% (4/23) and 11% (1/9) during NAT, after NAT and after surgery, respectively. None of the patients with positive ctDNA after NAT achieved pathological complete response (chi-square test, $p = 0.01$). A HER2+ patient with baseline ctDNA positivity who had multiple interruptions of NAT due to intolerance of the initial treatment regimen maintained a positive ctDNA at cycle 5 of NAT. After modification of the treatment regimen, the ctDNA became negative at cycle 7 of NAT and remained ctDNA negative after NAT with a pathological complete response. This patient has been followed up for 629 days with no signs of recurrence or metastasis. In one triple-negative BC patient, ctDNA was negative during NAT, but became positive on day 6 after NAT. Imaging progression and another ctDNA positivity were observed simultaneously on day 23 after NAT. The patient underwent radical resection three days later. Despite negative ctDNA at 3 months postoperatively, the patient experienced metastasis at 349 days postoperatively.
Conclusion: Stage II-III breast cancer suffers from a high proportion (93%) of VUS and a high degree of genetic heterogeneity (91% of unique single nucleotide variant-associated genes). These factors predict a significant advantage of tumor-informed personalised panels for dynamic monitoring of MRD in BC patients undergoing NAT. Positive ctDNA after NAT was significantly associated with efficacy of non-pathological complete response. Individual case analyses suggest that personalised MRD testing can predict treatment efficacy and recurrence in advance, offering valuable insights for clinical decision-making.

P3-05-23: Single nucleus chromatin accessibility and transcriptome analyses reveal aberrant inter-cellular communication in inflammatory breast cancer

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International efforts focused on discovery of biological targets in inflammatory breast cancer (IBC) have suggested that aberrant inter-cellular relationship instead of genomic aberrations is the key driver of IBC. To identify such IBC-specific aberrations, we generated single nucleus chromatin accessibility and transcriptome atlas of IBCs (9,628 nuclei from three donors) and compared IBC atlas with a similar atlas of the healthy breast (81,735 nuclei from 92 donors). We recently reported single nucleus atlas of breast tissues of healthy women of diverse genetic ancestry (Nature Medicine in press). In that report, we described markers that identify three major epithelial cell subtypes [Basal-myoepithelial (BM), luminal adaptive secretory precursor (LASP), luminal hormone sensing (LHS)], two

endothelial cell subtypes, two adipocyte subtypes, fibroblasts, macrophages, and T cells of the healthy breast. We also showed that LHS cells are the likely cells-of-origin of Luminal A, Luminal B and HER2+ breast cancers. Unlike these breast cancer subtypes, IBC does not appear to have a cell-of-origin, as epithelial cells in IBCs shared gene expression pattern across BM, LASP and LHS cells. Similar results were obtained when comparison was restricted to ancestry-specific healthy breast atlas. However, individual gene level expression differences affecting specific signaling pathways were observed in epithelial cells of IBCs. LASP, and LHS cells of IBCs overexpressed GPR137C, a positive regulator of mTORC1 signaling, and HS6ST3, an enzyme required for heparan sulfate synthesis. LASP and LHS cells of IBCs displayed activation of mitotic and matrix metalloproteinase signaling, respectively. Endothelial cells of IBCs, which showed significant gene expression differences compared to endothelial cells of the healthy breast, displayed enhanced integrin cell surface interaction but loss of cell junction organization. Most critically, BM, LASP, LHS, and endothelial cells of IBCs compared to their counterparts in the healthy breast showed downregulation of TEX14, an inter-cellular bridge forming factor in germ cells and a regulator of mitosis. Downregulation of TEX14 expression in these cells was accompanied with changes in the chromatin accessibility patterns of this gene. These results reinforce the notion that defective inter-cellular communication is a hallmark of IBC and TEX14 expression levels may serve as a biomarker of this defect. We suggest that greater attention to vascular biology and vascular-epithelial cell communication has to be given for better understanding of IBC biology and therapeutic targeting.

P3-05-24: Mapping the Genomic Landscape: Comprehensive Profiling of Diverse Histological Types of Breast Cancer

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Introduction: Breast Cancer (BC) is a highly heterogeneous tumor consisting of various histological types. Data on genomic alterations (GAs) in special types of breast cancer, which account for about 10% of all breast cancers, remain limited. We aimed to clarify genomic profiles of BC, especially focusing on breast cancer of special type.
Methods: We retrospectively reviewed data on female patients with unserectable / metastatic BC who underwent comprehensive genomic profiling (CGP) tests and were registered for the Center for Cancer Genomics and Advanced Therapeutics in Japan from June 2019 to June 2024. CGP tests were conducted by NCC Oncopanel System, FoundationOne CDx (F1CDx), or GenMineTOP. We examined somatic GAs, microsatellite instability (MSI) status, and tumor mutation burden (TMB) of BC. TMB-high was defined as ≥10 mutations/Mb. Actionable GAs were counted if they were classified as "pathogenic/oncogenic" or "likely pathogenic/likely oncogenic variants". Druggable GAs were defined as actionable GAs that are the targets of drugs approved for BC or other types of cancer, or that have been shown to be effective in clinical trials for BC or other types of

cancer.
Results: 3763 patients were identified, of which cases in which detailed histology could not be determined or non-epithelioid tumors were excluded, and finally 2915 patients were included in the analysis. The most used CGP test showed F1CDx (89.7%). Histological types included invasive ductal carcinoma (IDC), invasive breast cancer of no special type (IBC-NST), invasive lobular carcinoma (ILC), metaplastic carcinoma (MpBC), mucinous carcinoma (MuBC), solid papillary carcinoma, adenoid cystic carcinoma (ACC), inflammatory breast cancer (IBC), secretory carcinoma (SBC). The number of each histological subtypes were 2212, 410, 169, 81, 22, 7, 6, 6 and 2, respectively. There were statistically significant differences regarding the PI3K/AKT/mTOR pathway (IDC vs ILC: 57.8% vs 72.8%; p = 0.0045, IBC-NST vs ILC: 55.4% vs 72.8%; p = 0.0031) and BRCA1/2 alterations (IDC vs ILC: 8.7% vs 4.1%; p = 0.039). The frequency of ESR1 alterations was the highest in mucinous carcinoma (36.4%), and the lowest in ACC, MpBC, IBC, and SBC (all of them: 0%), but did not show statistically significant differences by histological type. The proportions of MSI-high and TMB-high were the highest in ILC (1.2% and 18.3%, respectively), but the differences were not statistically significant by histological type. The frequency of druggable GAs was the highest in SBC (100% for NTRK fusion), and the lowest in ACC (33.3%), but did not show statistically significant differences.
Conclusions: Despite the limited number of cases in some histological types, this study revealed genomic characterizations among many histological types of BC. This information will provide an important basis for considering genome-based precision medicine in the future.

P3-05-25: Dynamic increases in T cell repertoire diversity determine palbociclib efficacy and improved antitumor immune response

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Background: Inhibitors of cyclin dependent kinase 4 and 6 (CDK4/6i) are the current treatment of choice in hormone-receptor positive breast cancer patients. In addition to their well-described role in cell-cycle regulation, it is now clear that they also impact T-cell immunity. Single cell transcriptomic profiling of circulating immune cells enables high sensitivity detection of rare cells and may identify T cell antigen receptors (TCR) involved in the anti-tumor response, potentially leading to a non-invasive mean of monitoring treatment-induced immune activity. In our study, we perform TCR single-cell sequencing in advanced breast cancer patients undergoing palbociclib treatment to explore changes in T-cell repertoire and monitor antitumor immune responses.
Methods: Longitudinal blood samples of 9 advanced breast cancer (ABC) patients treated with palbociclib were collected and peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque density gradient centrifugation. To characterize T cell

response, profiling of peripheral CD3+ T cells was performed using 10x Genomics VDJ single-cell sequencing. The study cohort consisted of 9 patients diagnosed with HR+HER2- advanced breast cancer, with metastatic lesions present only in bone and treated with palbociclib in combination with hormonal therapy as first-line treatment. Blood samples,were collected at three different time-points, pre-treatment initiation, after 3-6 months while on response (On-treatment) and at progression or last follow-up (LFUP), after 24 months of sustained clinical response. Patients were clinically stratified in good (n=4) or bad (n=5) efficacy based on our own algorithm, that takes into account clinical and survival data according to data from pivotal studies.
Results: Eleven T cell subpopulations, including naïve and memory CD4+ and CD8+ T cells, activated CD4+ and CD8+ T effector cells, activated CD4+ T regulatory cells, were characterized in all samples from the nine enrolled patients based on their transcriptomic profiles, at baseline and longitudinal timepoints. T cell subpopulation distribution was similar in pre-, post- treatment and progression/last-follow up samples in all nine patients. Similarly, no significant differences in TCR diversity and clonality were observed in basal samples from good and bad efficacy patients, albeit TCR repertoire evolved during palbociclib. Indeed, there was a significant increase in the diversity of TCR repertoire in post-treatment samples of patients from the good efficacy group (32% mean increase), compared to pre-treatment samples. On the contrary, TCR repertoire diversity decreased in samples from the bad efficacy group (25% mean decrease) when compared to baseline. At progression/LFUP, TCR diversity decreased in all cases, although it was less pronounced in the good efficacy group, who presented higher TCR diversity index in all time-points studied. Importantly, tracking over time the expanded T cell clonotypes reveals growth of pre-existing effector populations in all patients, but the emergence of novel expanded clones was more frequent in the good efficacy patients, suggesting that Palbociclib treatment increases the antitumor T cell memory pool, resulting in a more effective response and prolonged progression free survival.
Conclusions: Overall, our results indicate that efficacy of Palbociclib treatment is associated with increased TCR diversity and enhanced T cell clonal expansion, both characteristic of improved antitumor immune response. These data are consistent with the model that T cells play a key role in palbociclib-mediated tumor control, and warrant further investigation in a larger sample population.

P3-05-26: Efficacy of first-line CDK4/6 inhibitors stratified by ER, PR, HER2 expression levels and Ki-67 in hormone receptor-positive, HER2 negative metastatic breast cancer: Real-world outcomes

Maya Gogtay, Olivia L. Makos, Lina Elsayed, Robin R. High, Meghana Kesireddy

Introduction: Cyclin-Dependent kinase 4 and 6 (CDK4/6) inhibitors (palbociclib/ abemaciclib/ ribociclib) combined with endocrine therapy (ET) as first-line treatment has significantly improved progression-free survival (PFS) in hormone receptor-positive (HR+), HER2 negative (HER2-) metastatic breast cancer, with ribociclib also showing statistically

significant improvement in overall survival (OS) in phase III studies. However, these phase III studies have not assessed efficacy differences based on estrogen receptor (ER), progesterone receptor (PR), HER2 receptor, or Ki-67 levels. Our study aims to evaluate the impact of ER, PR, HER-2, and Ki-67 on PFS and OS in metastatic HR+, HER2- breast cancer treated with CDK4/6 inhibitor and ET in their first line.

Methods: We conducted a retrospective study of 146 patients with metastatic HR+ HER2- breast cancer treated with first-line CDK4/6 inhibitor and ET at our institution. We have recorded ER, PR, HER2, and Ki-67 expression levels from their first metastatic site biopsy, along with age, gender, ethnicity, menopausal status, location of metastatic sites, date of initiation of CDK 4/6 inhibitor, and date of progression or death. The primary endpoints were PFS and OS stratified by ER (≤50% vs >50%), PR (≤50% vs>50%), HER2 (0 or 1+ vs 2+ on IHC) and Ki67 (≤20% vs >20%) levels. Survival distributions were estimated using the Kaplan-Meier method, and independent group t-tests were used to compare the difference in median between two groups. We also calculated OS and PFS using restricted mean survival time (RMST) which is the area under the survival curve upto a specific time. Results: The ≤ 50% ER group had a median time for PFS of 15.5 months, RMST for PFS of 29 months, median OS of 47 months, RMST for OS of 48.3 months; whereas the > 50% group had significantly longer median PFS of 49 months, RMST for PFS of 47.3 months, median OS of 80.3 months, RMST for OS of 64 months (p=0.002, 0.016, 0.006, 0.05 respectively).

The ≤ 50% PR group had a median PFS of 27.3 months, RMST for PFS of 60.5 months, median OS of 63.7 months, RMST for OS of 93.2 months; while the >50% PR group had significantly longer median PFS of 77.3 months, RMST for PFS of 99.4 months, median OS of 116.7 months, RMST for OS of 133.9 months (p= 0.013, 0.024, 0.014, 0.039 respectively).

The 0 or 1+ HER2 IHC group had a median PFS of 45.4 months, RMST for PFS of 53.9 months, median OS of 71 months, RMST for OS of 69.9 months; while the 2+ HER2 IHC group had a median PFS of 49 months, RMST for PFS of 48.4 months, median OS of 77 months, RMST for OS of 71.4 months (p=0.25, 0.50, 0.74, 0.86 respectively).

The ≤ 20% Ki-67 group had a median PFS of 75.9 months, RMST for PFS of 96.3 months, median OS of 225.3 months, RMST for OS of 140.9 months, while the >20% Ki-67 group had a significantly shorter median PFS of 37.3 months, RMST for PFS of 56.4 months, median OS of 68.8 months, RMST for OS of 82.6 months (p=0.005, 0.006, 0.006, <0.001 respectively).

Conclusion: Higher ER and PR levels were linked to significantly longer PFS and OS, while higher Ki-67 levels were associated with significantly shorter PFS and OS. HER2 IHC levels had no significant impact on PFS and OS. Our study highlights ER, PR and Ki-67 levels as predictive factors for HR+ HER2- metastatic breast cancer patients on CDK4/6 inhibitors. Larger studies are warranted to confirm the utility of these markers as predictive factors for CDK4/6 inhibitor therapy across different treatment lines.

P3-05-27: Real-world evidence on the use of the 21-gene assay in prognostically intermediate luminal breast cancer (BC): experience from a French multicenter cohort (N=1,126)

Natacha Joyon, Caroline Charles, Nadine Dohollou, Loic De Poncheville, Barbara Pistilli, Magali Lacroix-Triki, Elie El-Rassy, Joana Ribeiro Mourato, Chayma Bousrih, Alessandro Viansone, Bruno Cutuli

Background: Real-world data on the use of Oncotype DX® testing (RS) and its impact on treatment choices in early-stage ER+/HER2– breast cancer (BC) pN0/1 patients are limited in settings where broad access for all is not available.
Methods: We conducted a comprehensive retrospective observational study of the use and impact of RS in 4 French public/private practice comprehensive cancer care centers where RS was proposed according to National guidelines.
Results: 1,126 pts underwent RS testing, including 577 across 3 private clinics (Apr 2014 – June 2024) and 549 at Gustave Roussy Cancer Campus (Nov 2020 – June 2024).
Pt characteristics were as follows: age distribution at diagnosis, ≤50y 32.7%, 51–70y 59.4%, >70y, 7.9%; menopausal status, 64.0% postmenopausal, 35.2% premenopausal; male 0.8% (n=9).
Pts received breast-conserving surgery or underwent a mastectomy in 79.8% and 20.2% of cases, respectively. Sentinel lymph node biopsy and axillary dissection were performed in 84.0% and 19.3% of cases.
Clinicopathological features were as follows: main histological subtypes, invasive ductal carcinoma (IDC) 76.6%, invasive lobular carcinoma (ILC) 13.5%, mixed IDC/ILC 5.9%, other 4.0%; pathological tumor size, pT1a 0.4%, pT1b, 7.8%, pT1c 56.9%, pT2 33.4%, pT3 1.5%; tumor grade, grade 1 9.9%, grade 2 73.6%, grade 3 16.4%; mitotic count: 1 46.8%, 2 42.2%, 3 11.0%; multifocality, unifocal 78.0%, bifocal 11.1%, multifocal (≥3 lesions) 10.9%; Ki67 <20% 37.8%, 20–30 47.4%, >30% 14.8%; node status, pN0 63.7%, pN1mi 11.3%, pN1 25.0%.
Retrospective analysis showed that the primary reason for testing was decision uncertainty due to discordant/intermediate histoprognostic factors (69.1%). Other reasons included the need to confirm CET or endocrine therapy (ET) only indication (20.9% and 8.3%, respectively).
RS testing led to major changes in therapeutic choices, with a 68.9% reduction in the indications of chemotherapy (CT) in our cohort. In pN0, pN1mi and pN1 pts, CT indication was reduced by 62.6%, 67.9% and 83.9%, respectively.
In specific patient subgroups (all with a p<0.001), RS impact (% decrease in CT indication) was as follows (CET de-escalation):
- Age groups: ≤ 50y / 51–70y / > 70y, 55.4%/76.5%/64.3%
- Menopausal status: Premenopausal/Postmenopausal/Men, 56.3%/75.1%/88.9%
- Hormone receptors: ER≥90 PR≥10 / ER<90 PR≥10 / ER≥90 PR<10 / ER<90 PR<10, 75.1%/54.6%/51.3%/36.0%
- Histological subtype: IDC/ILC/Mixed/Other, 66.4%/81.6%/64.7%/79.1%
- Grade: 1/2/3, 84.9%/72.0%/49.2%

- Ki-67: <20%/20–29%/>30%, 84.8%/62.3%/56.0%

Excluding men (small sample size), the highest de-escalation rates were seen in:

- Grade 1 tumors in postmenopausal pts: 96.1%
- Grade 1 pN1 tumors: 96.1%
- pN1 tumors with Ki-67 <20%: 95.2%
- Ki-67 <20% tumors in postmenopausal pts: 93.3%
- pN1 ILCs: 92.3%

RS result (continuous variable) was strongly correlated with histological grade (Kruskal-Wallis and ANOVA, $p < 0.001$). RS (continuous variable) was also correlated with Ki-67, with a linear distribution across Ki-67 index ranges (Pearson correlation, $r=0.375$; $p<0.001$). Similar trends were observed for ER and PR (≥90 vs <90 and ≥10 vs <10, respectively), with RS being inversely correlated with staining (% tumor cells) ($p=0.005$ and $p<0.001$, respectively [t-test]).

Conclusions: RS In this real-life, purpose-selected population, RS decreased the indications of CT by a mean of 68.9%. These results confirm the test's utility in refining management strategies and avoiding overtreatment in our daily practice according to ongoing guidelines of use.

The distribution of RS and its impact on adjuvant CT decisions will be detailed during the presentation, which will showcase this updated data on over 2,000 cases from 8 centers and present a multiparametric model.

P3-05-28: A clinical risk prediction model for subsequent invasive breast cancer after ductal carcinoma in situ

Charlotta Mulder, Will Harley, Petra Kristel, Lennart Mulder, Sten Cornelissen, Renee Menezes, Michael Schaapveld, Marjanka K. Schmidt, Jelle Wesseling, Esther H. Lips

Upon the inception of population-based screening programs, the incidence of ductal carcinoma in situ (DCIS) increased 6-fold. DCIS is a non-obligate precursor lesion of invasive breast cancer (IBC), of which the majority does not progress. This implies that women with non-progressive DCIS are overtreated. Within the Cancer Grand Challenge PRECISION project, we identified prognostic markers, including immunohistochemical, morphological markers, and an RNA-seq classifier holding promise in distinguishing progressive from non-progressive DCIS. We now aim to validate these externally and build a clinical prediction model.

We conducted a case-cohort study nested in a Dutch population-based cohort of 8987 patients with DCIS treated with breast-conserving surgery (BCS) between 2005 and 2015. Women who subsequently developed ipsilateral IBC were considered cases and controls were those who did not.

Our study population consisted of a random sample of 10.7% of the full cohort as our subcohort, and all other additional cases, totaling 1237 women (cases n=308; controls n=929). Tissue blocks were requested for all women, of which 940 were received and eligible for immunohistochemical analysis of ER, HER2, COX-2, Ki67 and P16 1. A new H&E

slide was cut for the measurement of adipocyte size 2, level of periductal fibrosis 3 and a ductal morphometric analysis 4. RNA-sequencing was carried out on a selection of BCS-only patients (cases n = 100; controls n = 100). To assess the association of each marker and iIBC risk, prentice-weighted Cox proportional hazards models, with age as the underlying time variable, were implemented. Multivariable models were also implemented and adjusted for treatment, margins status, DCIS grade and size. To account for multiple comparisons, a false discovery rate (FDR) < 0.05 was used to define statistical significance.
The median follow-up time for the case-cohort was 7.2 years (interquartile range (IQR): 5.2-10.1). The age at primary DCIS diagnosis was similar for breast cancer cases and controls (58.0, IQR: 51.0-64.0 vs 58.0, IQR: 51.0-66.0), as was the size of the tumor (p = 0.06). After final surgery, 10.4% of cases and 6.7% of controls had involved margins of <2mm (p = 0.06). Cases were treated more frequently with radiotherapy than controls (76.0% vs 68.4%, p = 0.01) and were more often high grade DCIS (46.4% vs 38.0%, p = 0.04).
In December, we will present the validation of immunohistochemical, morphological markers, and an RNA-seq classifier for predicting subsequent ipsilateral IBC after DCIS and the benefit of using these markers in a clinical prediction model. Ultimately, this will aid individual risk stratification of women with primary DCIS, and can be used to diminish the current overtreatment of harmless, low-risk DCIS.

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P3-05-29: Seven in Absentia Homolog (SIAH) expression and clinicopathological correlates in early-stage triple-negative breast cancer (TNBC)

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Background: TNBC is an aggressive breast cancer subtype with higher rates of disease recurrence and mortality. Clinically and biologically distinct TNBC subtypes have been described, offering the potential of personalized, targeted therapy in subsets expressing

targetable biomarkers. SIAH is an E3 ligase involved in ubiquitination and proteasome-mediated degradation of specific proteins. Elevated SIAH expression has been associated with poor prognosis in early-stage TNBC and is indicative of persistent EGFR-K-RAS-SIAH pathway activation. We evaluated the association of SIAH expression with TNBC clinicopathologic characteristics in early-stage TNBC.
Methods: We measured SIAH protein expression using immunohistochemistry (IHC) in tumors from 280 patients who underwent upfront surgery for early-stage TNBC. SIAH expression was quantified according to staining intensity (none, weak, moderate, or strong) and to the percent of nuclear staining (0-100%). SIAH expression was dichotomized as high (moderate to strong staining in >25% of nuclei) or low/negative (low: weak staining in any % nuclei or moderate/strong staining in ≤25% of nuclei, negative: no staining in any nuclei). We evaluated the association of SIAH expression with clinicopathologic characteristics of interest.
Results: Among 280 patients with upfront resected TNBC, 212 (76%) tumors exhibited moderate to strong SIAH staining in >25% of nuclei (SIAH-high). Most patients (76%) were younger than 65 years old and 57% were postmenopausal. On pathologic staging, most tumors were > 2 cm (54%) and N0 (60%). Compared to SIAH low /negative, SIAH-high TNBC tumors occurred more often in women < 65 years old (80% vs 63%, p=0.006) and were more often > 2 cm (58% vs 40%, p=0.008), node-positive (43% vs 29%, p=0.044), grade 3 (97% vs 85%, p<0.001), with high (>15%) Ki-67 proliferative index (89% vs 55%, p <0.001), and PD-L1 positive (54% vs 36%, p=0.022). Co-expression of high levels of the androgen receptor (AR) was less common in SIAH-high TNBC (IHC <10% in 76% vs 63%, p=0.053). Consistently, molecular subtyping revealed that SIAH-high TNBC was more commonly of a non-luminal androgen receptor (LAR) phenotype (89% vs 73%, p=0.063, assessed via RNA-seq). We observed no differences in menopausal status or stromal tumor-infiltrating lymphocytes (sTILs) between SIAH high vs. low/negative tumors. Associations of SIAH expression with survival outcomes will be presented at the meeting.
Conclusions: High levels of SIAH expression in early-stage TNBC were associated with younger age, larger tumors, higher frequency of nodal involvement, and more aggressive pathologic features (e.g. higher grade and proliferative index). Furthermore, SIAH expression was inversely associated with AR expression and correlated with a non-LAR molecular subtype. Further evaluation of this biomarker and its potential interaction with AR signaling in larger cohorts of TNBC are warranted.

P3-05-30: Clinically Advanced Neuroendocrine Carcinoma of the Breast (CANEBC): A Genomic Landscape Study

Melissa Taylor, Dean Pavlick, Ethan S. Sokol, Julia C. F. Quintanilla, Mia A Levy, Jeffrey S. Ross, Neal A Fischbach, Lajos Pusztai, Maryam Lustberg

Background: CANEBC is a rare form of breast cancer that exhibits neuroendocrine features on histology. The genomic features of CANEBC are currently not well described.
Methods: A series of 86 predominantly Stage IV CANEBC and 1138 CA non-neuroendocrine

ductal breast carcinomas (CAIDC) underwent comprehensive genomic profiling (CGP) to assess all classes of genomic alterations (GA). Microsatellite (MSI)-high status, tumor mutation burden (TMB), homologous recombination deficiency signature (HRDsig), genomic ancestry, and single base substitution genomic signatures were all determined by hybrid capture-based sequencing of extracted DNA from FFPE tissues. ER, PR and HER2 expression were determined by immunohistochemistry (IHC) with HER2 Low defined as either 1+ or 2+/FISH- staining. Results were compared using the Fisher exact method with the false discovery rate (FDR) corrected using Benjamini/Hochberg adjustment.
Results: By design, synaptophysin IHC expression was positive in all (100%) CANEBC. Comparing CANEBC vs CAIDC, the median age (57.5 v 57.0 yrs) and frequencies of EUR ancestry (77.9% vs 68.1%) and APOBEC signature (3.5% vs 4.9%) were similar. The frequencies of ER+ status (60.2% vs 63.3%) and PR+ status (39.8% vs 45.5%) were similar. On IHC, CANEBC were more frequently HER2 negative (0+) (65.0% vs 34.1%; p<.0001), less frequently HER2 Low (1+) (20.0% vs 32.6%; p<.0001) and less frequently HER2 positive 3+ IHC and gene amplified (0% vs 12.9%; p<.0001). Using the CGP data, ERBB2 was less frequently amplified in the CANEBC group (0% vs 14.1%; p=.005). The CCND1/FG3,4,19 amplicon was more frequently amplified in the CANEBC group (25.6% vs 14.6%; p=.037) as were GA in FGFR1 (29.1% vs 13.8%; p=.005) and RB1 (31.4% vs 8.7%; p<.0001). GA in PIK3CA were less frequent in the CANEBC (19.8% vs 34.8%; p=.023). GA in ESR1 and PTEN were similar in both groups. Biomarkers for immune-oncology (IO) drug response including MSI-high and TMB≥10 mut/Mb status were similar in both groups.
Conclusions: CANEBC is an uncommon form of breast cancer that shares features with classic non-neuroendocrine CAIDC with similar frequencies for the endocrine and immunotherapy biomarkers but with lower frequencies of targeted therapy indications for drugs targeting ERBB2 and PIK3CA.

P3-06-01: Occupational exposure to ionizing radiation in female physicians and risk of breast cancer: a systematic review and meta-analysis

Milena Martello Cristófalo, Yedda Nunes Reis, Jonathan Yugo Maesaka, Bruna Salani Mota, José Maria Soares-Jr, Edmund Chada Baracat, Michail Ignatiadis

Background: Recent advancements in health technologies have enabled minimally invasive procedures with the increasing use of ionizing radiation for radioscopy and fluoroscopy by orthopedic surgeons, radiologists, urologists, cardiologists, vascular surgeons, and plastic surgeons. Concurrently, there appears to be an increased incidence of breast cancer among these professionals. In addition to various other risk factors to which they are exposed, it is likely that ionizing radiation also plays a role in carcinogenesis. This study is a systematic review and meta-analysis conducted to evaluate the prevalence of breast cancer in female physicians exposed to ionizing radiation in the workplace.
Methods: This systematic review was conducted following the PRISMA protocol and was registered in PROSPERO (ID: CRD42024553635). PubMed, Embase, and LILACS databases

were searched. Also, reference lists from other articles were searched. Mesh terms used were: breast neoplasms, physicians and prevalence. Articles that described the prevalence of breast cancer among female physicians exposed to ionizing radiation in the workplace were included. We restricted the publication languages to English, Spanish, or Portuguese. Article screening was performed independently by two evaluators (MM and YR) using the Rayyan platform. Methodological quality assessment and biases of included studies were assesed using the Newcastle-Ottawa Scale (NOS) for cohort studies. All statistical analyses were performed using the Review Manager (RevMan) Web v. 8.0.0 (The Cochrane Collaboration).
Results: As of January 2024, 2597 studies were retrieved, with seven studies meeting the inclusion criteria. One study was excluded due to duplicate data. Six cohort studies were included, evaluating a total of 34,744 female participants, including 8,103 female physicians exposed to ionizing radiation, and 26,641 participants in the control group, with a minimum follow-up of 10 years (ranging from 10 to 30 years). According to the NOS instrument, five studies were rated as good and one as fair. Meta-analysis assessment revealed an increased risk among female physicians exposed to ionizing radiation in the workplace, with an odds ratio (OR) of 1.84 (95% CI 1.11 to 3.06; p = 0.02; I^2 = 71%; six studies; 34,744 participants). A sensitivity analysis excluding the study rated as fair showed that the risk of breast cancer in the exposed group still increased, with an OR of 1.42 (95% CI 1.06 to 1.89; p = 0.02; I^2 = 0%; five studies; 23,854 participants).
Conclusions: Female physicians exposed to ionizing radiation during procedures such as radioscopy or fluoroscopy, appear to have an increased risk for breast cancer compared to female physicians not exposed to radiation. However, currently, there are no mandatory regulations for measuring radiation exposure, recommendations for the use of protective equipment according to the physician's body type, or specific screening policies for these professionals. Therefore, the assessment of this exposure and the associated increased risk of breast cancer should be further investigated to enhance worker safety measures and to guide specific screening strategies.

P3-06-02: Prenatal diethylstilbestrol (DES) exposure and risk of benign breast disease

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Introduction/Background: Diethylstilbestrol (DES), a synthetic estrogen and potent endocrine disruptor, was prescribed between the 1940s and 1970s to reduce risk of pregnancy complications and loss. DES was later found to be associated with increased risk of vaginal and other cancers among those who were prenatally exposed and is thus no longer prescribed during pregnancy. Whether in utero DES exposure is also associated with subsequent risk of developing benign breast disease (BBD), a risk factor for breast cancer, is unknown, and evaluating the impact of DES may serve as a model of potential

intergenerational chemical toxicity. We assessed the risk of developing BBD, overall and by histologic BBD subtype, associated with prenatal DES exposure.
Methods: From the National Cancer Institute Combined DES Cohorts Follow-Up Study, we examined associations of prenatal DES exposure with BBD risk among 4208 DES-exposed and 1830 unexposed women born between 1933 and 1976. Prenatal DES exposure status was abstracted from medical records or physician notes, and cumulative high or low DES dose was assigned in cohorts with recorded regional prescribing patterns. BBD diagnoses were self-reported on questionnaires from 1990 to 2017. Pathology-confirmation of BBD was available for 600 out of 1086 self-reported BBD cases. Cox proportional hazards models with age as the time scale were used to estimate adjusted hazard ratios (aHRs) and 95% confidence intervals (CIs) for the association of DES exposure (DES unexposed, exposed) with BBD risk. Follow-up started at birth and ended at the earliest of a diagnosis of BBD or breast cancer, death, or loss to follow up; the baseline hazard was stratified by year of birth (1933-1951, 1952-1953, 1954-1957, 1958-1976) and cohort, and further adjusted for baseline questionnaire year (1994, 1997 or 2001) and number of questionnaires a woman had answered in a time-varying fashion. We estimated cumulative incidence of BBD by DES exposure status. In additional analyses, we censored women with a BBD diagnosis only obtained by self-report at the age of BBD, and considered risk by histologic BBD subtype (nonproliferative, proliferative without atypia, proliferative with atypia) separately. To test for heterogeneity of associations of DES exposure by histologic BBD subtype, we used case-only polytomous logistic regression models adjusted for follow-up time and year of reported BBD.
Results: 6038 women contributed a total of 333,455 person-years with a median follow-up of 43.0 (range: 13.8-74.0) years for cases (n=1086) and 59.9 (range: 19.7-80.3) years for non-cases (n=4952). BBD diagnoses were confirmed by pathology among 225/350 (64%) unexposed women and 375/736 (51%) DES-exposed women. For the 600 BBD cases with pathologic confirmation, histology did not vary meaningfully by DES exposure: 54.7% nonproliferative, 32.1% proliferative without atypia, and 9.5% proliferative with atypia. Comparing women who were prenatally exposed to DES to unexposed women, we found no evidence of an association between DES exposure and risk of BBD (aHR: 0.98; 95%CI: 0.85, 1.12). There was no evidence of a dose-response relationship or difference in cumulative incidence by exposure status. The aHRs for nonproliferative BBD, proliferative BBD without atypia, and proliferative BBD with atypia were 0.83 (95%CI: 0.64, 1.07), 0.94 (95%CI: 0.67, 1.32), and 0.60 (95%CI: 0.33, 1.09), respectively, with no evidence of statistical heterogeneity by BBD subtype (p-heterogeneity: 0.86).
Conclusions: The risk of BBD was not elevated among women who were exposed to DES in utero, suggesting that previously observed increases in breast cancer risk associated with DES are not likely meditated through this pathological mechanism.

P3-06-03: Multi-drug adherence and breast cancer recurrence: A nationwide study of US veteran women with breast cancer and depression

Maya Aboumrad, Kala Visvanathan

Background: In a prior study, we demonstrated a strong relationship between major depressive disorder (MDD) and recurrence in veteran women diagnosed with breast cancer (BC). We now hypothesize that low adherence to both endocrine therapy (ET) and antidepressants may contribute to disease progression in this population.

Objective: To investigate whether adherence to either antidepressants or ET modifies the association between pre-diagnostic MDD and BC recurrence.

Methods: We used the Veterans Health Administration's electronic medical record database to evaluate treatment adherence in a retrospective cohort of women (age ≥ 18 years) with surgically resected early-stage invasive estrogen receptor positive BC diagnosed between 2010 and 2019 with follow-up through 2022. Our primary outcome of interest was BC recurrence. Using dispensed prescription records, we evaluated adherence to antidepressants during the two-years prior to BC diagnosis and adherence to ET at two-years following ET initiation. Women who had a BC recurrence or death during the two-year treatment period were excluded. Treatment adherence was calculated based on the Proportion of Days Covered (PDC): (number of days covered by a drug / two-year treatment period) x 100%. Multiple PDC thresholds were evaluated to identify an adherence threshold which we defined as the adherence level that was associated with the lowest risk of recurrence for either antidepressants or ET independently. Multivariable proportional hazards regression was used to examine associations between pre-diagnostic MDD and treatment adherence, accounting for interactions and adjusting for demographic, socioeconomic, clinical, and prognostic factors. Wald's test was used to evaluate significance of interactions.

Results: Our cohort was comprised of 4,757 women with estrogen receptor positive BC, of whom 1,359 (29%) had a pre-diagnostic MDD diagnosis. Based on a threshold for antidepressant adherence of 90%, women with MDD who were non-adherent to antidepressants had a 76% (HR=1.76; 95% CI: 1.44, 2.14) higher risk of recurrence compared to women with BC alone, whereas those with MDD who were adherent to antidepressants had a much lower increase in recurrence risk at 25% (HR=1.25; 95% CI: 1.02, 1.54), p-interaction<0.001. Based on a threshold for ET adherence of 80%, women with MDD who were non-adherent to ET had a 58% (HR=1.58; 95% CI: 1.01, 2.45) higher risk of recurrence compared to women with BC alone who were adherent to ET, whereas those with MDD who were adherent to ET had a lower increase in recurrence risk at 28% (HR=1.28; 95% CI: 0.93, 1.74), p-interaction<0.001. In secondary analyses, we examined the impact of adherence to both treatments on recurrence using the same adherence thresholds. Among the subgroup of women who were adherent to ET, women with MDD

who were non-adherent to antidepressants had two times (HR=2.00; 95% CI: 1.45, 2.77; p-interaction<0.001) the risk of recurrence compared to women with BC alone. Among the subgroup of women who were adherent to antidepressants, women with MDD who were non-adherent to ET had a 78% (HR=1.74; 95% CI: 0.95, 3.35; p-interaction<0.001) higher risk of recurrence compared to women with BC alone.

Conclusion: Our findings highlight the need for providers and patients to optimize adherence to both antidepressants and ET in women with MDD and estrogen receptor positive BC to maximize reduction in BC recurrence. Integrated care strategies could help support treatment adherence in this population.

P3-06-04: The effect of socioeconomic status on late-stage breast cancer by race and estrogen receptor status: Insights from the Carolina Breast Cancer Study

Joel Begay, Matthew R. Dunn, Brittney A. Gedeon, Melissa A. Troester, Marc A. Emerson

Background: Late-stage breast cancer (LSBC), defined as stage 3 or 4, significantly contributes to breast cancer (BC) mortality, underscoring the importance of pre-diagnostic care and screening. Understanding the factors contributing to LSBC, overall and by race and estrogen receptor (ER) status, could lead to strategies for timely diagnosis. OBJECTIVE: To evaluate the association between individual demographic and socioeconomic status (SES) characteristics and LSBC, overall and by race and ER status.

Methods: Using data from the Carolina BC Study, a population-based cohort of women diagnosed with invasive BC from 2008-13, we assessed the frequency of LSBC (n=545) compared to stage 1 diagnoses (n=1,225) using individual-level, self-reported demographic and SES data (marital status, education, poverty status, health insurance, pre-diagnostic care, screening history, and a multivariable SES latent class variable). Stage at diagnosis and ER status were abstracted from medical records. Age-adjusted relative frequency differences (RFDs) and 95% confidence intervals (CIs) were estimated separately for Black (n=835, 47.2%) and non-Black (n=935, 52.8%) women, and further stratified by ER status (negative vs positive). Results: Black women had more frequent LSBC diagnoses compared to non-Black women (38.7% vs 23.7%) and were more likely to be diagnosed with ER negative BC (32.7% vs 19.4%). Black women also lacked pre-diagnostic care (17.4% vs 7.4%) and were more frequently under-screened (25.6% vs 21.1%). Overall, marital status, education, poverty status, health insurance, pre-diagnostic care, screening history, and the multivariable SES latent class variable were significantly associated with higher LSBC frequency. Most associations persisted by race[JB1] , but lower screening history had a greater age-adjusted effect on LSBC frequency among Black women (RFD: 28.2, 95% CI: 20.0, 36.3) compared to non-Black women (RFD: 14.7, 95% CI: 7.1, 22.3). Among Black women, marital status and the multivariable SES latent class variable were significantly associated with LSBC frequency, but these associations were not observed among non-Black

women. ER-stratified analyses showed that marital status, poverty status, pre-diagnostic care, and screening history remained statistically significant for both ER positive and ER negative BCs, with greater effects of marital status and screening history among ER negative BCs. Health insurance was significantly associated with LSBC frequency among women with ER negative but not ER positive BC. Among ER positive BCs, poverty, pre-diagnostic care, and screening history were significantly associated with LSBC frequency in both Black and non-Black women, with a greater effect of under-screening among Black women. Among women with ER negative BC, pre-diagnostic care and screening history were significantly associated with LSBC frequency among Black women only.

Conclusion: Access to both pre-diagnostic care and screening services is crucial in reducing LSBC frequency, especially for Black women with ER negative BC where the effects were more pronounced. Further investigation is needed into the factors that protect non-Black women with lower SES and into the specific combinations of SES factors that delay diagnosis among Black women. Addressing SES-related disparities in preventive care is essential for achieving health equity.

P3-06-05: Real-world impact of remote symptom monitoring program on hospitalizations and emergency room visits in women with breast cancer

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Background: Clinical trials have shown remote symptom monitoring (RSM) using electronic patient-reported outcomes (ePROs) reduces healthcare utilization among patients with different cancer types. However, the impact of RSM is not well understood in real-world, standard-of-care patients diagnosed with early stage or advanced breast cancer.
Methods: This analysis of a hybrid, type 2 implementation-effectiveness trial evaluated the impact of RSM on healthcare utilization (hospitalizations & emergency room [ER] visits) among patients with breast cancer receiving chemotherapy, immunotherapy, or targeted therapy at the University of Alabama at Birmingham. We compared patients receiving RSM with historical controls who met RSM eligibility criteria yet received care prior to RSM implementation. Healthcare utilization was assessed 3 and 6 months post-RSM enrollment or date of first RSM qualifying treatment for controls. Unadjusted analyses included crosstabulations and estimation of effect sizes using Cramer's V (V: 0.10~small, 0.30~ medium, and 0.50~large effect size). We estimated risk ratios (RR) and 95% confidence intervals (CI) of healthcare utilization between patients receiving RSM and controls using modified Poisson regression with robust standard errors. Penalized logistic regression was used in subgroup analysis by stage (early [I-III] vs. metastatic [de novo or recurrent]) to estimate odds ratios (OR) and CIs of healthcare utilization for RSM-enrolled patients compared to controls. Models controlled for age at RSM enrollment, race, sex, cancer stage (Poisson models only), insurance status, prior treatment, number of comorbidities, rurality

of residence, area deprivation index, and an indicator for follow-up time during COVID-19 pandemic (April 2020-July 2021).
Results: From May 2021 to February 2024, 328 patients with breast cancer were enrolled in RSM; 30% were Black, 13% resided in a rural area, and 24% lived in a highly disadvantaged neighborhood. Patients receiving RSM were similar to controls treated from January 2017 to January 2021 (n=494) in terms of race and residence (28% Black, 18% rural residence, 27% lived in a highly disadvantaged neighborhood). However, patients receiving RSM more often had metastatic disease at diagnosis compared to controls (25% vs. 11%). Compared to controls, patients receiving RSM were less often hospitalized at 3 months (10% vs. 13%; V=0.02) and 6 months (19% vs. 26%; V=0.07) post-enrollment. Patients receiving RSM visited the ER less often compared to control patients (3 months: 15% vs 16%; V=0.02; 6 months: 21% vs. 25%; V=0.04). In adjusted models, 6-month risk of hospitalizations and ER visits were 30% and 31% lower, respectively, for patients receiving RSM compared to controls (RR 0.70, CI 0.52-0.93; RR 0.69, CI 0.53-0.90). Though not statistically significant, the 3-month risk of hospitalizations and ER visits were also 21% and 24% lower, respectively, for patients receiving RSM compared to controls (RR 0.79, 95% CI 0.52-1.21; RR 0.76, 95% CI 0.55-1.06). In subgroup analyses, 6-month odds of hospitalizations and ER visits were 37% and 39% lower for early-stage patients receiving RSM compared to controls (OR 0.63, 95% CI 0.41-0.96; OR, 0.61, 95% CI 0.39-0.94, respectively). Though not statistically significant, 6-month odds of hospitalizations or ER visits also trended lower for metastatic breast cancer patients receiving RSM compared to controls (OR 0.49, 95% CI 0.18-1.33; OR 0.49, 95% CI 0.18-1.30, respectively).
Conclusions: The use of ePRO-captured RSM reduced the risk of hospitalizations and ER visits for breast cancer patients in real-world settings, with the greatest impact seen for patients with longer engagement in RSM. Further work to expand this intervention nationally is needed.

P3-06-06: Associations between social drivers of health and breast cancer stage at diagnosis among US Black women

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Introduction: In the US, Black women who are diagnosed with breast cancer experience disproportionately high disease-specific mortality. This is, in part, because US Black women are more likely to be diagnosed with advanced-stage breast cancer. We sought to understand how social drivers of health (SDOH) relate to breast cancer diagnostic stage among US Black women.
Methods: This analysis included self-identified Black or African American women diagnosed with breast cancer who were participants in the Black Women's Health Study (BWHS; n=1,777), the Women's Circle of Health Study (WCHS; n=1,725), or the Carolina Breast Cancer Study Phase 3 (CBCS; n=1,493). SDOH were self-reported at enrollment in WCHS

and CBCS, and on pre-diagnosis questionnaires in the BWHS. Data on stage at diagnosis and tumor characteristics were abstracted from medical records and state cancer registry records. We used polytomous logistic regression adjusted for age at diagnosis, insurance status, and household income below the federal poverty line to estimate odds ratios (OR) and 95% confidence intervals (CI) for stage at diagnosis in association with each categorical SDOH exposure. Two categories of advanced stage (stage 2; stage 3 or 4) were compared to stage 1. Stages 3 and 4 were combined due to small numbers of stage 4 participants (BWHS n=75, WCHS n=50, CBCS n=109). Study-specific ORs were combined using fixed effects meta-analysis.
Results: Higher odds of late stage (3 or 4) versus stage 1 cancer were significantly associated with underutilization of mammographic screening (OR=2.97, 95% CI 1.85-4.77) and household income below the federal poverty line (OR=1.90, 95% CI 1.17-3.11). ORs were above 1.0 for lack of health insurance (OR=1.48, 95% CI 0.82-2.67) and lower educational status (i.e., did not graduate from high school; OR=1.24, 95% CI 0.76-2.03) but were not statistically significant. Marital status was not associated with late stage.
Discussion: SDOH that reflect economic instability and lower access to preventive healthcare were associated with later stage at diagnosis among US Black women with breast cancer. Interventions to increase screening utilization must contend with financial and insurance barriers to reduce late-stage diagnoses in this historically underserved population.
Acknowledgements: The Breast Cancer Research Foundation's Health Equity Initiative was supported by the Estée Lauder Companies Charitable Foundation.

P3-06-07: Plant-based Diets and Risk of Breast Cancer: A Pooled Analysis of 21 Cohorts

Emily Riseberg, Stephanie A. Smith-Warner, on behalf of the investigators of the cohorts

Background: Investigating relationships between dietary patterns and molecular subtypes of breast cancer may lead to a greater understanding of their pathogenesis. In the Nurses' Health Studies, the healthy plant-based diet index (hPDI), which considers the quantity and quality of the plant foods consumed, was associated with lower breast cancer risk, particularly estrogen receptor negative (ER-) tumors. These associations need to be studied in larger and more diverse cohorts.
Objective: We investigated relationships between the hPDI and unhealthy PDI (uPDI) and breast cancer risk overall and for subtypes defined by ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status in a pooled analysis of participant-level data in the Pooling Project of Prospective Studies of Diet and Cancer.
Methods. These analyses included 21 cohorts (N=1,446,309 women) that assessed usual diet at baseline using validated food frequency questionnaires or diet histories. Data on ER/PR status were provided by all studies; 10 studies provided HER2 status. Three overarching food categories each including ≥5 food groups were included in each index: healthful plant foods (e.g., vegetables, nuts), unhealthful plant foods (e.g., fruit juices,

refined grains), and animal foods (e.g., meat, eggs). For the hPDI, healthful plant foods received a positive score, and unhealthful plant foods received a negative score. For the uPDI, unhealthful plant foods received a positive score, and healthful plant foods received a negative score. In both scores, animal foods received a negative score. Indices (score range=18-90) were modeled using study-specific quintiles. Multivariable Cox proportional hazards models were used to calculate study-specific hazard ratios (HR) and 95% confidence intervals (95%CI). Models were adjusted for breast cancer risk factors and potential confounders (e.g., parity, body mass index). The random-effects meta-analysis method was used to pool log-HR estimates.
Results: During a median follow-up ranging from 8 to 28 years across cohorts, 70,099 breast cancers [9,305 ER-; 15,694 ER+ and/or PR+/HER2- (luminal A-like); 2,512 ER+ and/or PR+/HER2+ (luminal B-like); 971 ER-/PR-/HER2+ (HER2-enriched); and 2,092 ER-/PR-/HER2- (triple-negative)] were documented. Comparing the highest vs. lowest quintile, the hPDI was significantly associated with lower risk of overall (HR=0.94; 95%CI=0.92, 0.97), ER+ (HR=0.96; 95%CI=0.93, 0.99), ER- (HR=0.84; 95%CI=0.78, 0.91; p-heterogeneity with ER+=0.003), and triple-negative (HR=0.80; 95%CI=0.69, 0.94) tumors. The uPDI was significantly associated with higher risk of overall (highest vs. lowest quintile HR=1.04; 95%CI=1.01, 1.07), ER- (HR=1.19; 95%CI=1.10, 1.28; p-heterogeneity with ER+=0.001), and triple-negative (HR=1.21; 95%CI=1.04, 1.42) tumors. Weaker and nonsignificant results were observed for the remaining subtypes.
Conclusion: Healthful plant-based diets were associated with a slightly lower risk of breast cancer overall, with stronger inverse associations observed for ER- and triple-negative tumors. Unhealthful plant-based diets were associated with higher risks of the same subtypes. Following a healthy plant-based diet may aid in preventing breast cancer subtypes with fewer treatment options and poor survival.

P3-06-08: Chemotherapy decision making and physical and psychological symptoms in young breast cancer survivors

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Background: When young women are diagnosed with breast cancer, they often present with more advanced and/or aggressive forms of the disease. Given the use of chemotherapy is the recommended for women with triple-negative and HER2-positive subtypes of breast cancer (BC), as well as for locally advanced and genomically higher risk ER+, HER2- disease, most young women receive chemotherapy for early breast cancer. Despite clinician recommendations, patients may not have confidence in the decision to be treated with chemotherapy. Understanding different facets pertaining to this and its potential impact on physical and psychological symptoms is essential to improve patient care.
Methods: Using data from the Young Women BC Study (YWS), a prospective cohort of 1,302 women who were ≤40 and diagnosed with BC from 2006-2016. We evaluated the

association between confidence in chemotherapy decision (as assessed on the 6-month survey, rated by participants on a 10-point Likert scale) for women with stage I to III BC treated with chemotherapy, and physical symptom burden at 1-year survey (SF-36 and CARES), fear of recurrence at 1-year (Lasry Fear of Recurrence index), and anxiety and depression at 1-year (HADS). Logistic regression models used cut-offs chosen apriori with ratings of 7-10 grouped as 'high' confidence and ratings of 0-6 grouped as 'low to moderate' confidence. Ratings of ≥11 for both HADS subscales (depression and anxiety) was used, and indicates a significant number of symptoms that likely correspond with a clinical diagnosis. This analysis controlled for tumour subtype, stage, education, and hormonal therapy treatment.
Results: A total of 681 women were analysed due to eligibility and completeness of the data. The majority were non-Hispanic white (n = 602, 88.4%); 3% were non-Hispanic Black (n = 18) and 5% Hispanic (n= 33). The median age at the 6-month survey was 37 years (Range = 18-42). Most respondents (n=452, 66.4%) expressed a high level of confidence (rating it 10 out of 10), while smaller percentages had lower confidence ratings, ranging from 0 to 9. Individuals with less confidence in chemotherapy reported worse outcomes on the general health ($t(680) = 2.34$, $p = .012$) and mental health ($t(680) = 3.03$, $p = .003$) SF-36 subscales at one year, as well as the CARES psychological ($t(680) = 3.45$, $p < .001$) and medical domain subscales ($t(680) = 5.91$, $p < .001$). Individuals who reported less confidence in chemotherapy at six months reported greater anxiety ($t(680) = 2.78$, $p = 0.006$) and depression ($t(680= 3.53$, $p < .001$) at one year. No significant associations were found for fear of recurrence. In the logistic regression model, grouping by high confidence (indicating 7-10 on the Likert scale, n=637, 93.5%) and low to moderate confidence (indicating 0-6, n=44, 6.5%), participants with low to moderate confidence had higher odds of depression at one year compared to those in the high confidence group (OR 3.83, 95% CI 1.57 - 9.34) ($p = .003$). Confidence was not significantly associated with anxiety (OR 1.20, 95% CI 0.60 – 2.41) ($p = .603$) or fear of cancer recurrence (OR 1.20, 95% CI 0.57 – 2.51) ($p = .629$) in the multivariable models.
Conclusion: In this analysis of young breast cancer survivors who received chemotherapy, we found that most indicate high confidence in their chemotherapy decision. Our finding of an association between lower confidence in the chemotherapy decision and depressive symptoms at 1 year suggests this group may need targeted intervention to improve psychosocial outcomes.

P3-06-09: Effect of a 6-Month Tailored Exercise Program According to Cancer Trajectories in Patients with Breast Cancer: Preliminary Results of a Randomized Controlled Trial

Su Jin Yeon, Justin Y. Jeon, Seho Park, Jiwon Kang, Rosa Park, Susanna Jun

Purpose: This randomized controlled trial (RCT) aims to explore the short-term effects of a tailored home-based exercise program for breast cancer patients, individualized according to the phase of treatment and patient-specific conditions. The study seeks to assess the

impact of this intervention on physiological outcomes, including fasting insulin levels and shoulder function, during the first 6 months of the 12-month intervention period. These preliminary findings contribute to understanding how phase-specific exercise interventions influence recovery and overall health in breast cancer patients.

Methods: 96 patients with stage 1–3 breast cancer were planned to be enrolled in this 12-month RCT. Participants were randomized into either a tailored exercise group or a usual care group. The exercise program was home-based and tailored to the patient's treatment phase. In Phase 1 (post-surgery), the focus was on improving shoulder strength and range of motion (ROM). In Phase 2 (during chemoradiation therapy), the focus shifted to maintaining physical function and preserving muscle mass. This interim analysis presents preliminary data from 49 patients who completed the 6-month follow-up. The primary outcome was fasting insulin levels, with secondary outcomes including shoulder strength and ROM.

Results: Among the 49 participants analyzed (mean age 53.02 ± 7.46 years), fasting insulin levels in the exercise group showed a downward trend from baseline (8.80 ± 5.54) to 6 months post-intervention (6.05 ± 4.55), though this reduction did not reach statistical significance ($p = 0.149$). In the usual care group, fasting insulin levels exhibited a slight increase over the same period (7.49 ± 5.20 to 8.53 ± 8.47), but this change was also not statistically significant ($p = 0.896$). While the impact on insulin levels was inconclusive, improvements in shoulder strength were more pronounced. Shoulder strength recovery, analyzed through separate movements (flexion, abduction, extension), was significantly better in the exercise group (93.41 ± 27.2) compared to the usual care group (74.19 ± 16.55) at 1-month post-surgery ($p = 0.013$). Additionally, 33.33% of participants in the exercise group achieved full shoulder strength recovery by 1-month post-surgery, compared to none in the usual care group.

Conclusion: Preliminary findings suggest that early, tailored exercise interventions can significantly improve shoulder function in breast cancer patients. While changes in fasting insulin levels were not statistically significant at this stage, the improvements in shoulder strength highlight the potential benefits of integrating individualized exercise programs into clinical care. Further analyses of long-term outcomes, including cardiopulmonary fitness and insulin resistance, will provide additional insights into the efficacy of tailored exercise regimens throughout the cancer treatment trajectory.

*The protocol for this study has been registered with the Korean Clinical Trials Registry (KCT0007853); DOI: https://doi.org/10.1186/s12885-023-10664-1

P3-06-10: Triple Negative Breast Cancer: The standpoint of Mexican population

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Introduction: Breast cancer is the malignant neoplasm with the highest incidence and mortality rate among Mexican women, accounting for 28% of cancer cases and 16 deaths per 100,000 women over 20 years of age. Triple-negative breast cancer (TNBC) is a subtype of breast cancer that does not express estrogen and progesterone receptors, nor human epidermal growth factor receptor 2 (HER-2). Conventionally it presents with a poor prognosis compared to other subtypes and usually relapses within the first 3 years after diagnosis.
Objective: The aim of this study was to describe sociodemographic, epidemiologic, clinical, histopathological and prognostic characteristics of TNBC in a northeast Mexican-based cohort.
Methods: Original, retrospective, descriptive and analytical data were analyzed.The study was approved by the Research and Bioethics Committee, 18-year-old patients and older with TNBC diagnosis were included, treated at our University Hospital "Dr. José Eleuterio González" between 2014-2019. Sociodemographic, epidemiologic, histologic, biochemic, diagnosis, treatment and prognosis data were obtained from clinical records.
Results: One-hundred thirty-five patients were enrolled. The mean age at diagnosis was 48 ±12.9 years and the most common histological type was invasive ductal adenocarcinoma in 80.7% of cases. Regarding clinical stage, 24.4% were classified as stage IIA, followed by stage IIIA in 21.5% of the patients. Stage IV accounted for 11.9% of the patients. Thirty-four percent of the tumors had a Ki67 index of more than 20% and 11.4% presented BRCA mutations, with BRCA1 being the most prevalent. Platinum based chemotherapy was prescribed in 20.8% of patients. Surgical treatment was performed in 92.4% of patients and 84.9% received radiotherapy. Sixty-two patients reached clinical surveillance and 13 had disease recurrence.
Discussion: TNBC represents 23.1 % of breast tumors in México in comparison to USA reports with approximately 15% cases. In our study we observed that the age of diagnosis with a predominance above 40 years was greater than reported in developed countries where TNBC is more commonly diagnosed in premenopausal women; this is probably associated with poor health access and lack of public health education in secondary prevention of breast cancer. Twenty-nine (21.48%) patients had a history of menarche under 12 years. Age of diagnosis, histologic subtype and risk factors tendencies correlate to literature worldwide reports.
BRCA mutations in México are estimated at approximately 23% of cases and the incidence of TNBC has been reported substantially in patients with BRCA1 mutations. Reproductive risk factors including early menarche and non-parity regardless of its general relation with breast cancer independently of histologic subtype were frequent among our patients. Unhealthy lifestyles such as sedentarism, overweight, high carbohydrate intake, alcohol

consumption and tobacco have been strongly related to breast cancer development and were the predicted lifestyles of our cohort patients. Outlining the epidemiologic and sociodemographic characteristics of patients with TNBC in our population and its influence in the prognosis highlights the importance of reinforcing screening policies and implementing programs that promote healthy lifestyles.
Conclusion: The incidence of TNBC in Mexico exceeds the statistics reported in the United States by approximately 8%, with an 8-year greater mean age of diagnosis in comparison to American women. This is due to poor access to healthcare services and the lack of education regarding breast cancer screening, leading to a diagnosis at later stages and, consequently, to a worse prognosis and fatal outcomes. The BRCA prevalence mutations reported in our study was almost 12% lower than reported worldwide, probably due to lack of resources to obtain genetic testing and poor medical follow-up.

P3-06-11: Efficacy of a Structured Educational Program in Enhancing Breast Cancer Knowledge Among Caregivers in Egypt: A Quasi-Experimental Pilot Study

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Background: In low- and middle-income countries, breast cancer (BC) awareness and education are often hindered by misinformation, cultural barriers, and low literacy rates. Effective patient education is essential for improving health outcomes, yet structured interventions remain limited. This study assesses the impact of a comprehensive educational program designed to enhance BC knowledge among caregivers at Cairo University's National Cancer Institute (NCI).
Methods: A quasi-experimental design was employed in this pilot study involving 59 participants. The intervention consisted of a three-hour educational session focused on BC awareness, treatment options, misconception correction, and psychological health. Pre- and 30 days post-intervention knowledge assessments were conducted using a structured questionnaire. The statistical significance of the knowledge gains was evaluated through paired sample t-tests.
Results: Participants demonstrated significant improvements in key knowledge areas. Post-intervention scores showed marked increases in understanding BC symptoms (mean score: 1.1 ± 0.7 pre-test vs. 1.9 ± 0.3 post-test; $p < 0.001$), risk factors (2.7 ± 1.6 vs. 5.7 ± 1.4; $p < 0.001$), nutrition misconceptions (0.6 ± 0.6 vs. 1.6 ± 0.7; $p < 0.001$), and breast self-examination techniques (1.3 ± 0.87 vs. 2.2 ± 1; $p < 0.001$).
Discussion: The educational program effectively addressed gaps in BC knowledge, reflecting the urgent need for structured patient education in similar settings. Cultural and educational backgrounds were considered, with tailored content ensuring relevance and accessibility. The program also highlighted the importance of patient advocate groups and the role of healthcare professionals in ongoing education.
Conclusion

This study underscores the potential of structured educational interventions to significantly improve BC knowledge among caregivers in low- and middle-income countries. By correcting misconceptions and enhancing understanding, such programs contribute to better health knowledge. Future research should explore scaling these interventions to larger populations and diverse settings.

P3-06-12: Breast Cancer and Mortality Rates: An Analysis Using the NIS 2021 Database

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Background: Breast cancer remains a significant public health concern worldwide, with numerous factors influencing patient outcomes. This study aims to evaluate the mortality rates among breast cancer patients in the United States, utilizing data from the National Inpatient Sample (NIS) 2021 database. The analysis focuses on demographic, socioeconomic, and healthcare-related variables to understand their impact on mortality rates.
Methods: Data was extracted from the NIS 2021 database, and interpreted using STATA software. Inclusion criteria were set for adult patients (aged ≥18) diagnosed with breast cancer, identified by ICD-10 code C50. Variables studied included age, sex, race, Charlson Comorbidity Index (CCI), income quartile, insurance status, hospital bed size, teaching status of the hospital, and geographic location of the hospital. The Charlson Comorbidity Index was categorized as 0 (CCI=0), 1 (CCI=1), 2 (CCI=2), and 3 (CCI≥3). Survey-weighted analysis was conducted to generate totals and proportions, and linear regression was applied to assess the association between variables and outcomes.
Results: Among the 6,666,752 observations, 140,855 breast cancer patients met the inclusion criteria. The majority were female (98.66%) with an average age of 66.1 years (SE = 0.144). Racial distribution indicated 67.82% were White, 17% Black, 9.11% Hispanic, 3.13% Asian/Pacific Islander, 0.43% Native American, and 2.51% of other races. Socioeconomic status, represented by the median household income quartile, was evenly distributed among the patients.
The majority of patients (83.77%) had a Charlson Comorbidity Index of 3 or more. Insurance coverage showed 60.17% had medicare, 11.78% were medicaid, and 26.7% had private insurance. Hospital characteristics revealed that 51.6% of patients were treated in large hospitals, 76.27% in teaching hospitals, and 92.98% in urban settings.
The overall mortality rate among breast cancer patients was 5.65% (SE = 0.00147). The survey-weighted total estimate of deaths was 7,949.98 (SE = 234.21), with a 95% confidence interval of 7,490.80 to 8,409.16.
Conclusion: This analysis provides a comprehensive overview of the mortality rates and demographic factors among breast cancer patients in the U.S. hospital setting using the NIS 2021 database. These insights could provide additional information to identify vulnerable populations and guide healthcare policies and targeted interventions to improve outcomes for breast cancer patients.

P3-06-13: Prevalence and Effect of Comorbidities on Survival of Breast Cancer Patients by age

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Purpose: More than 30% of breast cancer patients have comorbidities unrelated to cancer before and after diagnosis, and there is a lack of research on the impact of this on the survival rate of breast cancer patients. This study aimed to examine the impact of comorbidities on the survival rate of breast cancer focusing on variation according to age.

Method: This study utilized data from the Cancer Public Library Database (CPLD), part of the Korean Clinical Data Utilization for Research Excellence project (K-CURE), to analyze breast cancer patients' treatments and comorbid conditions. Female patients diagnosed with breast cancer (ICD-10 code C50) between 2014 and 2019 were included. We estimated survival according to Age-adjusted Charlson Comorbidity Index (ACCI) and survival was determined by univariate and multivariate Cox proportional hazard regression analysis.

Results: A total of 128711 patients were included in this study. The mean ACCI for patients under 40 and in their 40s before and after diagnosis increased from 1.2 to 1.6 and from 2.3 to 2.7, respectively, and the most common comorbidities in both age groups were COPD (under 40 7.5%, 40s 6.9%, respectively) before diagnosis and liver disease (under 40 20.8%, 40s 23.1%, respectively) and gastric ulcer (under 40 18.2%, 40s 15.0%, respectively) after diagnosis. The mean ACCI for patients in their 50s increased from 3.7 to 4.0, with high blood pressure (21.7%) and liver disease (10.4%) being the most common before diagnosis and the same after diagnosis (25.5%, 25.9%, respectively). The mean ACCI for patients in their 60s increased from 5.3 to 5.6, and the most common cases were high blood pressure (55.1%) and diabetes (21.8%) before diagnosis, and high blood pressure (48.5%), liver disease (27.0%) after diagnosis. The mean ACCI for patients over 70 increased from 7.0 to 7.2, and the most common diseases were diabetes (35.5%) and hypertension (28.4%) before diagnosis, but hypertension (72.8%) and diabetes (37.7%) after diagnosis. The diseases that significantly increased breast cancer-related deaths were heart failure (aHR 1.5, 95% CI 1.2-1.8), dementia (aHR 1.6, 95% CI 1.0-2.2), paralysis (aHR 1.5, 95% CI 1.0-2.1), and kidney disease (aHR 1.5, 95% CI 1.1-1.8). The comorbidities with the highest proportion of untreated patients were dementia and paralysis, and these increased the risk of death the most across all age groups (HR 6.9, 95% CI 5.8-8.1, HR 6.4, 95% CI 5.4-7.4, respectively). Excluding dementia and paralysis, the diseases that most significantly increased mortality by age were cardiovascular disease and kidney disease in those under 40 and in their 50s, myocardial infarction and kidney disease in those in their 40s, and kidney disease and heart failure in those over 60s.

Conclusion: As age increased, the rate of age-related comorbidities increased. Dementia and paralysis have most significantly increased the mortality rate among the elderly so management at the national level is needed to ensure that these patients receive

appropriate treatment. Although there were differences by age group, kidney disease and heart failure also showed a significant increase in mortality, making appropriate management necessary.

P3-06-14: Evaluating “Spinning Science” A Patient-Led Health Literacy Educational Program for People Living with Metastatic Breast Cancer

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Background: There are currently over 150,000 people living with metastatic breast cancer (MBC) in the United States, a number projected to increase to over 169,000 people living with MBC by 2025. MBC treatment requires patients to digest an overwhelming amount of technical medical information to understand and participate fully in shared decision-making regarding testing and treatment. There have been limited efforts to increase MBC patient health literacy to improve self-efficacy systematically. This study aimed to evaluate “Spinning Science,” an MBC patient-led and delivered educational intervention designed to improve MBC-specific health literacy amongst people living with MBC.
Methods: Spinning Science was developed by an MBC patient advocate with doctoral training in biology, and the objectives were reviewed by other patient advocates, an oncologist, and a researcher. It consists of five online sessions covering basic scientific and technical topics related to MBC treatment and survivorship, such as breast cancer subtyping, genetic and genomic testing, clinical trials, ctDNA, information literacy, and more. The workshops were intentionally kept small (>20 people). Small group work, chat monitoring, time for questions throughout, and polling were used to encourage participation and deeper engagement with the material. The program was offered twice, in the Fall of 2023 and Spring of 2024, to members of Project Life, a virtual wellness community for people living with MBC and their loved ones. Participants in the program were asked to complete pre- and post-program surveys to gain an understanding of their change in knowledge as a result of the program, as well as the most salient aspects of the program. Paired 2-sample T-Tests were conducted to assess change in mean scores in the pre- and post-program surveys. Thematic qualitative analysis was conducted on free text responses in the post-program surveys.
Results: 54 people living with MBC participated in the Spinning Science program from Fall 2023-Spring 2024. 14 of the 17 Fall 2023 participants and 32 of the 37 Spring 2024 participants responded to both the pre- and post-program surveys, for a total of 46 complete survey respondents. In the pre-test 43% (n=23) of participants reported that they agreed or strongly agreed with the statement “I don’t know enough to make my own medical decisions” compared to only 13% (n=6) reporting agreeing with that statement in the post-test ($p<0.05$), demonstrating significantly improved self-efficacy. Qualitative analysis of free-text responses revealed that Spinning Science was responsive to participant feedback from the Fall 2023 to the Spring 2024 program. Specifically, the updated program addressed participants’ desire to learn more about clinical trials included a stand-alone workshop on how to read and understand academic posters, abstracts, and literature. The

Spring program also provided more MBC-specific content and addressed participant concerns about program pacing, workshop duration, and accessibility of program materials both during and after the program has finished.
Conclusion: Overall, Spinning Science improved participating MBC patients' health literacy and self-efficacy and responded to the feedback and needs of the Project Life community by addressing areas for improvement from one iteration to the next. Future Spinning Science iterations should implement validated survey-based evaluation metrics and focus on recruiting Project Life members with lower baseline health literacy and self-efficacy to evaluate the program's impact on MBC patients fully.

P3-06-15: Trajectories of Daily Step Counts in Breast Cancer Survivors in the Breast Cancer Weight Loss (BWEL) Trial

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Background: Step count is one of the most accessible and widely used device-measured metrics for monitoring physical activity levels. Step counts are associated with hospitalizations during cancer treatment and with other health outcomes in cancer patients (pts). The BWEL (Alliance for Clinical Trials in Oncology A011401; NCT02750826) trial is a phase 3 randomized trial testing the impact of a supervised weight loss intervention (WLI) on invasive disease-free survival in 3180 women with stage II-III breast cancer (BC) and a body mass index (BMI) ≥27 kg/m2. This secondary BWEL analysis describes 2-year (yr) trajectories of daily step counts in the BWEL WLI group.
Methods: Eligible pts were within 16 months (mos) of diagnosis of stage II-III HER2-negative BC, had completed chemotherapy and radiation, and were randomized 1:1 to a telephone-based WLI plus health education (HE) or an HE alone control group. WLI pts were assigned a trained coach at the BWEL call center and were provided 42 phone calls over the 2- yr intervention period. Calls were delivered weekly for the first 12 weeks (wks), biweekly for the remainder of the 1st yr, and monthly in the 2nd yr. The WLI focused on caloric restriction and exercise, with an activity goal of 150 minutes (min) of moderate to vigorous-intensity exercise per wk in the first 6 mos of WLI, increasing to 225 min/wk in mos 6-24. WLI pts were provided with a Fitbit for motivation and assessment of activity. Valid daily step counts (defined as ≥ 100 steps/day) were extracted from the BWEL portal and averaged (avg) weekly to capture habitual patterns. Linear mixed-effect models accounting for individual variances and Joinpoint regression were used to evaluate trajectories of daily step counts over the 104-wk WLI. Univariable and multivariable (including baseline age, race/ethnicity, and BMI) mixed-effect regression models were used to examine difference in daily step counts over 104 wks by pt factors.

Results: Of the 1591 WLI pts, 1240 provided Fitbit data with 466,400 records of daily step counts. Pts with Fitbit data had mean baseline BMI of 34.4 (±5.6) kg/m2 and mean age of 53.2 (±10.3) yrs; 10.8% self-identified as African American (AA) and 6.1% as Hispanic. A higher proportion of individuals without Fitbit data identified as AA (19.1%) or Hispanic (10.5%) compared to those with Fitbit data (P<.001). Trajectory analysis indicated that step counts increased from 4864 (95% CI: 4631-5096) to 5663 (95% CI: 5444-5882) avg steps/day from wk 1 to wk 12 (P for trend <.001) and to 6647 (95% CI: 6476-6818) avg steps/day between wks 26 and 52 (both P for trend <.001). Step counts decreased to 6058 (95% CI: 5782-6334) avg steps/day at wk 104 (P for trend <.001) but remained significantly higher than baseline (P<0.001). Patients aged ≥65 yrs had significantly lower avg daily step counts (5402 [95% CI: 5033-5772]) than those aged <65 (6389 [95% CI: 6225-6552]). Avg daily step counts were also significantly lower in pts with BMI ≥35 (5503 [95% CI: 5267-5739]) vs those with BMI <35 (6691 [95% CI: 6502-6879]). AA pts had lower avg daily step counts (5793 [95% CI: 5334-6252]) than pts who did not identify as AA or Hispanic (6297 [95% CI: 6128-6466]). Each 1-yr increase in age was associated with an avg 30 (95% CI: 16-44) fewer steps/day over the WLI period in a multivariable model. Similarly, each 1-unit increase in BMI was associated with 117 (95% CI: 91-143) fewer avg steps/day.
Conclusions: Daily step counts significantly increased in early BC survivors during a telephone-based WLI. Further follow-up of the BWEL trial will evaluate relationships between daily step trajectories and disease and pt-reported outcomes.
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P3-06-16: Heterogeneity of Tertiary Lymphoid Structures Predicts the Response to Neoadjuvant Therapy and Immune Microenvironment Characteristics in Triple-Negative Breast Cancer

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Background: Tertiary lymphoid structures (TLS), as functional microenvironments for immune responses, are closely associated with the prognosis of various cancers. In the treatment of triple-negative breast cancer (TNBC), neoadjuvant therapy (NAT) is a primary strategy. However, the role of TLS in the immune status of TNBC and its interaction with NAT remains insufficiently understood.
Methods: This study utilized single-cell RNA sequencing (scRNA-seq), multiplex immunofluorescence staining (mIF), and radiomic techniques to comprehensively assess TLS and the tumor microenvironment (TME) in paired samples from TNBC patients before and after NAT.
Results: In TNBC, the presence of TLS was associated with B-cell maturation and T-cell activation. Tumors with high TLS expression exhibited stronger cytotoxic expression of immunoglobulin family genes (IGHM and IGHG1) in B-cells and neoantigen-specific CD8+ T-

cells (neoTCR8) compared to tumors with low TLS expression. Furthermore, mIF assessments revealed significant heterogeneity between TLS and TME in TNBC. Interestingly, total CD8+ T-cell levels were not effective predictors of NAT response. Instead, the maturity of TLS correlated significantly with NAT response and improved prognosis ($P<0.05$). Finally, we developed an imaging biomarker scoring system to predict TLS status and the efficacy of NAT.
Conclusion: Our findings reveal changes in TLS and TME in TNBC patients post-NAT, confirming the predictive capability of mature TLS. Additionally, the study elucidates the immunomodulatory effects of NAT, enabling personalized immunotherapy based on immune characteristics of post-chemotherapy samples, ultimately improving clinical outcomes for breast cancer patients.

P3-06-17: IAPP mediated anti-breast cancer function of CD8+T cells via targeting cuproptosis

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Abstract: Objective: Breast cancer (BRCA) is the most common cancer worldwide, characterized by high heterogeneity and recurrence rates. It has been found that the biological behavior of tumors can be regulated by immunity and cuprotosis. Therefore, exploring potential targets or prognostic biomarkers to mediate immunity and cuprotosis is urgent for the therapy of BRCA.
Methods: By mining the TCGA database, we identified immune-related genes and immune-cuprotosis-related differentially expressed genes (ICR-DEGs). Prognostic analysis, differential expression analysis, univariate and lasso regression analyses were used to determine independent prognostic values for ICR-DEGs. Then, the role of the tumor immune microenvironment (TIME) in breast cancer was explored. To evaluate the relation between ICR-DEGs and immune scores, we constructed a prognostic risk model to evaluate immune checkpoints. Mitochondrial morphology was examined via electron microscopy. T cell functionality was assessed through flow cytometry and ELISA, and the anti-tumor effect of T cells was evaluated in a subcutaneous tumor model via RNA-seq analysis of related signaling pathways.
Results: 16-gene prognostic signature was constructed. The high-risk group had lower prognosis than the low-risk group. The AUC for the 1-, 3-, and 5-year risk scores were 0.733, 0.709, and 0.704, separately. The levels of CD8+T cell immune cell infiltration of high-risk group was lower than that of low-risk group, and the high-risk patients had lower Stromal Score, Immune Score, and ESTIMATE Score than the low-risk patients. In addition, prognostic features were significantly associated with immune checkpoint inhibitor (ICI)-associated genes. Downregulation of IAPP reduced cuprotosis of CD8+T or Her2-CAR-T cells while promoting cytokine release, thereby enhancing in vitro and vivo anti-BRCA functionality. IAPP mediated copper promotion of CD8+T viability and function through the NF-κB pathway
Conclusion: This study has provided novel insights into the role of ICRSig in the

development and progression of BRCA, laying a foundation for the identification of new markers and therapeutic targets for breast cancer. Furthermore, our research had clarified the function and mechanism of IAPP in T cells, providing new ideas for improving the diagnosis and treatment of BRCA.

P3-06-18: Novel regulators of PDGF-BB secretion in breast cancer

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Platelet-derived growth factor-B (PDGFB) is necessary for normal cell growth and development, with major roles in angiogenesis, differentiation, and migration. PDGFB is the preferred ligand for the receptor tyrosine kinase platelet-derived growth factor receptor-beta (PDGFRß) and is a known oncogene that our lab has reported to mediate breast cancer (BC) primary tumor growth and metastatic spread to the brain. As the mortality rate of metastatic BC patients, especially those with BC-associated brain metastasis, is extremely high, our current research focuses on identifying and targeting mechanisms that direct differential tumor-specific PDGFB overexpression and secretion. In normal physiology, PDGFB is expressed by epithelial and endothelial cells, and via paracrine signaling, activates PDGFRß on neighboring mesenchymal cells. While these interactions are well known, both the secretory cascade and extracellular regulation of PDGF-BB is grossly understudied in BC. In particular, it is known that PDGF-BB is associated with positively charged heparin sulfate proteoglycans on the cell surface. This cell-associated form can undergo proteolytic processing, where C-terminal cleavage releases the mature PDGF-BB dimer. Yet, the enzyme responsible for this cleavage is unknown. Here, we hypothesized that the endoglycosidase, heparanase (HPSE), was capable of cleaving extracellular heparan sulfate proteoglycan chains, thereby releasing PDGF-BB bound to heparan sulfate. We show that silencing HPSE in PDGF-BB secreting BC cell lines diminishes its secretion by ~40%. We are currently investigating other candidate regulators of PDGF-BB secretion through protease inhibitor screening and have identified the serine protease superfamily and MMPs as crucial regulators of PDGF-BB secretion, perhaps by direct cleavage of the C-terminus. Ongoing studies aim to delineate the precise roles of the superfamily members and study the in vivo roles of HPSE, serine proteases, and MMPs in PDGF-BB secretion. Importantly, PDGFB and HPSE expression are synergistically prognostic in patients. Together these findings suggest a crucial need to understand PDGF-BB secretion in BC.

P3-06-19: Spatial mulitomic assay for assessing immune cell phenotype and function in the tumor microenvironment

Anushka Dikshit, Debia Wakhloo, Julia Yu, Paul Liu, Sonali Deshpande

Tumormicroenvironment (TME) is a complex milieu of multiple cell types mainly including tumor cells, immune cells, endothelial cells and stromal cells. Immune cell profile within the TME can determine success of immunotherapies, indicate therapeutic efficacy and determine any potential therapeutic toxicity in various malignancies including breast

cancer. Spatially interrogating these immune cells is crucial in studying immune-immune and immune-tumor interactions. Characterizing immune cells require assessing cell marker expression using protein detection and activation markers using RNA detection. To address this, we have developed a high throughput RNAscope™ assay on the BOND RX to spatially visualize RNA and protein markers on the same slide.
We utilized the new RNAscope™ Multiomic LS assay that can detect up to 6 RNA and protein targets in breast cancer FFPE tissue. The TSA-based amplification strategy offers signal boost for both RNA and protein targets. To optimize protein detection, the workflow is completely protease-free there by preserving antigen integrity and tissue morphology. The assay can be customized to include any target RNA and proteins of interest. Here, we demonstrate the use of our pre-conjugated antibody panel which includes CD8, CD4, FoxP3 and PanCK to visualize tumor infiltrating lymphocytes (TILs). We also utilized unconjugated primary antibodies for CD68 and CD163 to detect tumor macrophages in multiple tumor tissues.
Infiltrating Cytotoxic T cell lymphocytes were detected using CD8, GZMB and IFNG co-expression. Regulatory T cells were detected using CD4, FOXP3 co-expression. PanCK was used as a tumor marker to delineate tumor from stroma. Similarly, tumor associated macrophages were detected using CD163, CD68 , IL10 and IL-1B expression. We also identified distinct M1 and M2 macrophage populations in the breast tumor sample.

P3-06-20: Lean adipocyte-secreted oxylipin, 9S-HODE, triggers ferroptosis and protects against obesity-accelerated breast cancer

Meghan Curtin, Guoying Wang, John Maschek, David Lum, James Cox, Keren Hilgendorf

Approximately 1 in 8 women will develop breast cancer in their lifetime, and over 40,000 women in the United States die from breast cancer every year. Post-menopausal breast cancer risk and mortality rates are exacerbated by those who are classified as obese, such that a 5-unit increase in body mass index is linked to a 12% increase in risk. The rising rates of obesity make understanding the link between obesity and the incidence, progression, and mortality of breast cancer of high priority. Obesity is marked by an expansion of white adipose tissue, which accounts for 90% of the breast cancer microenvironment. While several studies have identified paracrine factors secreted by obese adipose tissue that promote tumorigenesis, the role that lean adipose tissue plays in breast cancer progression remains underexplored.
In this study, I screened the secretome of primary adipocytes isolated from multiple fat pads from both lean and obese mice for the ability to modulate the growth of breast cancer cell lines across multiple different subtypes. I discovered a potent role of lean mammary adipocytes in selectively inhibiting breast cancer cells in vivo and in vitro. This inhibitory function is specific to lean mammary adipocytes, as the secretomes of obese mammary adipocytes or adipocytes isolated from visceral fat depots do not inhibit breast cancer cell growth. Further, I discovered that the lean mammary adipocytes, but not obese adipocytes, secrete an oxylipin called 9S-HODE, an oxidized metabolite derived from the essential fatty

acid linoleic acid. I show that lean adipocytes and 9S-HODE inhibit breast cancer cell growth by triggering ferroptosis. However, normal breast epithelial cells are protected from 9(S)-HODE-induced ferroptosis by transiently growth-arresting and rapidly forming lipid droplets. We propose that lean adipocytes actively inhibit the growth of breast cancer cells by secreting the ferroptosis inducer 9(S)-HODE, and that in patients with obesity, breast cancer is accelerated, at least in part, due to the loss of 9(S)-HODE-mediated tumor suppression.

P3-06-21: Needle Biopsy Accelerates Pro-Metastatic Changes and Systemic Dissemination in ER+ Breast Cancer: Implications for Mortality by Surgery Delay

Takemi Tanaka

Breast cancer is the most commonly diagnosed malignancy in the U.S. and over two-thirds of invasive breast cancer cases are diagnosed early stage. However, 15-year survival rates for early-stage breast cancer remain in the range of 70-77%. Mounting evidence showed an increased risk of disease progression and mortality among early-stage breast cancer patients whose surgery was delayed over 60 days after the diagnostic biopsy. Yet, the mechanism(s) underlying the rapidly increased mortality due to delay of surgery after diagnosis remains unknown.
Our cohort analyses of early-stage breast cancer patients reveal the emergence of a significantly rising mortality risk when the biopsy-to-surgery interval was extended beyond 53 days. Additionally, histology of post-biopsy tumors shows prolonged retention of a metastasis-permissive wound stroma dominated by M2-like macrophages capable of promoting cancer cell epithelial-to-mesenchymal transition and angiogenesis by secretion of transforming growth factor beta 1 (TGF-β1) and vascular endothelial growth factor (VEGF). We show that needle biopsy promotes systemic dissemination of cancer cells through a mechanism of sustained activation of the cyclooxygenase-2 (COX-2)/prostaglandin E2 (PGE2) cascade, which favors M2-polarization and its associated pro-metastatic changes, but are abrogated by oral treatment with COX-2 inhibitors in estrogen receptor-positive (ER+) syngeneic mouse tumor models.
Therefore, we conclude that needle biopsy of ER+ breast cancer provokes progressive pro-metastatic changes, which may explain the mortality risk posed by surgery delay after diagnosis. Also, our data may provide a rationale for a pharmacologic strategy to inhibit the COX-2/PGE2 cascade in cases when delay of surgery is unavoidable.

P3-06-23: Determining organotropic drivers of metastasis in a new genetically engineered mouse model

Eran Andrechek

The majority of transgenic and knockout / knock-in mouse models that develop mammary tumors also develop pulmonary metastasis. However, human breast cancer metastasizes to a variety of distal sites other than the lungs. It is unclear why mouse models are limited in this regard and there is a clear need for models that better reflect the human disease. Using a bioinformatic approach, we have predicted that the E2F transcription factor family is critical in mediating metastasis, with a particular role noted for E2F5. Testing an E2F5 gene signature, we noted that alterations to E2F5 were associated with lymph node metastasis in human breast cancer. In order to investigate the role of E2F5 in breast cancer, we proposed to interbreed the tumor strains with knockouts of E2F5. However, the E2F5 knockouts suffer from early lethality and we thus generated an E2F5 conditional knockout using MMTV-Cre to ablate E2F5 in the mammary epithelium.
The E2F5 conditional knockout mice have developmental abnormalities, including pubertal development delays and pregnancy proliferation delays. However, after a long latency these mice also develop mammary tumors with no other initiating oncogenic events. Indeed, we were surprised that loss of E2F5 alone in the mammary epithelium was sufficient to induce tumor formation. The tumors that developed in these mice were also highly metastatic to the lungs and importantly in other tissues, including the lymph nodes and livers of mice at endpoint. Given the long latency of the strain, all tumors and metastases were viably frozen. Reimplanting these tumors into syngeneic hosts allowed us to examine the tumors that metastasized to lymph node and liver. Repeated rounds of transplantation of metastases back to the mammary gland resulted in tumors that were enriched for their ability to metastasize to specific distal sites.
The role of particular genetic programs in these distal metastases was assessed through whole genome sequencing and through RNAseq analysis. Initially we noted differences in key pathways including cyclin D1 in these tumors. Studies into the role of specific pathways for the various organotropic lines are ongoing.

P3-06-24: Characterization of a Living Biobank of Patient-Derived Invasive Lobular Carcinoma Organoids that Retain Tumor Heterogeneity in 3D Culture and Tumorigenicity In Vivo.

Minakshi Gandhi, Melissa Kramer, Jill Habel, Zihua Wang, Raditya Utama, Suzanne Russo, Payal Naik, Karen Kostroff, David L Spector

Breast cancer (BC) is one of the primary causes of cancer related deaths among women worldwide. According to Cancer Statistics, over 310,720 new breast cancer diagnoses are expected in the United States in 2024 with an estimate of over 42,250 expected BC related deaths. Invasive Lobular Carcinoma (ILC) accounts for the 2nd most frequently diagnosed histological subtype of BC, constituting 10%-15% of all reported cases. ILC is hormonally driven (estrogen and progesterone receptors) with a hallmark characteristic of ILC being lack of E-cadherin (CDH1) and with mutations in the CDH1 gene reported in over 90% of cases. Mutations in the CDH1 gene have also been associated with 39%–52% increased lifetime risk of developing breast cancer in women. Patient-derived organoid (PDO) models

have been shown to offer significant advantages in terms of better recapitulating the tumor/microenvironment niche, however such models have not been available to study ILC.
With a vision to develop 3D models to better understand ILC and develop specific therapeutic targets, here, we describe the establishment and comprehensive characterization of a diverse biobank of ILC PDOs. We have performed in-depth genomic, transcriptomic and histologic profiling of the established ILC PDOs and validated the models to be true representatives of ILC tumors. Comprehensive genomic profiling of ILC PDOs using a custom 143 cancer driver gene panel (targeted exome sequencing) identified ILC specific mutations in CDH1, PIK3CA, TBX3, RUNX, etc. in PDOs. Short Multiply Aggregated Sequence Homologies (SMASH) identified widely reported copy number gains and losses (gains on Chr1q and losses on Chr16q and Chr17p among others) in established ILC PDOs. Transcriptomic profiling performed on matched tumor-normal pairs revealed the upregulation of ESR1 signalling-related pathways known to drive ILC carcinogenesis. Multiplexed single-cell RNA sequencing revealed that the established PDOs retained their cellular complexity and heterogeneity even after multiple passages and contained hormone responsive, hormone secretory, basal, fibroblasts, and proliferating populations of cells representing major cell types in ILC tumors. Lastly, we also show that the established ILC PDOs when xenografted (either in mammary glands or intra-ductally) in NOD-SCID gamma mice retain their tumorigenicity in vivo and the single file growth pattern, a characteristic of invasiveness in ILC tumors. In summary, we have established a living biobank of ILC PDOs often with matched normals (adjacent and/or distal) that are representative of different subtypes of ILC (classic, pleomorphic, tubule-lobular, mixed, etc.). Overall, this study has optimized a pipeline for establishment, culturing and validation of ILC PDOs as robust models available for studying ILC biology with a focus on basic, translational, and personalized medicine.

P3-06-25: Humanized patient-derived xenograft models of triple-negative breast cancer for analyzing the safety and effectiveness of mRNA lipid nanoparticle cancer vaccines

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Background. Triple-negative breast cancer (TNBC) has the highest rates of recurrence and relapse compared with other breast cancer subtypes. Chemotherapy alone, or in combination with immune checkpoint blockade is the current standard of care. However, many patients still do not benefit from these treatments. While T-cell targeting of tumor neoantigens through mRNA lipid nanoparticle (mRNA-LNP) cancer vaccines has become a truly personalized tool to elicit effective antitumor activity, more predictive preclinical models are needed to drive the rational design of cancer vaccine candidates and minimize failures in future clinical trials. Here we established a humanized patient-derived xenograft

(hu-PDX) mouse model of TNBC that recapitulates interactions between human immune cells and tumors to investigate the effectiveness and safety profile of mRNA-LNP cancer vaccines in vivo.
Methods. Tumor neoantigens were identified in the TNBC PDX model MC1 using a proprietary algorithm that prioritizes vaccine candidates on their likelihood to induce neoantigen-specific T-cell responses. mRNA encoding 20 selected HLA-A2-restricted neoantigens (Neo20 cancer vaccine) was synthesized and encapsulated into LNPs for in vivo administration. To assess the Neo20 cancer vaccine in vivo, we developed a hu-PDX mouse model of TNBC expressing human leukocyte antigen (HLA) class I. Briefly, human immunity was reconstituted in female HLA-A2 transgenic NSG mice through I.V. transplantation of human CD34+ hematopoietic stem cells from HLA-A2+ cord blood donors. Mice showing efficient human CD45+ (hCD45+) cell engraftment were implanted with MC1 tumors into the mammary fat pad. Once the tumors were established, MC1-hu-PDX mice received Neo20 mRNA-LNPs (10 μg mRNA/mouse) or empty LNPs as a control by intramuscular injection. Animals were treated every three days for one week and evaluated for vaccine immunogenicity and tumor growth. To analyze neoantigen-specific T cell responses, splenocytes were harvested 7 days after the last dose and pulsed with vaccine peptides for human interferon (IFN)-γ ELISpot assay. To examine the side effects of the Neo20 vaccine, major organs were collected for histopathological analysis and colorimetric detection kits were used to quantitatively measure biochemical markers [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and blood urea nitrogen (BUN)] in serum samples.
Results. Flow cytometry analysis of hCD45+ subpopulations of cells confirmed human reconstitution with T-cells, B-cells, myeloid cells/macrophages, and NK cells in blood, spleen, and bone marrow collected from MC1-hu-PDX mice. Two out of five mice treated with Neo20 vaccine generated functional human T-cell subsets with HLA-A2-restricted immune responses against multiple vaccine peptides as measured by ex vivo IFN-γ production. We observed a small but not significant change in tumor size between the Neo20 vaccine and control group after one week of treatment, suggesting that our treatment protocol should be further optimized to improve neoantigen-specific T cells and associated anti-tumor responses. Hematoxylin and eosin staining of tissue sections showed no histological differences between controls and Neo20 mRNA-LNP-treated mice, indicating no notable toxicity. ALT/AST and BUN levels were calculated to evaluate liver function and kidney activity, respectively. We found no obvious changes in these parameters in serum from mice after treatment with the Neo20 mRNA-LNPs, confirming negligible side effects.
Conclusions. This study provides evidence associated with mRNA-LNP cancer vaccines against TNBC supporting the use of hu-PDX mice as a model to preclinically validate early vaccine candidates, mRNA doses, and treatment schedules.

P3-06-26: p53 Haploinsufficiency Epistatically Masks A3B Tumor Phenotype in vivo

Joshua Proehl, Cameron Durfee, Yuan Zhao, Nuri Alpay Temiz, Reuben Harris

The single-stranded DNA cytosine deaminase enzyme, APOBEC3B (A3B), has been implicated as a mutational driver in multiple human cancers1,2. Breast cancer in particular shows high levels of A3B expression and positive associations with poor clinical outcomes3. Recent studies have also demonstrated that human A3B expression in mice is carcinogenic with significantly shortened life expectancies4. The only genetic factor in breast cancer thus far associated with A3B expression and APOBEC signature single base substitutions is p53 mutation5.
Therefore, here we test the hypothesis in vivo that human A3B expression will show pathlogical synergy in the form of accelerated mammary tumor penetrance upon combination with p53 haploinsufficiency due to a heterozygous deletion mutation spanning exons 2-10. Custom and commercially available animals were subjected to standard breeding practices to generate experimental (A3B, p53Delta exons 2-10/+) and control groups (p53Deltanexons 2-10/+). Surprisingly, in contrast to A3B-accelerated tumor development on a wildtype genetic background, A3B had no effect on the rates of tumor development in p53 heterozygous animals (median 13.5 months). The observed genetic epistasis was not due to a lack of A3B expression or activity, as evidenced by strong IHC positivity and a clear acculumlation of APOBEC signature single base substitution mutation in tumors. We conclude that A3B-accelerated tumor development requires full p53 function, and that a haploinsufficiency in p53 enables tumor cells (or their precursors) to better tolerate DNA damage lesions induced by A3B.

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P3-06-27: Immunotherapy and PI3K/mTOR inhibition combination to mediate metastasis and immunotherapy resistance in triple-negative breast cancer

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Background: Triple negative breast cancer (TNBC) tends to be highly aggressive, and almost 50% of patients develop distant metastasis to the brain, liver, and/or lung, at which point the median survival time is only 8-13 months. The average time to relapse in TNBC patients

is 19-40 months and the mortality rate within 3 months after recurrence is 75%. Although the addition of immunotherapy to neoadjuvant chemotherapy has improved the survival of patients with stage II–III TNBC, drug resistance remains a problem. Hence there is an urgent need to develop new therapy regimens and targets.
Methods: Extensive preclinical program initially starting with in vitro breast cancer cell lines (MDA-MB-231 and MCF-7) to evaluate various PI3K and PI3K-mTOR inhibitors using WST-1 cell proliferation assays, cell migration assays, qRT-PCR and immunofluorescence analysis.
In vivo studies utilised the highly aggressive, immunotherapy resistant, TNBC mouse model, 4T1. Briefly, six-to-eight-week-old female BALB/c mice were inoculated with 1 x 105 4T1 cells administered into the mammary fat pad. Once tumours reached ~50-100 mm3, the PI3K-mTOR inhibitor, Paxalisib, was administered daily by oral gavage in combination with either anti-PD-1 (pembrolizumab) treatment or PARP inhibitor (olaparib). Extensive pathology, nanostring and CODEX spatial analysis was used to evaluate primary tumour burden, metastases and inflammation.
Results: Dual targeting of the PI3K-mTOR pathway but not PI3K alone inhibited cancer cell proliferation and migration and promoted a favorable mesenchymal-to-epithelial phenotype. In vitro, the PI3K-mTOR inhibitor, Paxalisib, reduced metastasis-initiating cell signatures including the highly aggressive CD44high/CD24low CSC phenotype, persister cancer cell phenotype (p65, FOXQ1, NRF2, NNMT), and drug resistance markers (ABCB5, SNAIL, ALDH1). PI3K-mTOR blockade also reduced NFκB p50 and the pro-inflammatory downstream target IL-6 whilst inducing viral mimicry genes, making cancer cells more immune visible. Dose de-escalation of paxalisib in the highly aggressive 4T1 TNBC mouse model substantially reduced primary tumor burden, lung metastases, and liver inflammation in combination with immunotherapy +/- chemotherapy whilst overcoming toxicity complications and resistance associated with standard-of-care treatment. Paxalisib in combination with the PARP inhibitor olaparib also reduced primary tumor burden and metastases compared with olaparib monotherapy. NanoString analysis of paxalisib +/- immunotherapy-treated tumors showed a marked reduction in “pro-tumorigenic” immune populations whilst CODEX spatial analysis revealed an increase in the infiltrating adaptive immune arm following paxalisib + anti-PD-1 treatment.
Conclusions: The addition of paxalisib to immunotherapy in the 4T1 TNBC mouse model reduced primary tumor burden, lung metastases, and liver inflammation whilst overcoming toxicity complications and resistance associated with standard-of-care immunochemotherapy. Paxalisib in combination with the PARP inhibitor olaparib, but not monotherapy alone, also reduced primary tumor burden and metastases. Moving forward, work is ongoing to elucidate the PI3K-mTOR mechanism of overcoming metastasis and drug resistance as well as the translation of the data into a clinical development program for patients with TNBC and advanced breast cancer.

P3-06-28: Neo-adjuvant administration of MDNA11, a long-acting IL-2 Superkine, prevents metastasis, protects against tumor rechallenges and provides long-term survival in an orthotopic model of breast cancer

Abdalla Sheikh, Nina Merchant, Aanchal Sharma, Fahar Merchant, Minh D. To

Background: MDNA11 is an albumin-fused 'beta-enhanced not-alpha' IL-2 superkine that preferentially expands and activates immune effector cells over immune suppressive Tregs. MDNA11 effectively inhibited subcutaneously implanted syngeneic tumors with complete tumor regression and a potent memory response. Mice treated with MDNA11 demonstrated increased tumor infiltration of CD8+ T and NK cells and elevated circulating memory and antigen-specific CD8+ T cells. Given these results, we hypothesize that preconditioning of immune cells against cancer cells by MDNA11 prior to tumor resection can prevent metastasis and prolong survival. This study compared the efficacy of MDNA11 in neo-adjuvant and adjuvant settings in an aggressive orthotopic breast cancer model prone to distant metastasis following tumor removal.
Method: Female Balb/c mice with 4T1.2 tumors implanted in mammary fat pads were treated with a single dose of MDNA11 prior to or following tumor resection and survival was monitored. Excised tumors are being analyzed for immune cell infiltration. Surviving mice underwent re-challenges by subcutaneous implanting 4T1.2 cells without any additional treatment. Necropsy was performed to identify gross metastasis.
Results: All control mice that had tumors resected but received no treatment died by study day 54 with gross metastasis in multiple organs. In the adjuvant group where mice received a single dose of MDNA11 following tumor resection, 3 of 8 (37.5%) survived to study end (study day 134). Of the 5 mice in the adjuvant group that died while on study, 4 had distant metastasis. In contrast, of the 8 mice that were administered a single dose of MDNA11 prior to tumor resection, 7 (87.5%) survived to study day 134 with no observable clinical symptoms. The single mouse that was found dead had no metastasis. Remaining mice in the neo-adjuvant (n = 7) and adjuvant (n = 3) were re-challenged at day 65 by implanting 4T1.2 cells subcutaneously in their flanks. A second re-challenge was performed on study day 98. Mice did not receive any additional treatment and on both re-challenge occasions there was no tumor growth or metastasis whereas control naïve mice that were also implanted with 4T1.2 cancer cells showed robust tumor growth at the implanted site.
Conclusion: A single dose of MDNA11 in a neo-adjuvant setting confers immediate and long-term protection from metastatic disease in the 4T1.2 orthotopic breast cancer model.

P3-06-29: Circulating tumor cell expansion from frozen PBMCs enables drug screening against metastatic tumor cells

Shian-Jiun Shih, Yi-chun Han, David Hsieh

Background: Despite metastasis representing the foremost cause of mortality in cancer patients, current oncology drug development primarily relies on cell lines or primary tumor-derived models, which may not fully represent the complexities of metastatic

disease. We aim to develop a drug screening platform to enable the identification of drug candidates capable of targeting metastatic tumors.
Materials and Methods: Using a patented cell culture system from AcroCyte (Taiwan), we selectively cultivate circulating tumor cells from the peripheral blood of cancer patients with solid tumors. This process employs either 5 milliliters of fresh whole blood or equivalent frozen PBMCs, obtained from commercial sources or collaborations with patient consent. The cultured patient samples demonstrate formation of circulating tumor cell colonies, which often grow as three-dimensional organoid-like spheres within several weeks. These colonies can be subsequently expanded for drug screening, banked as frozen cells for future use, and have been employed to evaluate drug efficacy by assessing cytotoxicity over the course of 7 days. Drug concentrations used in assessing cytotoxicity were selected based on available human pharmacokinetic Cmax data; in the absence of such data, we relied on prior findings from cell line screening and/or in vivo studies. These colonies undergo comprehensive multi-omics analysis, including immunostaining and transcriptome profiling, to further characterize their properties before and after drug treatment.
Results: We have successfully established colonies from circulating tumor cells (CTCs) derived from frozen peripheral blood mononuclear cells (PBMCs) of patients with breast cancer, as well as other solid tumors, including lung, colon, prostate, and melanoma. These colonies exhibit expression of EpCAM and cytokeratin, confirming their identity as CTCs. Notably, CTC colonies from breast cancer patients generally exhibit slower growth compared to those from other solid tumors, often forming only cell clusters rather than three-dimensional organoids. Additionally, we have cultivated primary cancer colonies from dissociated tumor cells for comparative molecular characterization and drug sensitivity analysis. Our findings indicate that both CTC and primary tumor colonies display diverse sensitivities to standard-of-care chemotherapy drugs, as demonstrated by alterations in cell viability and morphology following drug exposure.
Conclusions: We demonstrate a robust drug screening platform against metastatic tumors using circulating tumor cell-derived colonies for solid tumors. The ability to bank these colonies and perform drug sensitivity characterization and multi-omics analysis suggests its potential for identifying effective therapies for the treatment of metastatic cancer. Our results affirm the potential of CTC colonies in understanding tumor biology of metastatic cancer and their role in personalized cancer therapy, particularly for breast cancer.

P3-06-30: Novel Humanized LIV-1 Antibody Based ADCs Conjugated with Topoisomerase I Inhibitor Payloads Displayed Significantly Higher Anti-tumor Activities than MMAE based ADCs in TNBC Tumor Models

Fei Teng, Huanhuan Guo, Hongjun Li, Lizhi Qin, Hanjing Mao, Sonny Yao, Xiaoli Zi, Lisa Zheng, Yi Gu, Xueming Qian

LIV-1 is a member of the zinc transporter family and an estrogen-regulated gene in metastatic breast cancer. While normal tissue expression is limited, LIV-1 was found to be

overexpressed in a high prevalence in breast cancer (93%), as well as in melanoma (82%), prostate (72%), ovarian (48%) and uterine (30%) cancers. LIV-1 is considered as one of the attractive cell surface targets for developing ADC therapeutics. To develop next generation LIV1 targeting ADC, we generated 48D6, a proprietary novel humanized anti-LIV-1 mAb with high affinity, specificity, internalization ability, unique epitope and improved pharmacokinetics profile in mice. In vitro studies indicated that breast tumor cells, such as MDA-MB-468 and MCF-7, are more sensitive to Topo I inhibitor than MMAE. Therefore, we generated two Topo I inhibitor-based ADCs (ADC-1 and ADC-2) using glycotransferase mediated site-specific conjugation. Both ADC-1 and ADC-2 have a drug-to-antibody ratio (DAR) of 4 but with different payload: a modified Topo I inhibitor for ADC-1 and Exatecan for ADC-2. A MMAE based ADC (ADC-3) with the same site-specific conjugation and DAR4 was also synthesized as the control. ADC-1 and ADC-2 displayed similar and specific cytotoxic activities against human LIV-1-expressing tumor cells in vitro, as compared to SGN-LIV1A analog (DAR8) or ADC-3. In the human LIV-1 transfected MDA-MB-468, a triple-negative breast cancer (TNBC) tumor model, ADC-1 or ADC-2 demonstrated dose-dependent anti-tumor activities and inhibited tumor growth more potently than the SGN-LIV1A analog or ADC-3. At 3 mg/kg the tumor growth inhibition (TGI)% are: ADC-1 92.4%, ADC-2 94.7%, ADC-3 68.5% and SGN-LIV1A analog 57.0% on Day 30; At 3 mg/kg, the overall response rate (ORR, 50% reduction of tumor volume from baseline) of SGN-LIV1A analog or ADC-3 was 0%, while ORRs of ADC-1 and ADC-2 were 40% and 70%, respectively. At 6 mg/kg, ADC-1 and ADC-2 had ORR of 100% and CR rate of 90% and100% on Day 29 respectively. And the body weight of mice didn't change significantly at either 3 or 6 mg/kg for ADC-1 or ADC-2. The enhanced anti-tumor activities of ADC-1 and ADC-2 are likely contributed by the high affinity binding of 48D6 to LIV-1 and high cytotoxicity of Topo I inhibitor in breast tumor cells. These data warrant further investigation of the lead LIV-1 targeting ADC (ADC-1 and ADC-2) as potential next-generation therapeutic agent in LIV-1 positive breast cancer and other solid tumors.

P3-07-01: Comparative effectiveness and safety of initial operation in inoperable locally advanced breast cancer—a real-world retrospective cohort study in China

Yan Lin, Bowen Liu, Ying Xu, Xin Huang, Yidong Zhou, Qiang Sun, Yu Song

Purpose: There are sparse data on the effect of initial operation for patients with locally advanced breast cancer (LABC), and even fewer data for inoperable LABC. This real-world cohort study aimed to present long-term outcomes of initial operation in women with inoperable LABC in China.
Methods: This is a retrospective cohort study of patients with LABC (stage III) who were treated from January 2017 to December 2018. Among them, inoperable patients (stage III, excepting T3N1M0) with limited locoregional involvement were included into our comparative survival analysis. All enrolled inoperable LABC were then divided into upfront surgery group and upfront chemotherapy group. Upfront surgery patients were matched

(2:1) to upfront chemotherapy patients with similar subtypes, nodal status, tumor status, age, and chemotherapy regimens. The primary objective was disease free survival (DFS) and overall survival (OS) in each group. Pathologic complete response (PCR) was secondary objective. Subgroup analyses included DFS and OS according to age, tumor status, nodal status and subtypes.

Results: Between Jan 1, 2017, and Dec 31, 2018, of the 3843 patients with breast cancer who were newly treated, 565(14.7%) patients presented with LABC. From them, 168 inoperable LABC patients were included into final analysis (112 for surgery-first group and 56 for neoadjuvant group). At data cut-off of Dec 1, 2023, median follow-up was 60.9 months (range, 40.0-85.1 months). No difference in OS was observed between surgery-first group and neoadjuvant group (hazard ratio [HR]=0.47; 95% CI, 0.19 to 1.17, P=0.11) and so do in DFS (HR=0.78; 95% CI, 0.42 to 1.45, P=0.43). The corresponding 5-year OS was 89.6% in the surgery-first group and 81.9% in the neoadjuvant group. The 5-year DFS was 73.0 % for surgery-first group and 67.1% for neoadjuvant group. Similarly, no significant difference in locoregional (7.9% versus 11.9%) and distant (22.1% versus 21.6%) recurrence were observed between patients who received primary surgery and their matched group. Multivariate analysis showed that patients with stage IIIA (HR, 0.22; CI, 0.06 to 0.86) and younger than 60 years old (HR, 0.32; CI, 0.11 to 0.96) had increased overall survival from initial surgery.
Conclusion: Initial operation was feasible in inoperable LABC with limited locoregional involvement and gave satisfactory long-term survival. However, this report acknowledges the limitations inherent in experience of management of LABC from a single center.

P3-07-02: Sentinel node biopsy alone versus sentinel node biopsy plus axillary dissection in cT2 cN0/1 breast cancer patients after neoadjuvant chemotherapy: 15 year results of a prospective interventional trial

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Background: There is no consensus on axillary management after neoadjuvant chemotherapy (NAC) in patients with clinically node positive breast cancer downstaged to pN0 after NAC. We investigated whether sentinel node biopsy (SNB) alone is adequate axillary treatment if the sentinel nodes (SNs) are clear (pN0) in cN0/N1 breast cancer patients given SNB after NAC.
Methods: From January 2007 to December 2023 we recruited 486 cT2 cN0/N1 breast cancer patients, median age 48 years (IQR 42-57), scheduled for NAC, to a prospective interventional study (NCT04436809). If the SNs were pN0 after NAC, patients usually received no further axillary treatment (SNB only); if the SNs were pN1, axillary dissection (AD) was usually performed. Primary outcomes were overall (OS) and disease-free survival (DFS) in SNB only versus SNB + AD patients, assessed by weighted Kaplan-Meier and log-

rank test, after estimating propensity scores to account for bias due to non-random assignment to SNB versus SNB + AD.
Results: Median follow-up was 102 months (IQR 59-162 months) in the SNB only group and 200 months (IQR 168-213 months) in the SNB + AD group. OS and DFS did not differ significantly between the groups by propensity score weighted comparison: 15-year OS: 89.4% (95% CI: 81797.8%) in SNB only patients versus 77.8% (95%CI: 66.6-91.0%) in SNB + AD patients (p=0.241); 15-year DFS: 75.1% (95% CI: 65.3-86.5) versus 67.6% (95% CI: 55.0-83.0%; p=0.604), respectively. Of 177 patients cN1 downstaged to SN pN0, 132 had SNB only. None of the 132 SNB-only patients developed axillary relapse after a median follow up of 100 months (IQR 56-166 months).
Conclusion: This prospective interventional study shows that, in cT2 CN0/1 breast cancer patients whose SNs are pN0 after primary chemotherapy, SNB can be offered with no further axillary treatment given if the SNs are negative, irrespective of axillary status beforehand, without affecting OS or DFS.

P3-07-03: Real-world comparison of safety and effectiveness of dose dense (2-weekly) versus weekly paclitaxel given with trastuzumab as neoadjuvant treatment in HER2-positive early stage breast cancer.

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Background: Traditionally, trastuzumab-based chemotherapy combinations used in HER2-positive early-stage breast cancer are delivered as 21-day cycles, either comprising weekly paclitaxel or 3-weekly docetaxel). In Ontario, Canada, an alternative dose-dense protocol allows the administration of 4 cycles of doxorubicin and cyclophosphamide (AC) followed by 2-weekly paclitaxel concurrently with trastuzumab over 4 cycles. Little is known about the effectiveness or safety of this schedule.

Methods: A single institution, retrospective chart review of patients with HER2-positive early-stage breast cancer treated with neoadjuvant AC-Paclitaxel chemotherapy between the years 2015-2019 was performed. Comparative effectiveness of AC followed by 2-weekly (dose dense) or weekly paclitaxel regimens was assessed as the proportion of patients achieving pathologic complete response (pCR). Safety was assessed as the proportion of patients completing the course of treatment and of patients with cardiac toxicity defined by the ESC guidelines. Differences between regimens were evaluated as the test of 2 proportions (Z-test). Statistical significance was defined as $p<0.05$.

Results: The initial analysis comprised 70 patients; 45 treated with 4 doses of paclitaxel 175mg/m2 every 2 weeks and 25 treated with 12 doses of paclitaxel 80mg/m2 weekly. Trastuzumab was given as an 8mg/kg loading dose and 6mg/kg maintenance dose, the latter either every 2 weeks or every 3 weeks based on paclitaxel schedule. The median age in the 2-weeks regimen was 54 years (30-77) and 55 years (35-71) in the weekly treatment.

The 2-weekly regimen demonstrated a pCR proportion of 68% (31/45), while for weekly treatment, pCR was observed in 40% (10/25). This difference was statistically significant (p=0.023). Treatment completion rates were 100% (45/45) for the 2-weekly group and 64% (16/25) for the weekly group (p<0.001). There were no differences in the cardiotoxicity profiles between the protocols (p=0.68). The 2-weekly regimen resulted in cardiotoxicity in 6% (3/45) of patients, moderate cardiotoxicity in 2% (1/45), and no cases of severe cardiotoxicity. The weekly regimen reported mild cardiotoxicity in 4% (1/25) of patients, no moderate cardiotoxicity, and severe cardiotoxicity in 8% (2/25).

Conclusion: In this retrospective study, both completion rates and pathologic complete response (pCR) rates were more favourable when AC was followed by 2-weekly paclitaxel/trastuzumab regimen compared to weekly paclitaxel/3-weekly trastuzumab. There was no difference between cardiac toxicity between the two schedules. An expanded dataset as well as an analysis of healthcare delivery costs, will be reported at the meeting.

P3-07-04: Postoperative upper Limb Edema & Dysfunction Generated by axillary nodes Excision w/ or w/out axillary vein branches reservation in breast cancer patients (PLEDGE-Surgery):a prospective,multiple-center,double-blinded, randomized controlled study

Xiangyun Zong, Yang Yu, Hongjian Yang, Xue Peng, Fen Tang, Wei Zhang, Ziwei Sun, Junhong Zhou, Fang Cheng, Kewei Sun, Qi Shao, Weiyun Pan, Yurong Zheng, Jiejie Hu, Chengdong Qin, Qianrui Xu

Background: Breast cancer-related lymphedema (BCRL) is a common complication after breast cancer treatment. The purpose of the study is to compare the effects of axillary lymph node dissection with or without axillary vein branches reservation on the incidence of postoperative upper limb edema and dysfunction in breast cancer patients.

Methods: The study was designed as a multicenter, prospective, double-blind, randomized, controlled clinical study, which was carried out in the following institutions: Shanghai Sixth People's Hospital, Shanghai Fengxian Central Hospital, and Zhejiang Cancer Hospital in China. Patients aged 18-69 years with breast cancer who were confirmed by histopathology and required axillary lymph node dissections before surgery were enrolled. Eligible patients were assigned to two groups to receive radical breast cancer surgery including axillary dissection by central stratified block randomization. The randomization table was generated by an independent statistician, and the random number was printed by the center. The allocation ratio was set at 1:2 in the study group and the control group. Participants, data collectors and data analysts were blinded, while surgeons will not be blinded in this study. In the control group, the axillary vein branches were routinely cut off during axillary dissection, while in the study group, they were preserved as much as possible. The primary endpoint of the clinical trial was the incidence of short-term(1-

month, 6-month and 12-month) upper limb lymphedema and dysfunction on the affected side after surgery. The secondary end points were tumor-free survival and the incidence of upper limb lymphedema and dysfunction at 5 years postoperatively. Data collection and initial analysis were conducted 6 months after all cases were enrolled and underwent planned surgical treatment. All intent-to-treat patients were included in the final follow-up and analysis. The trial was registered at ClinicalTrials.gov(NCT05120180) on January 3, 2022, closed to new participants on April 1, 2024.

Findings: From January 3rd, 2022 to April 1st, 2024, a total of 316 female breast cancer patients were recruited, 22 of whom were excluded because they did not meet the inclusion conditions. The remaining 294 patients received randomization, of which 36 patients voluntarily withdrew or did not continue to participate in the study plan due to meeting exclusion criteria. Finally, 258 patients completed randomization and received all planned treatments, including 87 in the intervention group and 171 in the control group. The average age of the patients was 52.81 ± 10.063 years, with a median age of 53 (47, 59) years. The physical state scores are all 0-1. One month after surgery, the incidence of upper limb edema in the intervention group and control group was 1/85 (1.18%) and 2/144 (1.39%), respectively, with no significant statistical difference(ARR 0.21%, Fisher's exact test, P>0.05, OR 0.845, 95% CI [0.075, 9.463]). The RR of upper limb edema in the intervention group was 0.897(95% CI [0.179, 4.483]), while the RR in the control group was 1.061(95% CI [0.474, 2.377]). Six months after surgery, the incidence of upper limb edema in the intervention group was significantly lower than that in the control group(0/59 (0%) vs. 11/102 (10.78%), ARR 10.78%, Fisher's exact, P=0.007, OR was not available). The RR of upper limb edema in the control group was 1.648(95% CI [1.449, 1.875]). At 12 months after surgery, the incidence of upper limb edema in the intervention group was significantly lower than that in the control group(1/42 (2.38%) vs. 11/68 (16.18%), ARR 13.8%, Fisher's exact, P=0.028, OR 0.126, 95% CI [0.016, 1.018]). The RR of upper limb edema in the intervention group was 0.199(95% CI [0.030, 1.320]); The RR in the control group was 1.576(95% CI [1.241, 2.002]). There were significant differences in upper limb dysfunction reported by patients and scored by researchers between the intervention group and the control group (DASH scores at 1, 6, and 12 months were 8.02 ± 2.983, 3.56± 2.611, 1.65± 1.881 in intervention group and 10.67 ± 3.756, 6.02± 3.422, 2.98± 2.439 in control group, respectively, analyzed using a general linear model). The DASH scores of each group decreased over time (Greenhouse Geisser, F=334.133， P=0.000) with similar trends(Greenhouse Geisser, F=0.954， P=0.371); The mean DASH scores of the intervention group were significantly lower than that of the control group (F=10.167, P=0.002). Both groups of patients did not experience any serious complications or deaths related to surgery. There were significant differences in the proportion of postoperative arm circumference changes between the intervention group and the control group (using a general linear model analysis, the proportion of arm circumference changes in the intervention group at 1, 6, and 12 months were 0.02 ± 0.022, 0.02 ± 0.019, and 0.02 ± 0.036, respectively, while the control group was 0.02 ± 0.028, 0.04 ± 0.041, and 0.04 ± 0.044). The

proportion of arm circumference increase in each group changed over time (Greenhouse Geisser, F=4.386, P=0.029) and the trend of change was different (Greenhouse Geisser, F=5.698, P=0.012）；The average increase in arm circumference in the intervention group was significantly lower than that in the control group (F=7.101, P=0.009).During the follow-up process, there were 2 deaths (2.30%) in the intervention group, who died from severe bone marrow suppression after chemotherapy (1/87, 1.15%) and fulminant hepatitis (1/87, 1.15%), respectively.

Interpretation: Lymphectomy with preservation of axillary vein branches is beneficial to reduce the short-term incidence of postoperative upper limb edema and upper limb dysfunction, which is worth promoting in breast cancer patients who need axillary lymph node dissection.

Keywords
Breast cancer, Lymphedema, upper Limb Dysfunction, axillary nodes Excision, axillary vein branches reservation
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P3-07-05: An Application of the Air Inflation Adjustment Technique in Reverseâ€‘Sequence Endoscopic Nippleâ€‘Sparing Mastectomy with immediate breast reconstruction : A prospective cohort study

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Background: One of the leading causes of suboptimal aesthetic outcomes following breast implant reconstruction is implant malposition, which is mainly caused by improper placement and fixation of the implant, pectoralis major muscle contraction after surgery, and the distribution of the skin and nipple-areola complex is not aligned with the position of the implant. Secondary or multiple surgeries are often required to correct the appearance of defects. Our center developed the air inflation adjustment technique (AIAT) by inflating air into the implant envelope with the patient in an upright position postoperatively to make the implant naturally align with the contralateral breast under gravitational influence, enhancing aesthetic outcomes. This prospective study aimed to evaluate the efficacy and safety of AIAT by comparing complications and aesthetic outcomes between patients who underwent AIAT or not (N-AIAT) after reverse-sequence endoscopic nipple-sparing mastectomy (R-E-NSM) with immediate breast reconstruction (IBR).
Methods: In this prospective, single-center, non-randomized study, we consecutively recruited women aged 18 years or older who underwent R-E-NSM with IBR at the Breast Center, West China Hospital, Sichuan University, between September 2020 and June 2023. Patients were divided into the N-AIAT and AIAT groups and were comprehensively

compared to surgical complications and early cosmetic outcomes. We conducted a prespecified risk-factor analysis using mixed-effect regression to investigate the relationship between surgery type and incidence of complication.
Results: A total of 323 patients were prospectively enrolled in the study, with 168 (52.0%) in the N-AIAT group and 155 (48.0%) in the AIAT group. Compared to the N-AIAT group, patients in the AIAT group demonstrated a shorter hospital stay, reduced costs, more significant breast ptosis, and a shorter median follow-up period ($P < 0.05$). After adjusting for clinical covariates, the AIAT group exhibited a significantly lower rate of deterioration in Ueda scores at 1 month (0.235 [95% CI, 0.067-0.831]; $P = 0.025$) and at 12 months (0.414 [95% CI, 0.222-0.772]; $P = 0.006$) post-surgery, compared to the N-AIAT group. Meanwhile, in both univariate and multivariate analyses, no significant differences were observed in the incidence of complications over three periods: 12 months postoperatively, within the first month, and between one and twelve months. Subgroup multivariate analysis further indicated that AIAT is more suitable for patients undergoing prepectoral implant-based breast reconstruction, unilateral implant reconstruction, and those requiring chemotherapy and radiotherapy.
Conclusion: AIAT is a safe, effective, straightforward, and easily applicable non-surgical technique that can significantly enhance postoperative aesthetic outcomes and effectively reduce the need for secondary surgery due to implant malposition, especially suitable for patients undergoing prepectoral implant-based breast reconstruction, unilateral implant reconstruction, and those requiring chemotherapy and radiotherapy.

P3-07-06: Axillary Lymph Node Dissection or Not Among Women with Breast Cancer and Sentinel Lymph Node (SLN) Metastasis in Upfront Surgery: A Meta-Analysis of Randomized Trials

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Background: In women with early breast cancer, axillary lymph node dissection (ALND), axillary radiotherapy (AR), and sentinel lymph node biopsy (SLNB) yield similar survival rates. In 2024, with the publication of a new clinical trial and updated data from previously published studies, an opportunity arises to update and pool the results on overall survival (OS), disease-free survival (DFS), and axillary recurrence (AxR). We conducted a systematic review (SR) and meta-analysis to compare and update the summarized effects of ALND versus AR and SLNB alone in upfront surgery.
Methods: This systematic review, registered with PROSPERO (CRD42024585305) and adhering to PRISMA guidelines, was driven by the following research question: In randomized controlled trials (RCTs), what are the outcomes of OS, DFS, AxR, and lymphedema when comparing SLNB alone with ALND or AR in women with cN0 early-stage breast cancer and 1-2 positive SLNs? A comprehensive search of Medline, Embase, and the Cochrane Central Register, covering 2010 to July 2024, was conducted using indexed terms

such as 'breast cancer,' 'RCT,' 'axillary radiotherapy,' and 'SLNB,' without language restrictions. Articles were retrieved, and data were extracted by two independent reviewers and validated by three experienced breast surgeons. Random-effects meta-analyses were performed using the DerSimonian and Laird method to calculate pooled risk ratios (RR) with 95% confidence intervals (CI) for all outcomes. Heterogeneity was assessed using I^2 values.
Results: A total of 2,462 papers were initially identified. After thorough review, 14 articles from 7 RCTs were included in the final analysis, representing 7,338 women with a mean age of 57 years, enrolled between 1999 and 2020, with follow-up periods ranging from 2.8 to 10 years. The 10-year OS did not differ significantly across RCTs, with a pooled RR of 1.02 (95% CI 0.98 to 1.06). Similarly, the 10-year DFS was comparable between the SLNB and ALND groups (RR = 1.0, 95% CI 0.95 to 1.06), as was regional recurrence (RR = 2.18, 95% CI 0.98 to 4.84). Lymphedema was significantly less frequent in the SLNB arm compared to the ALND arm (RR = 0.35, 95% CI 0.2 to 0.6).
Conclusions: This systematic review, including updated RCT data, demonstrates that SLNB, AR, and ALND provide comparable efficacy in reginal recurrence and survival outcomes. However, SLNB or AR is associated with a 65% lower risk of lymphedema compared to ALND, indicating that a more conservative approach could improve quality of life without compromising long-term survival or DFS in women with early breast cancer.

P3-07-07: 10-Year Local Recurrence Rates Following Selective Omission of Re-excision for Patients with Ductal Carcinoma in Situ and Margins

Cecily Stockley, Shiva Bahmanyar, Yuan Xu, Jeffrey Cao, May Lynn Quan, Alison Laws

Background: Current guidelines recommend ≥2mm margins for ductal carcinoma in situ (DCIS) with or without microinvasion to optimize local control following breast conserving surgery (BCS). There is some evidence to suggest it is acceptable to omit re-excision for "close" margins (i.e. not on-ink but <2mm), particularly when radiation therapy (RT) is planned. This study evaluates 10-year local recurrence (LR) rates following selective omission of re-excision in patients with DCIS and margins <2mm.

Methods: We conducted a retrospective population-based cohort study on patients undergoing BCS for DCIS with or without microinvasion from 2010-2014 in the province of Alberta. Final margin status was categorized as on-ink, <1mm, 1-1.9mm and ≥2mm. Given small sample size with on-ink margins, these were included with the <1mm group for analysis. As per provincial guidelines, use of RT boost was recommended for margins <2mm. The primary outcome was local recurrence of in situ or invasive disease in the ipsilateral breast. We generated Kaplan Meier curves and performed Cox proportional hazards analysis to evaluate the effect of margin status on LR. We used an interaction test to determine if the effect of margin status differed by RT use.
Results: 468 patients underwent BCS for DCIS with a median age of 59 years (IQR: 50.5-66). The majority were unifocal (n=409, 87.4%) with pure DCIS without microinvasion (n=424,

90.6%), and 51.5% (n=241) had grade 3 disease. Hormone receptor testing was not routine, but among tested patients, 88.9% were estrogen receptor (ER)-positive. The re-excision rate was 23.9% (n=112). Final margin status was on-ink in 13 (2.8%) patients, <1mm in 39 (8.3%), 1-1.9mm in 37 (7.9%) and ≥2mm in 379 (81.0%). Among those 89 patients with margins <2mm, most (94.4%) had only a single margin location <2mm. In 36 (40.4%) patients, the only margin that was <2mm was in the anterior or posterior location, and thus re-excision may not have been feasible. The majority (n=390, 83.3%) had adjuvant RT, whereas adjuvant endocrine therapy (ET) use was infrequent (n=43, 9.2%).

The only clinical characteristic that differed for patients with <2mm vs. ≥2mm margins was proportion of multifocality (20.2 vs. 10.8%, p=0.02). Otherwise, there was no significant differences in age, grade, pure DCIS vs. microinvasion, ER status, RT use (82.9% vs. 83.8%) or ET use (all p>0.05), including when those with only anterior/posterior margins <2mm were excluded from the <2mm group.

Median follow-up was 10.9 years (IQR: 9.8-12.3). 10-year rates of LR by final margin width were as follows: 23.0% (95%CI: 13.1-38.6) for <1mm, 8.8% (95%CI: 2.9-24.9%) for 1-1.9mm and 5.9% (95%CI: 3.9-8.9%) for ≥2mm (log-rank p<0.001). In Cox proportional hazards analysis, margins <1mm were associated with significantly higher risk of LR compared to margins ≥2mm (HR 4.22, p<0.001), while margins 1-1.9mm were not (HR 1.35, p=0.62). Results were unchanged when adjusting for multifocality (adjusted HR for <1mm margins = 4.16, p<0.001; adjusted HR for 1-1.9mm margins = 1.25, p=0.72), and also when those with only anterior/posterior margins <2mm were excluded from the <2mm groups.

There was no statistically significant interaction between margin status and RT use (p=0.52), though absolute LR rates were numerically higher in patients without RT: 18.9% (RT) vs. 46.4% (no RT) for <1mm, 6.7% (RT) vs. 25.0% (no RT) for 1-1.9mm and 6.0% (RT) vs. 5.5% (no RT) for ≥2mm.

Conclusion: For patients undergoing BCS for DCIS with or without microinvasion, our findings support that re-excision can be selectively omitted for limited volume 1-1.9mm margins, as the 10-year LR rates are comparable to margins ≥2mm when followed by adjuvant RT. However, we advise caution against omission of re-excision for margins <1mm given significantly higher rates of LR regardless of RT use.

P3-07-08: Breast-Conserving Surgery in US Young Women With Early-Stage, HR-Positive/HER2-Negative Breast Cancer: National Trends and Oncologic Outcomes after Neoadjuvant Systemic Therapy

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Background: In the United States, the incidence of breast cancer for young women has been rising in recent years. Neoadjuvant chemotherapy (NACT) or neoadjuvant endocrine therapy (NET) is often used to downstage tumors allowing for breast-conserving surgery

(BCS) among patients with early-stage, HR-positive/HER-negative breast cancer clinically. However, there is a paucity of data on BCS trends and associated treatment response and long-term survival as compared to mastectomy in the young patient population nationally.
Methods: We analyzed real-world data from the 2010-2020 National Cancer Database. This retrospective study was restricted to female patients aged <40 years with stage I-III, HR-positive/HER-negative breast cancer and included 2 treatment cohorts: NACT and NET. Surgery type was classified as mastectomy and BCS. Pathologic complete response (pCR) was defined as ypT0/Tis ypN0. We fit log-binomial regression models to assess trends in BCS and percent change per year (95% CI). Overall survival was event or censored at the time of death from all causes or last known patient contact. Estimated 5-year and 10-year probabilities of overall survival with 95% CIs were generated and compared using the Kaplan-Meier method and the log-rank test stratified by surgery type for both treatment cohorts.
Results: Of 4,561 patients treated with NACT, 35.0% underwent BCS, with a significant increasing trend from 31.0% in 2010 to 41.7% in 2020 (P for trend < 0.001; percent increase per year 2.6 [95% CI: 1.3-4.0]). Of 956 patients treated with NET, 33.7% underwent BCS and there was a significant increasing trend from 28.1% in 2010 to 44.1% in 2020 (P for trend = 0.001; percent increase per year 6.0 [95% CI: 2.6-9.5]). Young patients who achieved a pCR after NACT were more likely to have undergone BCS than those who did not (42.7% vs 33.9%; P < 0.001). Similarly, young patients who achieved a pCR after NET also had a greater likelihood of having undergone BCS than those who did not (46.9% vs 32.7%; P = 0.021). In the NACT cohort, compared to patients who underwent a mastectomy, those who underwent BCS had longer 5-year overall survival (89.9% [95% CI: 87.7-91.6%] vs 82.8% [95% CI: 81.1-84.5%]) and 10-year overall survival (80.1% [95% CI: 75.5-83.8%] vs 65.9% [95% CI: 62.1-69.5%]) (P < 0.001). In the NET cohort, patients who underwent BCS also had longer 5-year overall survival (93.3% [95% CI: 87.7-96.4%] vs 85.3% [95% CI: 80.9-88.8%]) and 10-year overall survival (91.0% [95% CI: 84.2-94.9%] vs 71.5% [95% CI: 62.6-78.6%]) than those who underwent a mastectomy (P = 0.002).
Conclusions: In this US national sample of young women with early-stage, HR-positive/HER-negative breast cancer, BCS rates increased significantly from 2010 to 2020, and achieving a pCR was associated with higher rates of BCS in both NACT and NET cohorts. Regardless of neoadjuvant treatment modality, young patients who underwent BCS had better long-term overall survival than those who underwent a mastectomy. Our findings highlight the benefit of neoadjuvant systemic therapy for BCS eligibility in young patients who achieve a pCR and improved survival among those who underwent BCS, which suggests the need for strategies to address disparities in treatment response and mortality. Etiologies of these disparities in the young patient population also require further investigation.

P3-07-09: Cost Differences of Hospital Admission vs. Home Recovery following Mastectomy

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Introduction: Home recovery (HR) after mastectomy promotes faster recovery and may improve patient experience. We examined the cost differences after mastectomy, comparing same day mastectomy discharge to inpatient admission amoung commercially insured women.
Methods: Merative MarketScan Commercial and Medicare Databases (2017-2019) were used to identify women ≥18 years old who underwent mastectomy. Mastectomy encounters were classified as home recovery (stays spanning a single calendar date) or inpatient stay (stays >1 calendar day) based on the length of the visit. Total payments (from both patients and insurers) for the mastectomy encounter were calculated by summing all paid claims falling within the start and end dates of the encounter. Payments were also summed separately for professional versus facility claims, and by patient versus insurer payments. Median costs were calculated stratified by home recovery status, and calculated by various encounter characteristics, such as patient region and occurrence of simultaneous breast reconstruction. Medians were compared between home recovery groups using rank-sum tests.
Results: Of 11,508 patients undergoing mastectomy, 4,683 (40.7%) were HR patients while 6,825 (59.3%) underwent hospital admission. Mean age of HR patients was 53.6 yo while admission patients was 51.8 yo ($p<0.001$). The mean length of stay for the inpatient group was 2.3 days. Overall, median total costs were significantly lower among women who underwent HR when compared to hospital admission ($23,173 vs $33,901, $p<0.001$); facility costs also differed ($16,721 vs $23,539, $p<0.001$) as well as professional surgical cost ($6,441 vs $7,962, $p<0.001$). The median cost difference was also present with simultaneous non-autologous reconstruction ($35,739 vs $41,028, $p<0.001$). Geographical location also affected median surgical costs: Northeast ($29,327 vs $41,233, $p<0.001$), North Central ($18,739 vs $28,455, $p<0.001$), South ($11,911 vs $32,819, $p<0.001$), and West ($23,912 vs $36,790, $p<0.001$). HR after mastectomy remained less costly than hospital admission regardless of Medicare ($7,322 vs $18,072, $p<0.001$) vs commercial insurance ($26,269 vs $34,691, $p<0.001$). The median 1-year all healthcare cost is less in the outpatient mastectomy group compared to admission group ($34,748 vs $38,410, $p<0.001$).
Conclusion: Following mastectomy, Home Recovery is associated with significantly lower costs when compared to hospital admission regardless of geographical region, simultaneous reconstruction, and insurance coverage. With fewer costs, faster recovery, and improved patient satisfaction, home recovery after mastectomy is a clinically and financially non-inferior alternative to conventional inpatient admission for the appropriate patient population.

P3-07-10: Omitting axillary dissection in Triple-negative and HER2-overexpressed cancers After neoadjuvant Chemotherapy: OTHER-NAC Study

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Even though there is an increasing evidence of published reports that omitting axillary lymph node dissection (ALND) following sentinel lymph node biopsy (SLNB) in patients with clinically node positive breast cancer could be oncologically safe in selected patients, those especially with a good response to neoadjuvant chemotherapy, more data is needed for those patients with an agressive tumor biology with high recurrence risk. We therefore investigated the outcome in patients with triple negative or HER2-neu positive node positive breast cancer who were treated with SLNB alone without axillary dissection.
Material and Methods: Clinically node positive patients (cT1-4N1-3M0) with triple negative or HER2-positive breast cancer were included into the study from 9 centers. All patients were treated with SLNB alone without ALND after neoadjuvant chemoterapy (NAC) followed by regional nodal irradiation. The primary end points were the axillary, locoregional and systemic recurrence rates, and 5-year disease free (DFS) and disease specific survival (DSS) rates.
Results: Between 2010 and 2021, 259 patients underwent SLNB without ALND after NAC. The median age was 46 (range, 38-55). The majority of the cohort presented with cT1-2 (76.2%) and cN1 (81.5%) disease. Of those, 63.7% underwent breast-conserving therapy (BCT). The median lymph node number retrieved was 4 (IQR, 2-5). The pathologic complete response rate was 47.1%, whereas breast and axillary pCR rates were 49.8% and 78.4%, respectively. Of 56 ypN-positive patients, the lymph node metastases were macrometastases in 24 (42.9%), micrometastases in 21 (37.5%), and isolated tumor cells in 11 (19.6%). Of 259 cases, 88 (34%) had triple-negative breast cancer (TNBC), 106 (40.9%) had luminal HER2-positive, and 65 (25.1%) had non-luminal HER2-positive cancer. The median Ki67-level was 40 (IQR, 23-66). Patients with TNBC were more likely to have younger age <50 (68.2% vs 55%, p=0.04), BCT (72.7% vs 59.1, p=0.03), ypN0-disease (85.2% vs 74.9%, p=0.055), and increased Ki67 scores (median; TNBC: 60, IQR, 40-80, vs HER2+: 35, IQR, 20-55, p<0.001) compared to patients with HER2-positive tumors. As expected, patients with non-luminal cancers were more likely to have higher Ki67 scores (median; non-luminal: 50, IQR, 30-75, vs luminal: 30, IQR, 20-50, p<0.001), higher pCR (52.9 vs 38.7, p=0.024), and axillary pCR (86.3% vs 67.0%, p<0.001) rates compared to those with luminal tumors.
At a median follow-up time of 46 months (IQR, 34-63), the axillary, locoregional, and systemic recurrence rates were 0.8% (n=2), 2.7% (n=7), and 7.7% (n=20), respectively. Since the present cohort consisted of selected patients who had a clinically excellent axillary response to chemotherapy, the advanced cT-stage (cT3-4) was the only significant factor associated with decreased DFS and DSS (DFS: cT1-2, 92.1% vs cT3-4, 75.3%, p=0.002).
Conclusion: Omission of axillary dissection could be safely considered in clinically node-positive patients with an aggressive tumor pathology with low locoregional recurrence rates as long as a good response to neoadjuvant chemotherapy has been achieved and effective local therapies including adjuvant radiotherapy in addition to the systemic therapies have been provided.

P3-07-11: Avoidance of axillary lymph node dissection in patients with 1-2 pre-operative positive lymph nodes undergoing upfront surgery: 'Primary targeted axillary dissection' early outcomes

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Introduction: NCCN guidelines support safe omission of axillary lymph node dissection (ALND) in clinically node negative (cN0) patients with 1-2 positive sentinel lymph nodes (SLNs) if they meet the eligibility criteria of ACOSOG Z0011 and AMAROS trials. For patients undergoing neoadjuvant systemic anti-cancer therapy (SACT), targeted axillary dissection (TAD) is recognised as a highly accurate technique to spare patients ALND whose cN+ status converts to ypN0.
However, there is no consensus on the optimal management of patients with low volume cN+ disease undergoing upfront surgery with ASCO guidelines recommending ALND and NCCN guidelines supporting primary TAD. In this context, 'primary TAD' combines these principles to de-escalate axillary surgery in patients with ultrasound-detected abnormal or biopsy-proven 1-2 positive lymph nodes (cN1) that are clipped for removal in combination with SLN biopsy to optimise further axillary surgical decisions. This method evolved after biopsy-proven involved nodes were missed during planned ALND.
Aims
To evaluate the early outcomes of primary TAD in a single high volume tertiary cancer unit in the United Kingdom (UK).
Methods
A prospective database of the first 126 patients ≥ 18 years with cT1-2cN1 undergoing primary breast surgery either as breast conserving surgery or mastectomy with primary TAD between 30/08/2018 and 12/03/2024 at the Royal Marsden NHS Foundation Trust was evaluated. We define primary TAD as removal of 1-2 marked axillary nodes using Magseed, either biopsy-proven or suspicious on axillary ultrasound scan but indeterminate biopsy, combined with SLN mapping with the use of Tc99m / SPIO / Patent Blue V. The clipped node(s) were sent to histopathology (as already known to contain disease), and additional SLNs underwent intra-operative assessment using one step nucleic acid (OSNA). If a total of 3 positive nodes (including marked nodes + those with macrometastasis on OSNA) were confirmed, the patient underwent completion ALND in the same operative sitting. If only 1-2 nodes, total from marked nodes and OSNA, contained macrometastatic disease, the patient was spared ALND and offered regional nodal irradiation (RNI). Patient demographics, tumor characteristics, treatment details and outcomes including breast and/or axillary recurrence, distant recurrence and overall survival were assessed. Descriptive statistics were used to report results.
Results
A total of 125 women and 1 man underwent primary TAD. The median age at surgery was 60.5 years. The majority of tumors (109/126) were hormone positive (HR+) and HER2 negative (86.5%), with 78/126 of the luminal A subtype (61.9%) and cT2 with a median tumor size of 24mm (IQR 17-34.8). Pre-operatively, 24/126 (19%) had suspicious nodes

unproven on biopsy, 1 abnormal and proven axillary metastatic node was detected in 74/126 (58.7%), and 2 abnormal with 1 biopsy-proven node in 28/126 (22.3%). Clipped node(s) were successfully removed in 125 patients. A median of 3 non-Magseed SLNs were excised for OSNA, of which median 0 (IQR 0-1) were positive. Of 126 patients, 121 (96%) had axillary metastatic Magseed node(s), 97 (77%) avoided ALND, 27 (21.4%) underwent immediate ALND, 2 (1.6%) underwent delayed ALND post SACT. Out of 126, 65 (51.6%) patients underwent RNI. Median follow-up was 21.2 months (IQR 11.2- 35.8); 1 patient developed axillary recurrence requiring further axillary surgery, no patients developed breast recurrence, and 3 patients developed and died of distant metastasis.
Conclusion
Primary TAD is feasible with reassuring early oncological outcomes but long-term follow-up is required. This method can be utilised to optimise axillary surgery in suitable patients to safely facilitate omission of routine ALND.

P3-07-12: Breast-conserving surgery versus mastectomy in patients with locally advanced breast cancer.

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Objective: Contemporary treatment strategies for locally advanced breast cancer (LABC) involve a multimodal approach that combines systemic and local treatments. However, the role of breast-conserving surgery (BCS) in locally advanced breast cancer following NAT remains uncertain.
Materials and methods: This study was a retrospective cohort study of 874 consecutive patients with LABC who received NAC and underwent BCS or mastectomy from Jan 2000 to Dec 2020in N.N. Blokhin National Cancer Research Center. Of these, 179 patients (21%) underwent BCS, while 695 patients (79%) underwent mastectomy. Outcomes examined included overall survival(OS), disease-free survival (DFS), and loco-regional recurrence rates (LRRs). The variables were compared using log-rank statistics and Cox regression model.
Results: The 3-years DFS was 92,2% in the BCS group and 90,7% in the mastectomy group (p = 0,41). In univariate analysis age of patients, size of tumor, clinical T stage, tumor grade, pCR, triple-negative subtype were significant variables related to DFS and were included in the multivariable analysis, type of breast surgery was also included in the multivariable analysis as a candidate factor. Multivariable Cox regression analysis showed that pCR (OR 0,523; 95% Cl: 0,382-0,715; p<0,001), triple-negative disease (OR 2,299; 95% Cl: 1,543-3,427; p<0,001), clinical T stage (OR 0,987; 95% Cl: 0,962-1,009; p=0,004) were independent predictors of DFS, but type of breast surgery was not independent predictors (p = 0,391).
LRR rate was 5.8% (10/179) for BCS and 10.2% (71/695) for mastectomy (p=0,061, OR 0,567; 95% CI: 0,292-1,099). Univariate analysis showed that triple-negative subtype was associated with a worse LRR-prognosis (p=0,011). Multivariate analysis also showed an

independent negative effect of triple-negative subtype (OR 2,367; 95% CI: 1,154-5,134; p=0,023) and tumor grade (OR 1,32; 95% CI: 0,963-1,809; p=0,085).
The 5-years OS was 94,8% in the BCS group and 92,8% in the mastectomy group (p = 0.667). Univariate analysis showed that OS was related to age of patients, tumor grade, PR and ER status, clinical T stage, ki-67, molecular subtypes and pCR. Type of breast surgery was non-significant variables in univariate analysis, but also included in the multivariable analysis as a candidate factor. Multivariable Cox regression analysis showed that triple-negative disease (OR 3,581, 95% CI: 2,017-6,354; p<0,001) and pCR (HR 0,387, 95% CI: 0,253-0,593; p<0,001), but type of breast surgery was not independent predictors.
Propensity-score analysis was performed for 179 BCS and 358 mastectomy patients. In univariate analysis 3-year local relapse free survival was 97,3% (95% CI 95,7-99,1%) for BCS group and 98,8% (95% CI 97,2-100,0%) for mastectomy group. 3-year OS for BCS group was 97,2% (95% CI 95,5-98,9%) and 97,2 (95% CI 94,8-99,7%) for mastectomy group (p = 0,834).
Conclusion: Our findings suggest that BCS is oncologically safe in LABC patients. The recurrence and survival outcomes are comparable with BCS and mastectomy. BCS can be performed in selected patients with small tumor size and good response to NAT.

P3-07-13: Preoperative Predictors of Nodal pCR for Potential De-escalation of Axillary Surgery in Inflammatory Breast Cancer

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Background: Despite successful de-escalation of aggressive surgical management for invasive breast cancer, similar efforts in inflammatory breast cancer (IBC) have been unsuccessful thus far. We sought to identify predictors of nodal pCR in IBC patients for whom future clinical trials of de-escalation of surgery might be considered.
Methods: This was a retrospective analysis of stage III IBC patients enrolled in a prospective registry from 2007-2023 at University of Texas MD Anderson Cancer Center. All patients underwent mastectomy and axillary lymph node dissection. pCR was defined as no invasive residual carcinoma in the resected breast specimen and all sampled regional lymph nodes (ypT0/Tis ypN0 per AJCC staging). ER- and PR-positivity was defined by staining of ≥ 1% of tumor nuclei by immunohistochemical staining. Student's t-test and chi-squared test were used to compare group differences. Univariate logistic regression was performed to identify predictors of pCR and multivariate logistic classification and regression tree (CART) was performed to investigate joint effects on pCR status and determine optimal cut-off points for predictors.
Results: Of the 290 patients included, median age was 52.0 years (IQR 44-61) and BMI was 31.0 kg/m2 (IQR 26.7-34.8). Patients were predominantly White (227, 78.3%), followed by Hispanic (36, 12.4%), and Black (17, 5.9%). The majority of patients were postmenopausal (175, 60.3%) with node-positive (275, 94.8%), ductal (259, 89.9%), and high grade (197,

67.9%) disease. Receptor subtypes were 39.3% (114) HR+/HER2-, 24.8% (72) triple negative, 19.7% (57) HR-/HER2+, and 16.2% (47) HR+/HER2+. Distribution of HR expression consisted of 52.1% ER+ (52.1%, 151) with 9.3% (27) ER-low (1-10%) and 36.2% (105) PR+ with 10% (29) PR-low (1-10%). The majority of patients had Ki-67 ≥ 20% (177, 92.2%), with a median of 50.0% (IQR 30.0-75.0). Almost all patients received trimodality therapy – neoadjuvant systemic therapy (289, 99.7%), modified radical mastectomy (271, 93.4%), and adjuvant radiotherapy (283, 97.6%). Patients were more likely to achieve pCR if they were ER- (pCR 50% vs. 23%, $p < 0.0001$) with lower ER% (median 0% vs. 30%, $p < 0.0001$), PR- (pCR 46% vs. 19%, $p < 0.0001$) with lower PR% (median 0% vs. 0.5%, $p < 0.0001$), HER2+ (pCR 48% vs. 28%, $p = 0.001$), or had higher Ki-67 (median 60% vs 45%, $p = 0.003$). Four iterations of CART analysis were performed, the first two using ten predictor variables – BMI, race, menopause status, AJCC stage, nodal status, ER and PR (status or %), HER2 status, grade, and tumor histology, the third using Ki-67 alone, and the fourth using Ki-67 with ER and PR %. When HR status was considered as a dichotomous variable, ER- status was the strongest predictor of pCR, followed by non-White race, PR-, and HER2+ status. Patients who were ER- and non-White had the highest pCR rate of 73.5% while those who were both ER+/PR+ had the lowest pCR rate (16.0%). Repeat CART analysis including HR status as a continuous variable identified a cutoff point of ER < 41% as predictive of pCR (48.8% vs. 15.2% when ER ≥ 41%). Additional CART analysis of Ki-67 alone identified a cutoff point of ≥ 35.7% as predictive of pCR (43.0% vs. 16.4% when Ki-67 < 35.7%).
Conclusion: We identified six potential predictors of nodal pCR in stage III IBC using CART analysis – ER negative, PR negative, and HER2-positive status, ER < 41%, non-White race, and Ki-67 ≥ 35.7%. These factors could be used to select appropriate patients for future trials of de-escalation of axillary surgery in patients with IBC.

P3-07-14: Omitting axillary dissection in Triple-negative and HER2-overexpressed breast cancers with positive sentinel Lymph nOdes during upfront surgery: OTHELLO Study

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Background: Prospective trials including ACOSOG Z011 or SENOMAC have shown no survival benefit of completion axillary dissection in patients with clinically node negative early breast cancer in the presence of 1 or 2 positive sentinel lymph nodes (SLNs). However, these studies mostly included patients with hormone receptor-positive breast cancer, but limited number of patients with aggresive tumor biology. Therefore, we investigated the oncological safety of omitting axillary dissection in selected patients with HER2-positive or triple-negative breast cancer (TNBC).

Method: The present retrospective cohort study included patients with clinically node-negative breast cancer who were treated with sentinel lymph node biopsy (SLNB) alone in the presence of 1 or 2 positive nodes and triple-negative or HER2-positive breast cancer. Patients with clinically node-positive breast cancer, receiving neoadjuvant chemotherapy, with axillary dissection or a lymph node pathology without any metastasis, any systemic metastasis were excluded from the study. All patients received regional nodal irradiation.The primary end points were axillary and locoregional metastases and disease free (DFS) and disease specific survival (DSS).
Results: Between 2015 to 2020, 118 patients with triple negative- or HER2-positive breast cancer were included into the study from 8 centers. Median age was 53 (34-73). Of those, patients with cT1, cT2, or cT3 were 37.3% (n=44), 57.6% (n=68) and 5.1% (n=6), and 72% (n=85) underwent underwent breast conserving surgery. The majority of the patients had invasive ductal cancer (n=109, 92.4%), and grade 3 tumors (n=85, 72%). The pathological subgroups were triple-negative in 33% (n=39), and luminal HER2-positive in 51.7% (n=61), and non-luminal HER2-positive in 15.3% (n=18). The majority of the patients (n=98, 83.1%) had 1 metastatic lymph node in SLNB. The lymph node metastases were macrometastasis in 50% (n=59), micrometastasis in 36.4% (n=43), and isolated tumor cells in 13.6% (n=16). At a median follow-up of 53 months (34-73), the locoregional recurrence rate was 2.5% (n=3), and the systemic recurrence rate was 11.9% (n=14) without any axillary recurrence. The 5-year disease free and disease specific survival rates were 86.4% and 88.0%. In cox regression analysis, factors associated with increased hazard ratio (HR) for DFS were cT2-3 (HR=7.77,1.01-59.54), and TNBC (HR=3.87, 1.01-14.81). Similarly, presence of TNBC was the only significant factor associated with increased HR for DSS (HR=9.61, 95% CI, 1.11-83.24).
Conclusion: Patients with cN0 HER2-positive and TNBC disease treated with upfront SLNB-alone with 1-2 lymph node metastases have shown a favorable outcome with excellent local control with omitting ALND as long as regional nodal irradiation is provided.

P3-07-15: VARIATION IN PRACTICE AND PROVISION OF CONTRALATERAL SYMMETRISING MASTECTOMY AFTER UNILATERAL MASTECTOMY FOR BREAST CANCER: A UK NATIONAL PRACTICE SURVEY

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Background: Up to 40% of the 56,000 women diagnosed each year in the UK undergo mastectomy and NICE recommend that breast reconstruction is routinely offered to restore symmetry. While not all women want or are suitable for reconstruction, most desire symmetry. For these women, a contralateral mastectomy to achieve 'flat symmetry' is a good option. While breast reconstruction is routinely offered, however, contralateral symmetrising mastectomy (CSM) is not and lack of equity in service provision has been raised as a concern.
We aimed to survey current UK practice and provision of CSM as an alternative to breast reconstruction for women undergoing unilateral mastectomy for breast cancer as the first

step to improving equity of access for this group.
Methods: An online survey was co-developed with patient advocates based on the literature and expert opinion. The survey explored the existence and components of current pathways for women seeking CSM, any local policies regarding the offer of and funding for CSM, professionals involved in the process and the information/support provided. It was circulated between July 2023 and May 2024 via social media and UK professional breast surgery associations with one survey completed per unit. Simple descriptive statistics were used to summarise results and content analysis for free text.
Results: 51 units completed the survey, including both small and large volume units (median number of cancers treated per year 401-600) with good geographical spread across the UK. Most units (n=45, 88.2%) reported experience managing women requesting CSM and commonly (n=33, 64.7%) estimated receiving 10 or fewer CSM requests per year. Less than 10% (n=4) units reported routinely discussing CSM as an option with patients and most units (n=36, 70.6%) only discussed symmetrising mastectomy if it was specifically raised by the patient. Funding for CSM was routinely available in most units (n=39, 76.5%). Just over a quarter of responding units (n=14, 27.5%) reported having a formalised pathway for the management of women seeking CSM. These varied widely in their requirements and included a combination of discussion at multidisciplinary team meetings (n=12, 85.7%), referral to psychology services (n=7, 50%) and/or plastic surgery (n=10, 71.4%) and/or inclusion of a cooling off period (n=7, 50%), but most had been developed exclusively by breast surgeons with little multidisciplinary involvement. None had involved patients. Units without pathways reported managing patients on a case-by-case basis. These units often still reported having a 'cooling off' period of between 6-12 months after completion of active treatment. Very few units (5.9%, n=3) said they would perform CSM at the time of the index mastectomy. Information and support for women seeking CSM was also variable. Respondents expressed concerns about decisional regret, a reliance on psychologist input, variation of practice, and a desire for standardised guidance to improve equity of access to CSM.
Conclusions: There is significant variation in the practice and provision of CSM in the UK resulting in a lack of equity in access to care which urgently needs to be addressed. This is as a UK research priority. The UK FLAME study (Co-development of an evidence-based pathway to improve access and outcomes for women seeking FLat symmetry and an Alternative to breast reconstruction after Mastectomy for brEast cancer) aims to use qualitative interviews with patients and professionals to explore experiences, concerns, and attitudes to CSM including timing of surgery, support required and views on potential pathways of care. This work will underpin development of consensus-based guidelines co-developed with patients to support professionals to equitably offer CSM as a valid alternative to breast reconstruction.

P3-07-16: Validation of a Novel Prognostic Staging System for De Novo Metastatic Breast Cancer: A Real-World Experience

Ibrahim Khamees, Himil Mahadevia, Kensey Gosch, Parth Sharma, Simran Chandra, Shelby Davis, Kelly Gast, Timothy Pluard, Whitney L Hensing

Background: De novo metastatic breast cancer (dnMBC) comprises 6–10% of new breast cancer diagnoses and 30% of metastatic breast cancers (MBC), with varying survival rates. The latest AJCC staging system integrates anatomic factors, including the tumor size, nodal status, and metastasis sites, and non-anatomic factors, such as biomarker status and tumor grade, to assign the prognostic stage. Based on this staging system, all patients with MBC are assigned to stage IV. Plichta et al. utilized a statistical approach from large population data sets to a novel staging system for dnMBC, stratifying patients with dnMBC into four prognostic stages (IVA-D) based on relevant clinical and pathologic variables, such as HER2 status and sites of metastasis at diagnosis. In this study, we presented an updated analysis aiming to validate this new staging system in an independent real-world population of patients with dnMBC.

Methods: This study utilized data from a retrospective cohort of patients with dn[GKL1] MBC who received treatment at the Koontz Center for Advanced Breast Cancer Center (KCABC) at St. Luke's Cancer Institute. The KCABC is one of few centers dedicated solely to caring for patients with MBC. These patients were grouped into prognostic stage IVA, IVB, IVC, and IVD according to the system designed by Plichta et al., JCO 2023. Overall survival (OS) was compared between groups using Kaplan-Meier analysis and the log-rank test. Given the association between age and survival, we performed a stratified analysis based on age <60 versus ≥60 years.

Results:131 patients with dnMBC were included in the analytic cohort. The mean age at diagnosis was 56.1 years, and most patients identified as either white (74.0%) or black (21.4%). 12 patients were assigned stage IVA (9.2%), 50 stage IVB (38.2%), 52 stage IVC (39.7%) and 17 stage IVD (13%). Most stage IVA dnMBCs were hormone receptor-positive (HR+), HER2-positive (triple-positive) (66.7%), most stage IVD dnMBCs were triple-negative (70.6%), and the majority of stage IVB-C were HR+, HER2-negative (74.5%). OS differed significantly between the four stage groups, both in the overall population (log-rank $p<0.0001$) and when patients were stratified by age at diagnosis (≥60 or <60) (both log-rank $p<0.05$). The overall population's overall 5-year survival rates were 88.9%, 68.4%, 49.5%, and 8.6% for patients with dnMBC stages IVA, IVB, IVC, and IVD, respectively.

Conclusions: The novel prognostic staging system that integrates subtype, grade, T stage, and sites of metastasis was able to stratify our real-world cohort of patients with dnMBC into four prognostic groups. These findings demonstrate the utility of this staging system in determining prognosis for patients with dnMBC.

P3-07-17: Adjuvant Therapy in Patients with Positive Lymph Nodes Post-Neoadjuvant Chemotherapy With and Without Axillary Lymph Node Dissection

Anthony Baez, Caitlin Marsh, Acacia Sharma, Alyssa Marmer, Lindsay Chevlin, Charles Dimaggio, Deborah Axelrod, Amber Guth, Freya Schnabel, Mary Gemignani

Introduction: The current standard of care for breast cancer patients with pathologic lymph node positivity (pN+) after neoadjuvant chemotherapy (NAC) is completion axillary lymph node dissection (ALND). However, recent data suggests a shift in patterns of care to less than standard surgical therapy. The aim of our study is to compare adjuvant treatment of patients with pN+ disease after NAC who underwent completion ALND with those who had sentinel lymph node biopsy (SLN) alone.
Methods: Retrospective review of IRB-approved prospective database was performed to identify patients with invasive breast cancer treated with neoadjuvant chemotherapy from 1/2010 to 12/31/2023. Clinical, pathologic and treatment data was collected and the Chi-square test was used to compare patients with pN+ disease undergoing ALND vs SLN only.
Results: Our study group consisted of 374 patients who received NAC and 148 (39.6%) had pN+ disease. There were no significant differences in age (p=0.379), gender (p=1.000), or histologic subtype (p=0.270) between patients with pN+ disease and those with no nodal disease (pN-). Patients with pN+ disease had larger tumors at diagnosis compared to pN- patients (2.60 cm (SD 2.20) vs 1.89 cm (SD 1.84), p=0.002). Treatment data was available for 138 patients with pN+ disease. Among these, 8 (5.8%) had SLN surgery only, while the remaining underwent ALND.
Patients with pN+ undergoing ALND, had higher rates of mastectomy compared to those who had SLN-only (76.1% vs 25%, p=0.002) resulting in a statistically significant difference in the rates of PMRT and WBRT between these groups (p=0.006). However, the rates of regional nodal irradiation were similar (SLN-only, 87.5% vs ALND, 84.6%, p=0.716). In addition, the SLN-only group had a mean of 1.4 (SD 0.5) positive nodes compared to a mean of 4.4 (SD 4.2) positive nodes in the ALND group, likely representing a larger burden of residual disease.
Conclusion: In our study group, we noted that most patients with positive nodes after NAC were treated with ALND. In the small subset of patients who had SLN only, no difference in adjuvant regional nodal irradiation was noted. Data regarding the equipoise in treatment of radiation (RT) only vs ALND/RT is forthcoming and warranted.

P3-07-18: Sacituzumab Govitecan in Metastatic Breast Cancer: Impact of Age and BRCA Mutational Status in a Real-World Cohort

Laurys Boudin, Anthony Gonçalves, Frederic Viret, Louis Tassy, Lucas Usclade, François Bertucci, Alexandre de Nonneville

Background: Sacituzumab Govitecan (SG) is an antibody-drug conjugate targeting Trop-2, showing efficacy in HER2-negative metastatic breast cancer. We assessed the real-world efficacy of SG, focusing on the impacts of age and BRCA mutation status on survival outcomes.
Methods: This retrospective cohort study included 97 female patients treated with SG at the Paoli-Calmettes Institute French Comprehensive Cancer Center from May 2021 to October 2023. Patient demographics, BRCA mutation status, hormone receptor (HR) status, HER2 expression, metastatic sites, treatment lines, and dose reductions were recorded. Progression-free survival (PFS) and overall survival (OS) were analyzed using univariate and multivariate analyses to identify significant predictors of treatment outcomes.
Results: The cohort had a median age of 60 years, with 24% aged 70 or older. TNBC was predominant (65%), and 11.3% had BRCA mutations. The median number of previous lines received in the metastatic setting was 3 (range 0 to 12). The median PFS was 2.8 months, and the median OS was 8.1 months. SG dose reductions were more frequent in patients ≥ 70y than in patients < 70 years (52.2% vs. 23%; p=0.008), but no age-related difference was observed in survival analyses with mPFS of 2.8 months in patients ≥70y vs. 2.7 months (p=0.072, log-rank test), and mOS of 7.8 months in patients ≥70y vs. 8.3 months (p=0.579). No difference in grade 3-4 toxicity prevalence was observed according to age (26.1% in ≥70y vs. 14.9%; p=0.216). mPFS and mOS for patients with and without BRCA mutations were 2.2 vs. 2.8 months (p=0.157), and 3.9 vs. 8.3 months (p=0.026), respectively. In multivariate analyses including HR and BRCA statutes, age was not associated with PFS or OS. While BRCA mutation did not independently affect PFS, it did have a significant negative impact on OS (HR 2.48 [95CI 1.01-6.11; p=0.047]).
Conclusions: SG demonstrates similar relative efficacy across different age groups of heavily pretreated patient without increased toxicity in the elderly population. This study highlights the need for further research into BRCA mutation-related cross-resistance mechanisms, which could inform future treatment strategies for BRCA-mutated metastatic breast cancer. These real-world data support SG's use in patients aged 70 or over.

P3-07-19: Survival Outcomes in Breast Cancer with Brain Metastasis Based on Prior Lines of Systemic Therapy for Metastatic Disease

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Background: Breast cancer brain metastases (BCBM) are often associated with short survival. However, specific prognostic factors for patients with BCBM are poorly understood. This single institution retrospective study compares Overall Survival (OS) in

patients with BCBM based on number of lines of prior systemic therapy (ST) for metastatic (M) BC at the time of their BCBM diagnosis.
Methods: This study included patients with BCBM, diagnosed from Jan 2010 to June 2021. Patients were classified into Early BM [BCBM that developed either de-novo (at the time of MBC diagnosis) or during 1st line of ST] and Late BM (BCBM that developed during or after 2nd lines of ST for MBC). Baseline characteristics were compared using Fisher's Exact & Wilcoxon Rank-sum tests. OS was estimated using Kaplan Meier methods and compared using Log Rank test. Important covariates were adjusted for using Cox-regression model.
Results: 123 BCBM patients were identified and divided into Early BM (n=83) and Late BM (n=40) groups.30% of Early BM were HR+/HER2- vs 60% of Late BM (p=0.005). 47% of Early BM had visceral metastasis vs 75% of Late BM (p=0.0004). Other baseline variables were not significantly different between the groups. Median OS (years, 95% CI) for Early BM was 2.8 (1.6-4.6) vs. 0.5 (0.4-1.5) for Late BM (p=<0.0001). In a Cox regression model, Late BM was associated with significantly higher risk of death vs Early BM [Hazard Ratio (95% CI) 2.246 (1.311-3.850)]. Older age, triple negative subtype (vs HR+/HER2- or HER2+) and worse performance status were also significantly associated with shorter OS. A reduced cox model for all factors significantly associated wtih OS obtained using a backward elimination procedure showed similar HR estimates.
Conclusion: This single institution study showed that OS in BCBM patients was significantly associated with number of prior line of systemic therapy for MBC at the time of diagnosis of BM. If validated, these results could provide important prognostic information for BC patients with BM.

P3-07-20: New Wine in Old Bottles - The Therapeutic Promise of the PARP Inhibitor Rucaparib as an N-WASP Inhibitor for Breast Cancer Metastasis

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Background: Rucaparib is an established PARP inhibitor known for its efficacy in treating BRCA-mutated cancers. Recent studies suggest its potential utility in targeting additional pathways involved in oncogenesis and metastasis, such as those mediated by the N-WASP proteins, which are essential in cell motility and invasiveness. This study investigates Rucaparib's capability to act beyond PARP inhibition by targeting N-WASP proteins, offering a novel approach to curb cancer metastasis.
Methods: Utilizing two breast cancer cell lines, MDA-MB-231 and MCF-7, along with HECV, an endothelial cell line, comprehensive in vitro biological assays included cytotoxicity tests, cell growth assay, Electric Cell-substrate Impedance Sensing (ECIS), wound scratch assay, immunofluorescence, qPCR and western blotting were conducted, to discerning Rucaparib's effects on cell proliferation, adhesion, motility, and its specific impact on N-WASP protein expression at both mRNA and protein levels. Chemical-target protein docking was carried out by computer-assisted modelling.
Results: The impact of Rucaparib on cellular dynamics was profound across the studied cell lines. Growth assays showed a statistically significant decrease in cell growth (P=0.0054,

P=0.0034, P<0.0001). Rucaparib effectively impaired the ability of cancer cells to adhere and migrate (P<0.0001, P=0.0214, P=0.0003). Rucaparib markedly decreased the RNA and protein levels of N-WASP (P=0.0019, P<0.0001, P<0.0001), indicating a potent inhibitory effect on this metastasis-related protein. These results not only reinforce Rucaparib's inhibitory role in essential cellular processes but also highlight the potential to disrupt mechanisms underlying metastatic progression, especially N-WASP, a key regulator of cytoskeletal reorganization. Finally, compound docking revealed that Rucaparib had an excellent binding profile with multiple amino acids of key domains of N-WASP protein. Conclusion: The study validates Rucaparib's potential as a multifaceted inhibitor, demonstrating substantial efficacy in inhibiting N-WASP protein expression, thereby reducing tumour cell proliferation, adhesion, and migration. It highlights Rucaparib's prospective role as a dual-action therapeutic in the management of metastatic breast cancer, presenting a promising avenue for further clinical exploration.

P3-07-21: Transcriptomic analysis of the tumor immune microenvironment (TIME) in patients with breast cancer with liver metastasis (BCLM)

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Background: Breast cancer with liver metastasis (BCLM) is a significant predictor of worse prognosis and poor overall survival. Liver specific homing of breast cancer is not well understood, and the tumor immune microenvironment (TIME) likely plays a critical role in the process of forming BCLM. This retrospective study was conducted to determine the clinical risk factors and transcriptomic biomarkers associated with BCLM.
Methods: A retrospective single institute chart review was conducted. Patient, disease characteristics and treatment variables were reviewed. Liver metastasis specific survival (defined by overall survival time from the diagnosis of liver metastasis) were compared across three breast subtypes (i.e., ER+/HER2-, HER2+, and triple-negative (TN) breast cancer) using Kaplan-Meier curves. Next generation sequencing (NGS) via commercial platforms were then used to obtain transcriptomic data. Transcriptomic analysis including differential gene expression analysis, gene set enrichment analysis (GSEA), as well as gene deconvolution analysis with TIMER2.0 were conducted for comparison of transcriptomic profiles and immune compositions across the three breast cancer subtypes.
Results: 176 patients with BCLM and available liver metastasis biopsy were identified through retrospective chart review. Liver metastasis specific survival was compared, HER2+ BCLM patients (n = 17) had the best outcome and TN BCLM patients (n = 46) had the worst outcome with ER+ BCLM patients (n = 112) in the middle ($p < 0.0001$, ER+ vs TNBC $p < 0.0001$, ER+ vs HER2+ $p = 0.038$, HER2+ vs TNBC $p = 0.00011$). Principle component analysis on the transcriptomic data of 30 liver metastatic samples revealed different expression profiles between ER+ (n = 20) and TN (n = 7) BCLM, while HER2+ (n = 3) BCLM did not show statistical differences with either group. B cell mediated immunity was

upregulated in TN BCLM compared to ER+HER2- BCLM by GSEA (normalized enrichment score = 2.35, adjusted P-value = 0.19). Consistently, plasma cells were relatively enriched in TN BCLM compared to ER+ and HER2+ BCLM (p = 0.037) based on CIBERSORT estimation. In contrast, monocytes and neutrophils were enriched in ER+ BCLM (p = 0.0093 & 0.018) based on TIMER2.0 estimation. Additional NGS analysis is currently underway for increased sample size.
Conclusions: BCLM in TNBC was associated with significantly worse overall survival. TIME in TN BCLM enriched for genes associated with B cell mediated immunity while ER+ BCLM had more monocyte/neutrophil mediated immunity.

P3-07-22: A miRNA expression profiling of breast cancer to develop a metastases predictor model and identify new molecular players of metastatic outgrowth.

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Background: Metastasis is the leading cause of breast cancer-related mortality. Current classification is based mainly on immunohistochemical markers and fails to reliably predict metastatic potentials. In recent decades, microRNAs (miRNAs) have emerged as promising clinical biomarkers due to their capacity to regulate key molecules involved in cancer progression and metastatic spread.
Methods: To identify novel miRNA-based biomarkers, we analyzed three retrospective cohorts. A miRNA Affymetrix Gene Chip 4.0 array was used to identify relevant miRNAs in the discovery cohort (n=40). Next, RT-qPCR analysis was performed to evaluate the accuracy of selected miRNAs in identifying patients who developed distant metastases in the extended cohort (n=223). A stepwise logistic regression model was used to construct a prognostic tool for metastases, including miRNA levels and clinicopathological features. The effects that both miRNAs may exert on tumor cell phenotype was preliminary assessed in a panel of breast cancer cell lines in terms of cell viability. Following ectopic modulation of candidate miRNAs by miRVANA miRNA mimics, cell viability was measured by PrestoBlue viability reagent.
Results: Global expression analysis of the discovery cohort identified eight miRNAs with differential expression between metastatic and non metastatic tumors. In the extended cohort, miR-3916 and miR-3613-5p were the best miRNAs for identifying patients who developed distant metastases. Increased expression levels of miR-3916 were associated with a reduced risk of developing distant metastases (OR=0.42, 95% CI: 0.23-0.70, p=0.002), while increased expression levels of miR-3613-5p were associated with an elevated risk (OR=2.06, 95% CI: 1.27-3.50, p=0.005). Importantly, by including the expression levels of miR-3916 and miR-3613-5p in a model with clinicopathological

covariates, the discriminatory power reached an AUC of 0.85 (95% CI: 0.79-0.91), outperforming a model with clinicopathological covariates only (AUC=0.76, 95% CI: 0.68-0.84) (delta-AUC p=0.001). As expected, the evaluation of the effects of miR-3613-5p and miR-3916 transfection in vitro showed that both miRNAs were able to impair cell viability in breast cancer cell lines.
Conclusions: In this study, we identified miR-3613-5p and miR-3916 as putative metastases associated miRNAs. A logistic regression model including both miRNAs and clinicopathological characteristics were able to predict the risk of metastases development supporting their potential utility in the clinical setting. Moreover, our initial in vitro studies suggest both miRNAs may affect tumor cell phenotype. Further in vitro and in vivo are currently ongoing to characterize miR-3613-5p and miR-3916 role in the mechanisms underlying metastases development in breast cancer.

P3-07-23: Incidence, Risk Factors, and Survival Outcomes of Brain Metastases in Breast Cancer Patients: Insights from a Tertiary Care Center

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Introduction: Breast cancer is one of the most prevalent cancers. Despite awareness efforts, many patients are diagnosed at an advanced stage. Some of these patients present with brain metastases, while others develop brain metastases throughout the disease trajectory.

Methods: This retrospective observational cohort study was conducted at King Abdullah Specialized Children's Hospital (KASCH), in Riyadh, including all female breast cancer patients diagnosed and treated between 2016-2022. The study aims to measure the incidence of brain metastases among breast cancer patients and its association with molecular subtypes, and their overall survival in comparison to those without brain metastases. Additionally, the study provides insights about the association of BM with other factors such as age, tumor grade, and disease stage.

Results: A total number of 954 BC females were identified, 89.7% were Saudi with median age of 52 (20-73) years at diagnosis. Among them, 59.4% had invasive ductal carcinoma (IDC), 8.3% invasive lobular carcinoma (ILC), and 2.3% had other subtypes. 717 (75.2%) HR-positive, 123 (12.9%) TNBC, 106 (11.1%) TPBC, and 96 (10.1%) HER2-enriched patients were reported. A total of 237 (24.8) BC patients were confirmed to have de novo metastatic disease with BM being declared as the fourth most common site of metastasis following the bone, lung, and liver. About 57 (6%) patients were found to have BM, 10 of them had BM at the time of diagnosis. The incidence of developing BM is 47(5%), after excluding the 10 cases.. The mean age at BM development was 45 ± 10.6, with the majority having G3 disease. Among BM patients, stage IV was the most predominant (50.2%), followed by stage III (28.2%), stage II (17.3 %), and stage I (4.3). Regarding BM incidence

based on the BC molecular features , HR- patients were more likely to develop BM n=23(10%) Vs n= 24(3.4%) P<0.001. her2-enriched were more likely to develop BM 19.8 Vs 3.4,P, 0.001. no statistically significant different were detected among the TPBC nor TNBC groups ..

Among the 47 patients who developed brain metastases during their treatment, 38 had stage IV breast cancer (BC), out of 227 other stage IV patients, resulting in an incidence of 16.7%. Of these, 33 (57.9%) patients had oligometastatic brain disease with five or fewer brain lesions. Additionally, 20 (44.4%) received whole brain radiation (WBR), 19 (42.2%) were treated with stereotactic radiosurgery (SRS), and only 4 (7%) patients underwent surgery.Kaplan-Meier survival curve was employed to estimate the overall survival (OS) of the whole population. The mean survival of all breast cancer patients was 7 years, Median OS was not reached . The OS among patients with BM at the time of analysis showed that 28 (15.6%) were dead, while only 16 (2.4%) were Alive (P<0.0001). The median survival for stage VI patients who did not develop BM Vs those who developed BM was 5.5 years 95% CI [4.4-6.6] and 3.1 years 95% CI [2.2-4.1] ,(P value =0.015).

Conclusion: This study showed a significant increase in brain metastases among HER2-positive breast cancer at 19.8% compared to 6.7% in TPBC, 4.1% in TNBC, and 3.4% in HR-positive subtypes. This result lines up with several prior studies that have also reported a higher risk of brain metastases among HER2-positive and TNBC in comparison to HR-positive breast cancer subtype. Based on our research, we conclude that age under 50, grade 3, and stage 3 are significant risk factors for BM. Patients with brain involvement had shorter survival compared to those without BM, 3.1 years and 5.5 years, respectively, which is consistent with previous literature demonstrating the negative prognostic impact of brain involvement in this patient population.

P3-07-24: Real-World Treatment Patterns and Clinical Outcomes of Sacituzumab Govitecan in HER2 negative metastatic breast cancer patients in China

Huanhuan Zhou, Zhanhong Chen, Xiying Shao, Yabing Zheng, Wenming Cao, Junqing Chen, XiaoJia Wang

Background: Sacituzumab govitecan (SG), a first-in-class Trop-2–directed antibody–drug conjugate, has been shown to improve prognosis of patients with metastatic triple-negative breast cancer (mTNBC) in ASCENT and EVER-132-001 trial. These led to NMPA (China health authority) approval of SG in June 2022 for mTNBC and then was available on June 13, 2023. Based on TROPiCS-02 study, SG also got FDA and EMA approval for unresectable locally advanced or metastatic hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative (IHC0, IHC 1+ or IHC 2+/ISH–) breast cancer who have received endocrine-based therapy and at least two additional systemic therapies in the metastatic setting. The new indication is accepted and under procedure by NMPA in early

2024. The study aims to investigate the real-world efficacy and safety of SG for HER2 negative mBC in China.
Methods: Data was retrospectively collected from 5 hospitals in China. Patient had a diagnosis of mBC, received at least two cycles of SG. Data cut-off was in July 2024. The primary endpoint is real-world progression-free survival (rPFS) estimated by the Kaplan-Meier method. Secondary endpoints are real-world overall survival (rOS) and safety. Physician-reported acute and late toxicities were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) 5.0.
Results: From June 2023 to July 2024, 44 patients were included in this study. The median age was 53 years (IQR range 31-80).Patients had previously received a median of 3 (1–10) prior lines of treatment in the metastatic setting. 77.3%(34pts） were TNBC , 22.7%(10pts ） were hormone receptor-positive, HER2-negative (HR+/HER2-) ;88.6%(39pts) had visceral metastases and 20.5%（9pts) had brain metastases. 68.2%（30pts）received SG monotherapy， 31.8%（14pts) received SG combined with other medications such as immunotherapy etc. Median (95% CI) real-world progression-free survival (mPFS) was 4.13 (3.86-5.80) months for all patients, 4.13（3.71-5.69） months in the mTNBC (n = 34) group and 5.25 months（2.78-6.91） in the HR+/HER2- group. 90% (9/10) had received treatment with CDK4/6 inhibitors and 70% (7/10) had received treatment with chemotherapy in the HR+/HER2- group. 18 (40.9%) Pts initiated low dose of SG treatment (less than 80% standard dose) considering the cost-saving and basic physical situation. The mPFS was 4.50 months (95% confidence interval [CI], 3.80-6.34) in standard dose group and 3.75 months (95% CI, 2.93-5.67) in low dose group (P=0.56). In this research group, no unanticipated toxicity was reported.
Conclusion: In the clinical practice of HER2 negative advanced breast cancer in China, for patients who have been rescued by CDK4/6 inhibitors or chemotherapy, the research results suggest that Sacituzumab govitecan (SG) has very good efficacy and safety. Despite limitations in the patient's PS status or economic conditions, the overall efficacy is still satisfactory.

P3-07-25: Current Treatment Strategies for PIK3CA-Mutated Metastatic Breast Cancer and Provider Perceptions of the INAVO120 Trial

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Background: The development of PI3K targeting therapies is transforming the approach to care for patients with PIK3CA-mutated metastatic breast cancer (mBC). The phase III INAVO120 trial (NCT04191499) is evaluating the addition of inavolisib, an investigational PI3Kα inhibitor, to first-line (1L) palbociclib + fulvestrant in participants with PIK3CA-mutated, HR+/HER2– mBC who relapse on or within 12 months of completing adjuvant endocrine therapy. Primary findings demonstrated that the addition of inavolisib to palbociclib + fulvestrant more than doubled progression-free survival (PFS), meeting the study’s primary endpoint. This survey-based study aimed to elucidate current treatment strategies within the PIK3CA-mutated mBC space and evaluate oncologists’ perceptions of

the INAVO120 data.
Methods: US-based oncologists convened at a live meeting in March 2024 to review clinical updates presented at SABCS 2023. Participant characteristics and demographic data were collected via an online survey prior to the meeting. Perceptions/reactions to queries on clinical updates were captured in real-time via an audience response system. Not all participants answered every question. Responses were aggregated and summarized using descriptive statistics.
Results: Among 61 respondents, 74% identified as community providers. Additionally, respondents indicated that they have 20.2 mean years of clinical experience and see approximately 18 patients per clinic day. Prior to reviewing the INAVO120 trial data, respondents selected alpelisib + fulvestrant (42%; N=50) and cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) + aromatase inhibitor (36%; N=50) as their top two preferred 1L treatment regimens for patients with PIK3CA-mutated mBC. Notably, half of respondents cited ribociclib as their preferred CDK4/6i for this patient population. After reviewing the INAVO120 trial, 84% of respondents (N=56) reported being likely to incorporate inavolisib into their patients' 1L CDK4/6i + hormone therapy-based treatment regimen, if FDA-approved and/or guideline-recommended. A majority of respondents (62%; N=55) cited the superior PFS associated with inavolisib + palbociclib + fulvestrant as the most compelling outcome from the trial. Hyperglycemia (52%; N=56) and stomatitis/mucosal inflammation (41%; N=56) were selected as the top two most concerning adverse events associated with the triplet regimen.
Conclusions: Overall, the INAVO120 data were viewed favorably by this cohort of physicians, as evidenced by most respondents indicating that they would incorporate inavolisib into their patients' 1L CDK4/6i + hormone therapy-based regimen. Although a minority of patients with HR+/HER2– mBC are both PIK3CA-mutated and rapidly progress on or after adjuvant endocrine therapy completion, respondents' willingness to adopt the INAVO120 treatment strategy suggests that it fulfills an unmet need for this niche patient population. Interestingly, the INAVO120 trial utilized palbociclib as its CDK4/6i backbone, whereas a majority of respondents prefer ribociclib in this setting. If approved by the FDA, the INAVO120 regimen will bolster the armamentarium of available treatment options for patients with PIK3CA-mutated mBC. However, it remains to be seen if physicians will opt for this triplet regimen over currently approved doublet regimens in the real-world setting given inavolisib's unique toxicity profile and it is unclear how much influence the CDK4/6i partner (i.e., palbociclib vs. ribociclib) will have on the adoption of the INAVO120 regimen into routine clinical practice if approved.

P3-07-26: Inpatient outcomes and predictors of mortality in Leptomeningeal carcinomatosis in patients with breast cancer

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Objective: To assess baseline characteristics, comorbidities, and factors associated with poor outcomes in hospitalized breast cancer patients with Leptomeningeal carcinomatosis
Introduction:
Leptomeningeal carcinomatosis (LC), defined as the infiltration of cancer cells into the leptomeninges, is a rare but severe complication occurring in up to 5% of breast cancer patients. It causes significant morbidity and mortality, with poor outcomes even in patients receiving aggressive treatment. Identifying risk factors can help with deciding management plans and improving physician-patient communication.
Method: We analyzed data from the National Inpatient Sample to identify patients who were admitted with a diagnosis of breast cancer in 2021. Among this population, we isolated patients with LC. To ensure that LC was secondary to breast cancer, we excluded other most common causes of LC such as primary central nervous system (CNS) tumors, lung cancer, and melanoma. We investigated the baseline characteristics of those with and without LC. The primary endpoint was in-hospital mortality, stratified by the presence of LC. We also examined other outcomes such as length of stay (LOS), total charge (TOTCH), sepsis, and mechanical ventilation. Additionally, we explored independent predictors of in-hospital mortality.
Result: A total of 659,989 admissions for breast cancer occurred between January and December 2021; out of this cohort, 22,664 (3.4%) had LC. The mean age of those with LC was 59.5 compared to 71.6 in those without LC ($p<0.01$). The in-hospital mortality rate for those with LC was significantly higher (8% vs 3%, $p<0.01$). After adjusting for demographic and patient-related variables, independent predictors of mortality with p-value <0.01 were lung metastases (aOR 1.95, CI 1.73-2.19), bone metastases (aOR 1.72, CI 1.57-1.88), protein-energy malnutrition (aOR 1.61, CI 1.46-1.78), cachexia (aOR 2.22, CI 1.90-2.60), CKD (aOR 1.30, CI 1.14-1.49), liver disease (aOR 1.33, CI 1.18-1.50), and CHF (aOR 1.26, CI 1.16-1.37). Patients with LC also had a longer length of stay (6.5 d vs 5 d), more palliative care referrals (25% vs 7%), and an increased cost of hospitalization ($90,163 vs $69,598).
Conclusion: Treatment of Leptomeningeal disease in breast cancer patients is exceedingly challenging. Identifying risk factors like nutritional status, presence of metastatic disease, and comorbidities including liver disease, CKD, and CHF remain crucial and can help in deciding the overall management plan. Considering the high inpatient mortality of patients with multiple comorbidities, utilizing early palliative care services might be beneficial. Studies on risk stratification will help in navigating care for these patients.
KEYWORDS: Leptomeningeal carcinomatosis, Breast cancerDESIGN: Retrospective cross-sectional studySETTING: National Inpatient Database 2021 using ICD 10 codesSTATISTICS: Logistic regression assessed patient and hospital factors influencing outcomes in patients with Leptomeningeal carcinomatosis (LC) and its association with inpatient mortality using the STATA BE 18.0 software. Results were adjusted for age, race, smoking, alcohol, liver disease, diabetes, hypertension, chronic kidney disease (CKD), and congestive heart failure (CHF). Nutritional factors like protein energy malnutrition (PEM) and BMI were also assessed and adjusted for outcomes.

P3-07-27: Selective De-Escalation of Surgery: Trends in Surgical Management of de Novo Stage IV Breast Cancer Over the Past Decade

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Background: Previous studies have demonstrated declining rates of surgery for de novo stage IV breast cancer given the lack of prospective randomized evidence in support of surgical resection. We aimed to characterize trends in utilization of breast and axillary surgery in a contemporary cohort of de novo stage IV patients at our institution.
Methods: This was a retrospective review of all patients treated for de novo stage IV breast cancer at University of Texas MD Anderson Cancer Center from 2014-2022. All patients received systemic treatment with or without surgical resection of the primary breast tumor (systemic therapy alone vs. surgery cohort). Chi-squared and Mann-Whitney U tests were used to compare group differences. Linear regression and mixed-effects model were used to identify temporal variability in patient and tumor characteristics among the surgery cohort.
Results: In total, 1290 patients with de novo stage IV breast cancer were treated at our institution over an 8-year period. Of these, 16.7% (215) underwent surgery of the primary breast tumor. The median age at diagnosis was 54 years (IQR 32.4-63.0) and BMI was 28.2 kg/m2 (IQR 24.4-33.1). Patients were predominantly White (903, 70%) with T2 or higher (999, 77.4%), node-positive (1020, 79.1%), ductal disease (1016, 78.8%). The most common site of metastasis was visceral (637, 49.4%), followed by bone-only (491, 38.1%), and soft tissue and distant lymph nodes (131, 10.2%). Compared to the systemic therapy cohort, patients in the surgery cohort were younger (median age 49 vs. 55, $p < 0.001$), had higher nodal burden (N2-3, 48.8% vs. 28.2%, $p < 0.001$), and were more likely to have higher grade (52.1% vs. 31.4% $p = 0.002$), HER2-positive disease (34.4% vs. 19.8%, $p < 0.001$). Patients with inflammatory breast cancer (IBC) were significantly more likely to undergo surgery (35.3% vs. 9%, $p < 0.001$). With regard to site of metastasis, patients with soft tissue/lymph node metastases were more likely to undergo surgery (30.5% vs. 6.4%, $p < 0.001$) while those with visceral metastases were less likely (33.3% vs. 54.1%, $p < 0.001$). Over the 8-year period, there were no significant changes in the overall percentage of stage IV patients undergoing surgery ($p = 0.46$); however, surgery rates significantly decreased for patients with clinical T2 ($p = 0.042$) and N1 tumors ($p = 0.02$) and increased for IBC patients ($p = 0.037$). Overall, rate of surgery significantly decreased for HR+/HER2+ tumors ($p = 0.003$) while there were no differences in surgery utilization by receptor subtype among IBC patients. There were no changes over time in age, BMI, race/ethnicity, histology, grade, type of metastasis, and type of breast surgery performed within the surgery cohort. However, the percentage of patients undergoing ALND significantly decreased over time ($p = 0.04$), with a 1.8% decrease annually.
Conclusion: We found temporal variability with an overall decrease in primary tumor resection in de novo stage IV breast cancer but significantly increased rates of surgery in patients with IBC. These findings suggest providers are increasingly more selective in offering surgical resection to stage IV patients, in accordance with current evidence, with the exception of stage IV IBC patients who were excluded from prospective randomized trials.

P3-07-28: Anxiety, Depression and Coping Mechanisms Among Young Women Diagnosed with De Novo Metastatic Breast Cancer

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Background: Prior research has demonstrated that young patients presenting with stage IV breast cancer (de novo metastatic disease) have a high prevalence of anxiety and, less commonly, depression. Little is known about factors that help young women to cope with the diagnosis and treatment of metastatic breast cancer. We sought to study coping mechanisms in young patients with de novo metastatic disease, and their associations with anxiety and depression, to inform future interventions.

Methods: The Young Women's Breast Cancer Study (YWS) is a multisite, prospective cohort that enrolled 1,302 women diagnosed with stage 0-IV breast cancer at age ≤40 from 13 North American academic and community sites from 2006-2016. Participants completed surveys at study baseline (median of 5 months post-diagnosis), 6 months, and 12 months after enrollment, then were surveyed every 6 months through 3 years, and annually thereafter. Coping was assessed at 18 and 24 months using an 18-item investigator-developed survey designed to understand how participants have coped with their diagnosis and treatment. Anxiety and depression were assessed at 12 months using the Hospital Anxiety and Depression Scale (HADS), a 14-item validated instrument. The current median follow-up is 10.5 (range 0.4-17.6) years. Medical record review was used to assess disease characteristics. We excluded women with stage 0-III disease (n=1,238) and women who did not answer at least one survey at the four time points (n=6). Descriptive analyses were used to characterize the baseline demographics. We used univariable analysis to examine the relationship between coping strategies and anxiety and depression.

Results: Among 58 patients with de novo metastatic breast cancer included in this analysis, most patients were white (n=52, 89.7%), 3.4% were Black (n=2), 3.4% Asian (n=2), and 3.4% Native American (n=2). Two (3.4%) patients were Hispanic. Median age at diagnosis was 37 years; 38 (65.5%) were married or living with a domestic partner, 6 (10.3%) were widowed, and 12 (20.7%) were never married at baseline. Emotional support from a partner/spouse/significant other was commonly reported to help with coping at both 6 and 18 months, 76% and 62%, respectively, followed by emotional support from parents at 6 months (15%) and taking care of close ones at 18 months (19%). Approximately 50% of the patients reported coping with exercising and/or diet at both 6 and 18 months. Four patients reported moderate or greater reliance on recreational drugs and one patient, on drinking alcohol. The mean HADS anxiety score at 12 months was 7.71 (SD, 5.09) and mean HADS depression score was 3.96 (SD, 3.33). Moderate or high anxiety (HADS score ≥11) symptoms were present in 18 patients (31.0%), and 2 patients (3.4%) scored moderate or high in the depression subscale. In univariate analysis, there was no statistically significant association between specific coping strategies and anxiety or depression.

Conclusion: Young patients with de novo metastatic breast cancer most commonly rely on emotional support from their partners and family/friends to cope with their disease. Lifestyle changes including diet and physical exercise were also commonly reported coping strategies. While approximately one third of patients experienced moderate or severe anxiety, we did not observe an association between specific coping strategies used and anxiety or depressive symptoms. Additional research may further inform interventions that support coping amongst young survivors living with de novo metastatic breast cancer.

P3-07-29: ACT-MBC: A Prospective Observational Impact Study of Circulating Tumor Cells in Metastatic Breast Cancer

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Introduction: The FDA-cleared CellSearch® Circulating Tumor Cell (CTC) assay is a non-invasive liquid biopsy approved for metastatic disease monitoring. In metastatic breast cancer (MBC), prospective phase III data supports a positivity threshold of ≥5 CTC. Positive CTCs (≥5 CTCs) at baseline (BL), persistent CTC positivity, and increases in CTC count over time outperform serum tumor markers such as CA 27-29 and CA 15-3 and predict radiographic disease progression. Nevertheless, CTC enumeration has not been utilized broadly in real-world clinical practice. Therefore, we sought to evaluate provider perception of clinical utility of CTC enumeration in routine clinical practice for treatment decisions and/or response assessment.
Methods: The ACT-MBC study is A Prospective Observational Impact Study of Circulating Tumor Cells (CTCs) in Metastatic Breast Cancer (MBC) in which patients receive CTC enumeration and biomarker testing. Provider perception of CTC testing is measured with questionnaires at baseline (PQ1), at each patient's restaging visit (PQ2), and at study end (PQ3). The study also evaluates if CTC results correlate with response assessment and disease progression as defined by standard of care imaging, and whether CTC results influence the timing of radiographic assessment. The study includes metastatic Hormone Receptor (HR)+/HER2- patients who are starting ≥2nd line and metastatic Triple Negative (TN) patients at any line of systemic therapy. We present the interim results from PQ1, PQ2 and patient baseline CTC results.
Results: Thirty providers completed PQ1 (16 from academic sites and 14 from three community US sites). In PQ1, most (83%) had no prior use of CTCs during routine practice, and the remaining 17% reported rare CTC test use. Eighteen (60%) agreed or strongly agreed that CTC offered prognostic information with the remaining 12 (40%) as neutral. The most common barriers to utilizing CTCs in routine practice included concerns about insurance reimbursement (90%), lack of familiarity with CTC testing (73%), uncertainty on logistics of test ordering (66%). Thus far, 34 subjects were enrolled; clinical data were

available for 24 subjects. The median age of subjects was 63 years (range 41-82 years); 80% had ductal, 20% had lobular histology; 75% were HR+/HER2- and 25% were TN; 83% of subjects had visceral metastases and 17% had bone only disease. Three subjects received 2 prior lines of therapy, 6 subjects 3 lines, and 15 subjects 4 lines or more. 14/24 subjects had a baseline CTC assessment of ≥ 5 CTCs, 4 subjects had 1-4 CTCs; and 4 subjects had zero CTCs (for 2 subjects the BL CTC results could not be analyzed).
Nineteen available provider questionnaires were completed after the first restaging visit (PQ2). Providers indicated that for 13 of 19 subjects, CTC results were concordant with imaging results (for 5 subjects the provider was unsure about concordance). For 12 subjects, providers indicated that assessing CTCs along with standard modalities enhanced the ability to assess treatment response [strongly agree (n=9); agree (n=3); neutral (n=5); disagree (n=2)]. For 10 subjects, CTC assessment helped guide physician decision to continue or change therapy (strongly agree (n=6); agree (n=4); neutral (n=3); disagree (n=5); strongly disagree (n=1). In those subjects without disease progression at first restaging, providers indicated that CTC assessment influenced timing of future restaging imaging assessments in 4 out of 10 subjects.
Conclusions: The ACT-MBC analyses to date show that assessing CTCs along with standard modalities enhanced the ability of physicians to assess treatment response, guide treatment decisions and may additionally alter the timing of restaging scans. In summary, these data suggest that assessment of CTCs may provide an important tool for clinical treatment decision making.

P3-07-30: Mesenchymal Stem Cells Regulate E-cadherin Level, Proliferation, and Immuno-Resistance on Breast Cancer Cells

Bei Dai

Breast cancer is the most common cancer in women worldwide. Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, while estrogen receptor-positive breast cancer (ER+BC) exhibits the longest dormancy phenomenon, wherein breast cancers recur as distant metastases even decades after removal of the primary tumor. Therefore, this study will focus on both TNBC and ER+BC.
The majority of breast cancer-related deaths are attributed to breast cancer metastasis, while E-cadherin is integral in the metastasis process with its downregulation enabling escape from the primary tumor site, and its re-expression enabling the survival of disseminated tumor cells (DTCs) when they seed the metastatic site. Mesenchymal stem cells (MSCs) were previously shown to be recruited to the primary breast cancer site and also to be present in the breast cancer metastasis. Their wide distribution allows them to act on both primary and metastatic tumor cells.
We are expanding this research area to determine how MSCs regulate the more epithelial-mesenchymal phenotypes including immuno-resistance, proliferation, and E-cadherin-mediated interactions with parenchymal cells. Our preliminary results demonstrate that MSCs inhibit the E-cadherin level of specific TNBC cell lines and enhance the proliferation of

TNBC cells and ER+BC cells; thus driving a more progressive phenotype. Fas ligand (FasL)-induced apoptosis will be measured to represent the non-specific immune responses. After that, whether this signal is paracrine or juxtacrine will be determined by the results of direct co-culture and indirect co-culture experiments including Transwell co-culture and conditioned media (CM) experiments. Signaling molecule screening will be performed by siRNA library screening, multiplex immunoassay, or small RNA sequencing to look for possible therapeutic targets, with the specific screening method is dependent on the co-culture results. Finally, we will confirm our conclusions including the effect on epithelial-mesenchymal phenotypes of BCCs and screened signal molecules in animal models and ex vivo MPS models which mimic human liver microenvironments. By studying the mechanism behind this regulation, we hope to define a new therapeutic approach to suppress the metastatic outgrowth of dormant breast cancer.

P3-08-01: Cardiovascular and Non-cardiovascular Risks among Female Breast Cancer Survivors in Japan: A Matched Cohort Study

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Background: With improvements in the detection and treatment of breast cancer (BC), the number of BC survivors has increased worldwide. However, the landscape of their non-cancer disease risks, including cardiovascular and non-cardiovascular risks remains unclear, especially among Asian women.

Methods: We conducted a matched cohort study using data from the JMDC claims database (https://www.jmdc.co.jp/en/) that covers company employees and their family members in Japan. Between January 2005 and December 2019, women aged 18–74 years with and without an incident BC (defined as the BC diagnosis and surgery) were matched with 1:4 ratio for age and entry timing to the database. Using stratified Cox regression analysis, we estimated and compared the risks for six cardiovascular diseases (acute myocardial infarction, acute heart failure, atrial fibrillation/flutter, ischemic stroke, intracranial hemorrhage, and pulmonary embolism) and six non-cardiovascular diseases (major osteoporotic fractures, other fractures, gastrointestinal bleeding, urinary tract infection, infectious pneumonia, and anxiety/depression) between the groups, overall and by follow-up time (<1 year and 1-10 year from the BC diagnosis, separately). In addition, risk by chemotherapy (anthracycline, taxane, anthracycline&taxane, and no anthracycline/taxane) and hormone therapy (tamoxifen, aromatase inhibitors, no tamoxifen/aromatase inhibitors) were estimated using non-stratified Cox regression analysis, with the reference group being women without BC. The treatment regimens were identified and classified during the first year from the BC diagnosis and follow-up was started at 1 year from the diagnosis. For this analysis, we adjusted for hormone therapy (tamoxifen and aromatase inhibitors) when analyzed by chemotherapy, and chemotherapy (anthracyclines and taxanes) when analyzed by hormone therapy.

Results: We included 24,017 BC survivors and 96,068 matched women in the analysis. The incidence rates of acute heart failure, atrial fibrillation/flutter, and all non-cardiovascular diseases were higher in the BC survivors than in the matched cohort group. For cardiovascular diseases, the highest adjusted hazard ratio was marked by heart failure (3·88, [95% confidence interval 2·55–5·90]), followed by atrial fibrillation/flutter (1·83, [1·40–2·39]). For non-cardiovascular diseases, the highest adjusted hazard ratio was marked by gastrointestinal bleeding (3·55, [3·10–4·06]), followed by anxiety/depression (3·06, [2·86–3·28]). Of the diseases with significant increases over the entire period, the hazard ratio at <1 year was larger than that at 1–10 years from the BC diagnosis in most outcomes; however, major osteoporotic fractures and other fractures had a larger hazard ratio in 1–10 years than in <1 year. For osteoporotic fracture and other fractures, the hazard ratio in 1–10 years was 1·77 (1·46–2·15) and 2·09 (1·86–2·34) respectively, whereas that in < 1 year was 1·30 (0·92–1·85), and 1·28 (1·05–1·56), respectively. By chemotherapy, hazard ratios for acute heart failure, atrial fibrillation/flutter, other fractures, gastrointestinal bleeding, infectious pneumonia, and anxiety/depression tended to be higher in the anthracycline&taxane group than in the other groups. By hormone therapy, a higher risk of fractures and gastrointestinal bleeding was observed among aromatase inhibitor users.

Conclusion: The BC survivors in Japan showed an increased risk of acute heart failure, atrial fibrillation/flutter, and six non-cardiovascular diseases than women without BC. Most risks increased more steeply during the first year of intensive multidisciplinary care of BC, whereas the risk of fractures increased later. It is important for healthcare providers and patients to understand the risks of these diseases and link them to screening, prevention, and early treatment.

P3-08-02: Real-world Evidence: Pegylated Liposomal Doxorubicin 40mg/m2 versus 50 mg/m2 in Patients with Metastatic Breast Cancer.

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Background: The use of anthracyclines in breast cancer treatment remains essential despite their known adverse effects, prompting the development of new formulations. Pegylated liposomal doxorubicin (DLP) encapsulates doxorubicin in a lipid coating, altering its bioavailability and side effect profile, notably increasing the incidence of hand-foot syndrome (HFS). Our experience in an oncology center in Brazil suggests that a standardized dose of 50 mg/m2 every four weeks in the metastatic setting is less tolerated, while the reduced dose of 40mg/m2 with the same interval has the same clinical benefit with better toxicity profile. The present study aims to assess whether dose reduction maintains clinical efficacy while minimizing adverse effects. Methods: This is a retrospective, observational, cohort study that analyzed electronic medical records of adult

female patients with metastatic breast cancer diagnosed between 2018 and 2023 at an oncology hospital in São Paulo, Brazil, treated with DLP at either dose of 40mg/m2 and 50mg/m2 every four weeks. Data were reviewed and collected from June to November 2023. Toxicities were classified using CTCAE v6.0. Logistic regression and Cox proportional hazards models, adjusted for potential confounders, were used to assess clinical response and progression-free survival. Results: A total of 193 patients were treated with pegylated liposomal doxorubicin (DLP) between 2018 and 2023. Among them, 98 patients received a standard dose of 50 mg/m2, while 95 patients received a reduced dose of 40 mg/m2, both every four weeks. The mean ages were 57.10 years (range 23-82) in the 50 mg/m2 group and 55.89 years (range 34-83) in the 40 mg/m2 group. In the standard dose group, 81 patients (85.3%) had an ECOG performance status of 0-1, whereas in the reduced dose group, 27 patients (28.4%) had an ECOG performance status of 2-3. Progression-free survival was 7.4 months (95% CI 5.3–11.3) in the 50 mg/m2 group and 6.7 months (95% CI 4.5–8.1) in the 40 mg/m2 group (adjusted HR 1.11; 95% CI 0.75-1.61, p = 0.623). Notably, prior re-exposure to anthracyclines in the reduced dose group had an HR of 1.72 (95% CI 1.02-2.90, p = 0.04), while in the standard dose group, the HR was 0.69 (95% CI 0.37-1.28, p = 0.25). Grade 3 toxicity related to the dose was more frequent in the 50 mg/m2 group with 33 cases (34.74%) compared to 10 cases (10.20%) in the 40 mg/m2 group. The most prevalent toxicity in both arms was hand-foot syndrome (18.88%). Dose reductions occurred more frequently in the 50 mg/m2 group, affecting 39 cases (41.05%). Conclusions: There was no difference in progression-free survival comparing 40mg/m2 vs 50mg/m2 protocols, emphasizing better tolerance with reduced adverse events for those treated with reduced dose. For patients previously treated with anthracyclines, the 50mg/m2 every four weeks might provide greater benefit by increasing progression-free survival.

P3-08-03: Trends in Presentation of HER2+ Breast Cancer: A Retrospective study of De Novo Versus Recurrent Metastatic Breast Cancer (MBC) in the Real-World French National ESME Cohort (2008-2022)

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Background: In HER2-positive (HER2+) metastatic breast cancer (MBC), the clinical presentation at metastatic diagnosis (dg), de novo (dnMBC) or recurrent (rMBC), is an independent prognostic factor. In contemporary clinical trials, the proportion of patients (pts) with dnMBC is high, likely due to improvements in the efficacy of (neo)adjuvant therapy. ESME-MBC, a national cohort of real-world data, recruiting pts consecutively treated for MBC in all comprehensive cancer centers in France since 2008, allows capturing of this evolution over time.
Methods: We selected pts with HER2+ (immunohistochemistry, IHC 3+ or ISH

amplified) MBC diagnosed between 2008 and 2022, excluding pts whose hormone receptor (HR) and HER2 status were unknown and those who did not receive systemic treatment for MBC. We compared clinicopathologic characteristics based on presentation at metastatic dg (dnMBC i.e. metastasis found within 6 months from initial diagnosis, or rMBC). We evaluated the trends in presentation according to year of MBC dg (YOD) using a Cochran-Armitage test, and studied factors associated with first-line progression-free survival (PFS1) and overall survival (OS) using multivariable Cox models.
Results: Among the 35,687 pts in the ESME MBC cohort, 5,573 pts were treated for HER2+ MBC, including 2,800 (50.2%) with rMBC and 2,773 (49.8%) with dnMBC. Most pts were women (99.4%) with a median age of 57 years (range, 19-97). Compared with rMBC pts, dnMBC pts had significantly more often a premenopausal status (41.6% vs 35.9%, p <0.0001), have a BMI ≥30 (23.5% vs 17.8%; p <0.0001) and have ductal tumors (86.8% vs 78.8%; p <0.0001). In dnMBC pts compared with rMBC pts, there were more visceral involvement in absence of central nervous system (CNS) disease (54.1% vs 42.6%; p <0.0001), and less CNS involvement (4.7% vs 16.9%; p <0.0001), respectively. HR-positive status was similar in both categories (dnMBC vs rMBC, 60.5% vs 59.7%, p= 0.54) while HER2 score 3+ on IHC was more frequent in dnMBC (86.8% vs 82.5%, p <0.0001).
Types of therapy by dnMBC and rMBC differed: first-line anti-HER2 treatment + chemotherapy +/- endocrine therapy (ET), anti-HER2 treatment + ET, anti-HER2 treatment alone or no anti-HER2 treatment in 88.4% and 66.6%, 3.2% and 6.6%, 1.6% and 7.6%, and 6.8% and 19.1% of pts, respectively.
The proportion of rMBC significantly decreased from 63.5% in 2008 to 32.9% in 2022 (Cochran–Armitage test; p < 0.0001), whereas the proportion of HR+ tumors significantly increased from 53.2% in 2008 to 68.3% in 2022 (Cochran–Armitage test; p < 0.0001) both in dnMBC (50.0% to 67.0%) and rMBC (55.0% to 70.9%) pts from 2008 to 2022 (Cochran–Armitage test; p=0.0197 and 0.0008, respectively).
With a median follow-up of 82.8 months (mo) (95%CI: 79.7-84.6), the median OS of dnMBC vs rMBC pts were 72.5 mo (95%CI: 66.4-77.3) vs 43.8 mo (95%CI: 41.6-46.4) and the median PFS1 were 18.9 mo (95%CI: 17.7-20.3) vs 9.4 mo (95%CI: 8.9-9.9). In the multivariable analysis, dnMBC was associated with better OS (adjusted hazard ratio (aHR): 0.65 (95%CI: 0.61-0.70); p < 0.001) and PFS1 (aHR: 0.64 (95% CI: 0.60-0.68); p < 0.001). In both categories of pts, HR- status, older age at metastatic diagnosis, presence of CNS disease, and multiple metastases were independently associated with poorer OS and PFS1. HER2 3+ status was an independent favorable factor for PFS1 in both dnMBC and rMBC pts, and for OS in dnMBC pts only.
Conclusion: Between 2008 and 2022, in a large national cohort of pts with HER2+ MBC, we observed an epidemiological shift from a majority of rMBC pts to a vast majority of dnMBC pts, along with an increase in the proportion of HR+ pts among those with HER2+ cancer. Patients with dnMBC experienced significantly longer PFS1 and OS than those with rMBC. Attention to dnMBC vs rMBC enrollment in studies of HER2+ MBC is needed in the design and contextualization of study results.

P3-08-04: Prevalence of HER2-ultralow subtype and outcome among patients with HR-positive (HR+) HER2-negative (HER2-) metastatic breast cancer receiving first line chemotherapy. A real-world single center study.

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Background: Recently, the DESTINY-Breast06 study has shown that Trastuzumab deruxtecan (T-DXd) outperforms standard of care (SOC) chemotherapy (CT) in terms of progression-free survival (PFS) as first-line treatment for HER2-low (L) and ultralow (UL) metastatic breast cancer (MBC) progressing after 1 or 2 lines of endocrine therapy including CDK4/6 inhibitor. Real world data on the prevalence of HER2-UL subtype and outcome with first-line SOC chemotherapy of HER2-L and HER2-UL MBC are needed to precise the medical need.
Methods: All cases of HR+/HER2-negative MBC (IHC scores of 0, 1+, and 2+/ISH-) and treated with first-line SOC chemotherapy at the Institut de Cancerologie de l'Ouest (ICO) were retrieved. All archived HER2 IHC slides of patients known as HER2-zero were subjected to rescoring by a pathologist according to ASCO CAP guidelines to distinguish HER2-null tumors (infiltrating cancer cells completely free of staining) and HER2-UL tumors (IHC 0 with incomplete and faint staining in ≤10% of tumor cells). Demographics, clinical characteristics, and treatments patterns were recorded. Kaplan-Meier method was used to estimate the real world PFS (rwPFS), time to next treatment (TTNT), and overall survival achieved with first-line SOC chemotherapy in the HER2-UL, L and null population.
Results: Between January 2016 and February 2023, 320 patients were included (HER2-L, n=198; HER2-zero n=122). After HER2 rescoring, historical HER2-zero group was finally reclassified in HER2-UL (n=50) and HER2-null (n=72). Overall, HER2-UL subtype accounted for 15.6% of the HR+/HER2-negative (IHC scores of 0, 1+, and 2+/ISH-) population. In the HER2-L and UL population (n=248), median age was 62.8 (IQR, 52.3-71.13) years, 73.0% and 16.9% of patients had visceral metastases or bone only disease respectively. Median number of previous endocrine therapy for MBC was 1 (IQR, 0-1) and 42.7% of patients had received previous CDK4/6i. First-line CT included taxane monotherapy (48.1%), combined with anthracyclines (1.6%), capecitabine (20.6%), anthracycline (19.7%), or other (10.0%). After a median follow up of 39.4 months [95% CI, 37.1-47.6], median PFS with first line CT was 7.9 months [95% CI, 4.6-19], 7.3 months [95% CI, 6.4-9.1] and 6.2 months [95% CI, 4.6-8.9] in the HER2-UL, L and null groups respectively. Median TTNT was 8.7 months [95% CI, 4.8-19.5], 8.2 months [95% CI, 7.0-9.9] and 7.0 months [95% CI, 5.1-10.3] in the HER2-UL, L and null groups. Patients with visceral metastases had a median PFS of 7.5 months [95% CI, 4.6-12.9], 6.6 months [95% CI, 5.6-7.8] and 5.1 months [95% CI, 3.8-7.6] in the HER2-UL, L and null groups. Median OS was 19.5 months [95% CI, 10.7-33.4], 21.5 months [95% CI, 19.0-25.8] and 16.6 months [95% CI, 11.9-23.6] in the in the HER2-UL, L and null groups respectively.
Conclusion: After HER2 rescoring following DESTINY-Breast06 trial presentation, HER2-UL MBC represents around 15% of HR+/HER2-negative MBC patients in our institution.

Outcome of HER2-UL and L MBC patients with SOC CT in a real-world setting is similar to those reported in the DESTINY-Breast06 trial.

P3-08-05: Cyclin inhibitors for breast cancer: A comparative real world data analysis

Julio Cesar Betiol, Beatriz Bueno, Pedro Exman

Background: More than 70% of patients (pts) with advanced breast cancer have hormone receptor positive disease and almost all pts will develop endocrine resistance during disease progression. CDK 4/6 inhibitors (CDK 4/6i) represent a new paradigm shift in treatment and currently ribociclib (Rib), palbociclib (Palb) and abemaciclib (Abem) are available. To date, Rib is the only to demonstrate a statistically significant (ss) median overall survival (mOS) gain in phase III trials, but data for Palb and Abem are still relevant despite the lack of statistical benefit. As there is no head-to-head study, we aim to evaluate the impact of these different CDK 4/6i as first-line therapy on mOS (median overall survival) in a contemporary real-world setting.
Methods: Data from TriNetX (a global dataset of electronic medical records of patients from 111 healthcare organizations) were analyzed and queried for patients with specific terms between 2004 and 2024. A propensity score matching (PSM) analysis balanced the cohort. Rib, Palb and Abem were compared using a 2x2 group selection method. Analysis 1 compared Rib versus (vs) Abem; Analysis 2 compared Rib vs Palb; and analysis 3 compared Palb vs Abem. mOS was evaluated with Kaplan–Meier method. Statistical comparison was made with a stratified log-rank test.
Results: No difference of risk for death were identified at Analysis 1 (n=271 pts in each arm), with a not reached mOS for both cohorts (5yrs OS = 61.82% vs 53.66%), x2=0.03, p 0.863, HR = 0.964, 95% CI = 0.634 – 1.467. Analysis 2 (n=980 pts in each arm) revealed a ss increased risk for death when receiving palb over rib, RR 2.42 (95% CI=-0.202, 0.280; p= <0.0001). with mOS of 1286 vs 1946 days (x2=15.447, p <0.0001, HR = 1.441, 95% CI = 1,200 – 1.731). Analysis 3 (n=318 in each arm), revealed a ss increased risk for death when receiving palb over rib RR 2.47 (95% CI=-0.231, 0.372; p= <0.0001) with mOS of 1124 vs 1706 (x2=9.025, p <0.003, HR = 1.56, 95% CI = 1,165 – 2.091).
Conclusions: Our study revealed that patients treated with Palb instead of rib or abem achieved a lower mOS with increased risk for death of breast cancer. Additionally, when comparing rib to abem, despite a percentual trend favoring rib, no ss difference were found in regards of overall survival analysis for risk of death of breast cancer.

P3-08-06: Clinical Activity of Sacituzumab Govitecan in Metastatic Triple Negative and Hormone Receptor Positive, HER-2 Negative Breast Cancer

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Background: Sacituzumab govitecan (SG) is an antibody-drug conjugate approved for the treatment of metastatic triple negative breast cancer (mTNBC) and hormone receptor positive (HR+), HER-2 negative breast cancer. Dose reductions may be necessary to manage adverse events (AE) such as neutropenia and diarrhea. We evaluated the clinical safety and efficacy of SG as well as incidence of SG dose reduction and utility of granulocyte colony stimulating factor (GCSF) support in patients with mTNBC and HR+, HER-2 negative breast cancer.
Methods: This study is a single institution retrospective review of patients with locally recurrent/inoperable or metastatic HR+/HER-2 negative (IHC 0, IHC 1+, or IHC 2+/ISH negative) or TNBC treated with SG at Rush University Medical Center from April 2020 to May 2024, with outcomes followed through July 2024. The use of GCSF with SG therapy for management of neutropenia, the need for dose reductions based on treatment-related AE, and survival data were collected.
Results: 49 total patients were included for retrospective chart review. Median age at the time of receiving SG was 58 years, while 41 patients (83.7%) had mTNBC and 8 (16.3%) shad HR+, HER-2 negative disease. Median number of lines of prior treatment in the metastatic setting was two (range 0-11). Patient race was 53.1% white, 28.6% African American, 10.2% Asian, 6.1% Hispanic/Latino and 2% identified as other race. Total of 18 (36.7%) patients required dose reductions at any time throughout course of SG treatment and 16 (32.7%) patients experienced delays or interruptions in treatment. The most common reason for SG discontinuation was disease progression (59%), followed by intolerable AE (12%), death/hospice enrollment (10%), and loss of follow-up (4%). Seven (14%) patients in the study cohort remain on treatment. Regarding neutropenia, the incidence of grade 1/2, grade 3 or higher and febrile neutropenia was 61.2%, 42.9% and 8.2%, respectively. One patient died as a complication of severe neutropenia. Twenty-seven (55.1%) patients required the use of GCSF due to treatment-related neutropenia. Of those patients that required GCSF, median number of cycles for which it was administered was over 4 cycles (range 1-48).
Among evaluable patients, the overall median progression free survival (mPFS) and median overall survival (mOS) were 4.25 and 9 months, respectively. Among 41 evaluable patients, the mPFS was higher in patients who received GCSF (n=23, mPFS 6.5 vs 3.89 months). Among 29 patients with OS data, those who received GCSF had longer OS (n=16; 9.0 vs 6.5 months). The mPFS among patients who required dose reduction (n=13) and patients who did not require dose reduction (n=27) were 5.75 and 3 months, respectively. The mOS among patients who required dose reduction (n=8) and patients who did not require dose reduction (n=20) were 13.53 and 9.63 months, respectively.
Conclusion: The safety and efficacy of SG for treatment of patients with mTNBC or metastatic HR+/HER-2 negative breast cancer in a real-world setting is comparable to results from the IMMU-132 and ASCENT trials. Guidelines for treating clinicians to manage side effects and optimize drug tolerability while limiting dose reductions/interruptions to treatment warrant further exploration, especially in a heavily pre-treated patient population.

P3-08-07: A real-world analysis of clinical characteristics and outcomes of early-stage, triple-negative breast cancer patients receiving anthracycline-sparing neoadjuvant chemoimmunotherapy: the Mayo Clinic experience

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Background: While anthracyclines are frequently utilized in the treatment of early-stage breast cancer (BC) patients (pts), they are associated with serious toxicities including dose-dependent cardiotoxicity and secondary myelodysplastic syndrome/acute myeloid leukemia. Neoadjuvant therapy (NAT) combining anthracycline-based chemotherapy and immunotherapy has become the standard of care for early-stage triple-negative BC per the pivotal KEYNOTE-522 study [1]. However, pts aged ≥65 represented <20% of KEYNOTE-522 pts. Here, we assessed the clinical and surgical outcomes of older adult pts who received anthracycline-sparing NAT for early-stage, triple-negative BC.
Methods: In this retrospective case series, 34 Mayo Clinic enterprise pts aged ≥65 who were documented to have 1) received anthracycline-sparing NAT with immunotherapy +/- chemotherapy and 2) completed surgery for nonmetastatic, HER2-negative BC between 6/2019 and 3/2024 were reviewed. We did include pts with low hormone receptor (ER +/- PR) expression. One pt who had a concomitant non-BC malignancy was excluded. Demographics, baseline clinical and tumor characteristics including stromal tumor-infiltrating lymphocytes (sTILs), reasons for anthracycline omission, NAT-related toxicities, and surgical outcomes were assessed. The primary endpoint was pathological complete response (pCR).
Results: Thirty-three pts were included. Median pt age was 75 (range, 65-89), and most pts (28/33, 84.8%) were Non-Hispanic White. Nine pts (27.3%) had a prior history of cancer, and 4/33 (12.1%) had a history of anthracycline use. Only 3/33 (6.1%) had documented LVEF ≤50% prior to NAT, one of whom had received an anthracycline previously. Most had grade 3 (24/33, 72.7%), cT2 (22/33, 66.7%), and cN1 (17/33, 51.5%) disease pre-NAT. Baseline sTIL % ranged from 0 to 80% for the 16 pts for whom these data were available. The most frequently administered NAT regimen was carboplatin, paclitaxel, and pembrolizumab (25/33, 75.8%). The most-often cited reasons for anthracycline omission were medical comorbidities including cardiac comorbidities (7/33, 21.2%), favorable clinical response to anthracycline-sparing NAT (7/33), and difficulty tolerating anthracycline-sparing NAT (7/33). Over half of pts (17/33, 51.5%) had a documented grade ≥3 adverse event (AE) of any type, of whom 6 pts had a grade ≥3 immune-related AE. Similarly, 17/33 pts had to discontinue all or part of NAT due to NAT-related toxicities. No pts had a documented grade 4-5 AE.
pCR was achieved by 11/33 (33.3%) pts, most of whom had cT2 (7/11, 63.6%), node-positive (7/11) BC pre-NAT. None had axillary nodal disease only. 2/11 (18.2%) had a BRCA mutation or variant of unknown significance, and 3/11 had ER +/- PR-low BC. All but 1 received carboplatin as part of NAT. Most pts (8/11, 72.7%) had a documented grade ≥3 AE, half of which were immune related, and 7/11 (63.6%) had to discontinue all or part of NAT due to NAT-related toxicities. Only 4/11 received adjuvant pembrolizumab (36.4%).
Conclusion: One third of elderly pts achieved pCR with a non-anthracycline-based NAT

regimen. However, over half of our sample (51.5%) experienced grade ≥3 AEs, including 72.7% of pts with pCR. Our institutional experience was limited by a small sample size. Analysis of pCR based on sTILs is ongoing.

Reference:
1. Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med. 2020;382(9):810-821. doi:10.1056/NEJMoa1910549

P3-08-08: Clinical and financial impact of implementation of Oncotype Dx in women with HR positive HER2 negative early breast cancer treated in a Chilean Cancer Center.

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Introduction: Fundación Arturo López Pérez Foundation (FALP) is a non-profit oncology institute located in Santiago, Chile. It serves patients from the public and private health system, associated or not to an oncology insurance offered by the same institution. Since 2022, this insurance covers Oncotype Dx genomic platform for its affiliates. We present real-world clinical and financial data associated with its application, with the aim of optimizing this resource in our institution.

Method: Ninety-one patients with HER2-negative hormone receptor-positive early breast cancer were prospectively analyzed between January 2022 and April 2024. In all of them Oncotype Dx was applied to define the indication for adjuvant chemotherapy (ChT).

The following clinicopathological variables were described in lymph node positive (N1) and negative (N0) patients: age, tumor size, estrogen and progesterone receptors, Ki67, grade and menopausal status. The association between the aforementioned variables and the recurrence score by Oncotype Dx (RS) for each group of patients was studied by Fisher's exact test. In addition, for both N0 and N1, the Predict score was calculated and its predictive ability to obtain a RS >25 was evaluated by means of ROC curves and area under the curve, identifying the best cut-off point.

From a financial point of view, costs were estimated by analyzing two scenarios. The first corresponds to the cohort with Oncotype. The second was simulated assuming that N0 patients with Predict ≥ 3 and 100% of N1 patients, in the absence of Oncotype Dx, would receive ChT.

Results: The median age was 61 years (48-77 y). 48% were diagnosed by screening. 59% were T1, with an average size of 1.8 cm (0.8 - 3.5 cm). 91% were ductal carcinomas and 22% were grade 3. 33 patients were N0. The mean age was 48.1 years, 33.3% were menopausal. There was a higher percentage of patients with T2 and grade 3 tumors. 58 patients were N1. The mean age was 61.1 years, all postmenopausal. A higher mean estrogen receptor and a lower mean Ki67 were observed. RS distribution had significant association with: progesterone receptors, Ki67, T and tumor grade, in both groups. The predictive ability of Predict for RS>25 was higher in N0 (AUC = 0.984) than in N1 (AUC = 0.793).

After analysis, in the N0 cohort, the best Predict cutoff point was 4. If tumor grade equal to 3 is also considered, the negative predictive value of Predict for SR>25 is 100%. In N1, a Predict cut-off point of 3.7 was calculated, obtaining a negative predictive value of 94.1%, leaving 2 patients with SR >25 undetected.
There was a 74% reduction in the total cost of ChT in the N1 cohort. When adding the cost of Oncotype Dx, the total expense is reduced by 32% in this group. When analyzing the N0 cohort, there is a 17% reduction in ChT costs. However, when including platform costs, there is a 41% increase in cost in this group. If we consider both cohorts, there is a 59% reduction in treatment costs, but only a 1% reduction in total costs.
Conclusions: There is a significant change in the indication for ChT by RS, de-escalating by 74% in the N1 group, and changing the indication by 32% in N0.
With respect to the financial analysis, and taking into account the costs in both groups, the savings are marginal.
Although more data are still required, in this study, the results suggest that Oncotype Dx should be indicated in N1 and only in N0 patients with Predict less than 4 and tumor grade 1 and 2, optimizing resources and improving cost-effectiveness.

P3-08-09: Clinicopathological feature based risk of recurrence in pT2N0 HR-positive and HER2-negative early breast cancer as included in NATALEE-trial: a retrospective, real-world, monocentric study

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Introduction: In the NATALEE-trial, Ribociclib added to adjuvant endocrine therapy significantly reduced breast cancer recurrence in the subgroup of women with high-risk, lymph node negative, hormone receptor (HR) positive, HER2 negative early stage disease. To assess the prognostic value of clinicopathological features alongside tumour stage, we compared breast cancer outcome between patients with high risk pT2N0 breast cancer (as eligible in the NATALEE-trial) and patients with a pT1-2N1 breast cancer.
Methods: This is a retrospective, monocentric study with real-world data of patients with HR-positive, HER2-negative early breast cancer treated in University Hospital Leuven (UHL) between January 2000 and October 2023. We defined high risk pT2N0 disease as grade 3 tumours or grade 2 with a K67 >20% or MammaPrint (MP) clinical high risk. pT1-2N1 patients were included regardless of grade, Ki67 index or MP clinical risk score. As per intern protocol, the Ki67 index was not determined for grade 3 tumours. All patients received adjuvant radiotherapy and systemic treatment according to institutional guidelines. Our endpoints were distant disease free survival (DDFS), invasive disease free survival (IDFS) and breast cancer specific survival (BCSS). We used Kaplan–Meier analyses for the estimation of the 5,10 and 15yr DDFS and IDFS and cumulative incidence function

(CIF) for BCSS, to evaluate the associations of the breast cancer's clinicopathological features with patients' outcomes. Analyses have been performed using SAS software.
Results: We included 711 patients with pT2N0 high risk breast cancer [median age: 62yrs; (neo-)adjuvant chemotherapy 42,5%]. 538 (75.7%) were grade 3 and 173 (24.3%) grade 2 with a high Ki67 or MP clinical high risk. 1902 patients with pT1-2N1 disease were included [median age: 58yrs; (neo-)adjuvant chemotherapy 49.2%]. 533 (28.0%) were grade 3 and 1356 patients had a lower grade of which 119 (6.2%) had a Ki67 >20% while 594 (31.2%) had a Ki67 <20% and 643 (33.8%) did not have Ki67 testing. Tumours that did not have Ki67 testing were MP clinical high risk. After 15 years, DDFS were 61.5% (95% CI [56.7;65.8]) and 64.0% (95% CI [61.2;66.7]) in the pT2N0 and pT1-2N1 groups respectively (HR 1.179 [1.008;1.378], p-value 0.0392). IDFS was 57.1% (95% CI [52.4;61.5]) for the pT2N0 group and 60.6% (95% CI [57.8;63.3]) for the pT1-2N1 group (HR 1.220 [1.052;1.414], p-value 0.0085). BCSS was 85.4% (95% CI [81.8;88.6]) in the pT2N0 group and 88.3% (95% CI [86.4;90.1]) in the pT1-2N1 group.
Conclusion: In this retrospective analysis DDFS, IDFS and BCSS were all numerically worse in a NATALEE eligible high risk pT2N0 population compared to a population with pT1-2N1 tumours of any grade or Ki67 index. These findings suggest that tumour biology may have a more important prognostic value than the tumour stage in HR-positive HER2 negative early stage breast cancer. Longer follow-up in the NATALEE-trial is needed to investigate the possible benefit of adding Ribociclib to adjuvant therapy based on clinicopathological features.

P3-08-10: Real-world treatment patterns and clinical outcomes for patients with metastatic triple-negative breast cancer (mTNBC) in the United States: an electronic health records observational study

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Background: Survival outcomes for patients with mTNBC have improved with the approval of immunotherapy for PD-L1-positive disease; however, chemotherapy remains the standard of care for patients who are PD-L1-negative and those who cannot receive immunotherapy. This study was initiated to assess real-world treatment patterns and clinical outcomes for patients with mTNBC in the United States.
Methods: This was a retrospective, observational study utilizing electronic health record data from the Flatiron Enhanced Datamart. Patients (≥18 years of age) diagnosed with mTNBC between January 01, 2018 and June 30, 2023, who received at least one line of therapy (LoT) in the metastatic setting were included. Patients diagnosed with other primary cancers (except non-melanoma skin cancer) ≤3 years prior to mTNBC diagnosis and patients enrolled in clinical trials were excluded. Patients were followed until date of death or last recorded activity in the Flatiron database, or data cut off (November 30, 2023). Baseline and clinical characteristics, treatments received, and clinical outcomes were captured. The primary endpoint of the study was the type of drug given for LoT1. Secondary endpoints included treatment patterns for LoT1–5, and clinical outcomes by LoT.

Results: The cohort comprised 1,044 patients with mTNBC. Most patients were female (99.2%); median age was 61 years (interquartile range 52–71) and 52.1% were White. The most common drug class in all LoTs was chemotherapy given as monotherapy or in combination with other agents: LoT1 87.5%, LoT2 76.8%, LoT3 69.8%, LoT4 70.7%, and LoT5 75.0%. Median (95% CI) real-world overall survival (rwOS) was 14.0 (12.9–16.0) months; real-world progression-free survival from mTNBC diagnosis was 5.8 (5.4–6.3) months. Among patients with confirmed PD-L1 status (n=367), 109 (29.7%) were PD-L1-positive and 258 (70.3%) were PD-L1-negative. Median rwOS (95% CI) was 18.6 months (15.2–24.4) in the PD-L1-positive cohort compared with 12.7 months (11.0–16.0) in the PD-L1-negative cohort. From 01 Jan 2021 onwards, immunotherapy was received by 21/39 (53.8%) patients who had PD-L1-positive tumors in LoT1, and by 33/39 (84.6%) patients with PD-L1-positive tumors across all LoTs. Median rwOS (95% CI) was longer for patients with PD-L1-positive tumors who received immunotherapy (18.6 months [15.2–26.3]) months than for those who did not (15.5 months [18.3–not estimable]).
Conclusions: In this real-world study in the United States, clinical outcomes in patients with mTNBC remain poor, particularly among those with PD-L1-negative disease. There is a high unmet need for more efficacious and tolerable treatments for mTNBC, including targeted agents that may be more effective and less toxic compared to conventional chemotherapy.

P3-08-11: Clinico-Pathological Features of Breast Cancer Patients Who Recured After Achieving pCR with Neoadjuvant Chemotherapy before Surgery (KBCSG-18)

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Background: Patients who achieve a pathologic complete response (pCR) after neoadjuvant chemotherapy (NAC) before surgery have been reported to have higher long-term survival rates. However, despite a better prognosis, pCR patients have a potential for local and/or distant recurrence. Herein, we report clinico-pathological features of breast cancer patients who recurred after achieving pCR with NAC.
Methods: This study retrospectively reviewed the data of patients diagnosed with breast cancer who experienced recurrence after achieving pCR following NAC between January 1999 and April 2022 from 13 cancer hospitals in the Korean Breast Cancer Society Group (KBCSG). The patients were identified in a database that had been managed in each hospital. pCR was defined as no residual invasive cancer in the breast and axillary nodes (ypT0 or ypTis with ypN0). Breast cancers were classified into three subtypes based on hormone receptors (HR) and human epidermal growth factor receptor 2 (HER2) status. The primary purpose of the study was to identify the clinicopathologic characteristics, recurrence patterns, and overall survival of patients who experienced recurrence after achieving pCR following NAC. This study was approved by an institutional review board in each hospital.
Results

A total of 131 patients were identified to have achieved pCR and relapsed thereafter. The median age of the patients was 48 years (range 27-72), with a median follow-up duration of 25.6 months (range 0.5 - 136.7). The median recurrence-free survival (RFS) was 17.0 months (95% CI 13.5-20.5). The distribution of initial clinical TNM stages was as follows: stage IB in 1 (0.8%), stage IIA in 16 (12.2%), stage IIB in 27 (20.6%), stage IIIA in 29 (22.1%), stage IIIB in 11 (8.4%), and stage IIIC in 45 (34.4%) patients. This study included 70 (53.4%) HER2-positive, 50 (38.2%) triple-negative and 8 (6.1%) HR-positive breast cancer patients. Among these populations, 81 (61.8%) had systemic recurrence, 33 (25.2%) experienced local recurrence, and 17 (13.0%) had both local and distant recurrence. Upon reviewing the first presented recurrence patterns of 131 patients, they showed brain (n=45, 34.4%), lung (n=26, 19.8%), liver (n=14, 12.2%), bone (n=14, 10.7%), locoregional lymph node (n=16, 12.2%), ipsilateral breast (n=24, 18.3%), and contralateral breast (n=8, 6.1%) recurrence. After recurrence, median overall survival was 76.7 months (95% CI 41.2-112.2) in the HER2-positive, 45.8 months (95% CI 16.5-75.1) in TNBC, and 40.3 months (95% CI 2.7-77.8) in HR-positive subtypes.
Conclusion: Given the limitation of retrospective study, such as selection bias, patients with pCR after NAC followed by relapse showed a high incidence of brain metastasis as the first relapse site and favorable overall survival compared to historical data.

P3-08-12: Poor Response of Metaplastic Triple-Negative Breast Cancer to Neoadjuvant Chemoimmunotherapy: An Unmet Oncological Need

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Introduction: Metaplastic breast cancer (MpBC) is a rare and aggressive subtype characterized by clinical and pathological heterogeneity, advanced stage at presentation, frequent chemoresistance, and distant spread. The majority of MpBCs are hormone receptor and HER2 negative and thus treatment protocols and guidelines tend to follow those of triple-negative breast cancer (TNBC). Oncology teams have begun to use the KEYNOTE-522 (KN-522) neoadjuvant chemoimmunotherapy regimen to treat TNBC MpBC. Real-world data on MpBC responses to this regimen are limited, and the KN-522 trial did not report the inclusion of patients with MpBC.

Methods: We conducted a real-world review of patients with biopsy-proven, non-metastatic, TNBC MpBC who were treated with the KN-522 neoadjuvant regimen at our institution. This regimen consists of neoadjuvant paclitaxel, carboplatin, doxorubicin, and cyclophosphamide, combined with neoadjuvant and adjuvant pembrolizumab, an immune checkpoint inhibitor monoclonal antibody against programmed cell death protein 1. Institutional Review Board (0430-23-EP) approval and consent from all patients were obtained. Demographic, clinical, and pathological data were collected. The primary outcome was pathological complete response (pCR) defined as ypT0/Tis ypN0. The secondary

outcomes were adverse events (AEs) and recurrence-free survival.

Results: Between July 2021 (KN-522 regulatory approval) and July 2024, seven female patients aged 39-78 presented to our cancer center with T2 (>2 cm) or larger, and/or node-positive, biopsy-proven TNBC MpBC. Three patients were Non-Hispanic White, two were Non-Hispanic Black, and two were Hispanic. One patient had a pathogenic variant on CHEK2 and another on BRCA1. Prognostic stages ranged from stage IIB to IIIC. Metaplastic carcinoma differentiation included squamous keratin-producing, chondroid, chondromyxoid, and mesenchymal spindle cell types. All patients were recommended to receive the KN-522 neoadjuvant regimen consisting of four cycles of pembrolizumab, paclitaxel, and carboplatin, followed by four cycles of pembrolizumab, doxorubicin, and cyclophosphamide, followed by nine cycles of adjuvant pembrolizumab. Three patients discontinued their neoadjuvant regimen and proceeded directly to surgery due to intolerance of AEs for two and local tumor progression for the other. Five patients underwent dose reductions of the regimen due to AEs, and one patient discontinued pembrolizumab due to grade III immune-related colitis. Another patient did not receive adjuvant pembrolizumab due to high-dose steroid treatments for kidney transplant rejection. Five patients underwent lumpectomy with sentinel lymph node biopsy (SLNB) and two underwent mastectomy with SLNB with targeted axillary dissection, with one of these undergoing a completion axillary lymph node dissection. No patient sustained a pCR on final pathology (0%), with residual cancer burden (RCB) classes of two patients with RCB-III, four patients with RCB-II, and one patient with RCB-I. At the last follow up, no patient had experienced breast cancer recurrence, with a median follow-up of only 9 months after completion of all loco-regional treatments (range, 2-19 months).

Conclusion: In this real-world experience, none of our patients with TNBC MpBC treated with the KN-522 neoadjuvant regimen achieved a pCR. These patients also exhibited poor tolerance, requiring frequent dose reductions and discontinuations. Despite no patient has experienced recurred to date, the follow-up period is still immature. This experience highlights the need to evaluate whether KN-522 is an appropriate neoadjuvant regimen for TNBC MpBC, and underscores the distinct heterogeneous biology of MpBC and its unmet oncological needs.

P3-08-13: Measuring the impact of Komen's Financial Assistance program on the treatment experience of those living with MBC

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Background: The American Cancer Society published that 61% of cancer patients find it difficult to pay for their care (2021). Additionally, lower income groups often delay or skip filling prescriptions to save money or cut pills to reduce care costs (ACS, 2021). Patients

also report making financial sacrifices to pay for their care or avoiding scheduling or cancelling previously scheduled appointments due to associated costs. Susan G. Komen's Komen Financial Assistance Program provides direct cash assistance to qualifying individuals diagnosed with breast cancer. Meeting the needs of those living with metastatic breast cancer (MBC) is a priority for Komen, and last year, 27% of those who received funds though the Komen Financial Assistance Program identified as living with MBC.
Methods: When a patient applies for assistance through the program, an application is completed that collects demographic information, the primary financial need, information about their diagnosis, and income information. To measure the impact of Komen's Financial Assistance program, an evaluation survey is distributed 30 days after the assistance is provided. Survey questions ask patients about their experience with the program and how the assistance was used. Questions included are meant to determine if the assistance provided by Komen affected the financial stressors experienced by patients that has been documented in peer reviewed literature. The survey asks if the assistance helped patients continue their care, if they were able to avoid skipping breast cancer related appointments, if they were able to avoid skipping medications, and if the assistance allowed them to make fewer financial sacrifices. For analysis, the data was sorted by breast cancer stage, and an in-depth analysis was completed for recipients who are living with metastatic breast cancer.
Results: Though analysis of the application data, it was found that, across all approved applicants (Stages 0 - IV), the highest reported financial need was for rent or housing (31.4%). When stratified by stage, it was found that the highest reported financial need of those living with MBC was oral treatment medications (40.6%). Of survey respondents living with MBC (N=1,668), 54.3% said that the assistance helped them avoid skipping medications. Sixty-one percent of respondents living with MBC indicated that the assistance helped them avoid delaying or skipping breast cancer related appointments. Additionally, 97.7% of those living with MBC agreed that, after receiving the assistance, they were better able to continue their breast cancer care. Finally, 92.3% of survey respondents living with MBC said that, after receiving the assistance, they had to make fewer financial sacrifices to cover the cost of their breast cancer care.
Conclusion: Through the evaluation of Komen's Financial Assistance Program, results show that direct cash financial assistance to patients helps alleviate financial stressors, particularly for those living with MBC. Komen's Financial Assistance Program substantially impacts those it serves who are living with MBC. However, financial toxicity is a complex issue and requires intervention at multiple levels for systemic change. This includes support from policy makers for expanded health care coverage, continued funding of programs like Komen's financial assistance program, and addressing the unique needs of specific groups.

P3-08-14: Analysis of the Occurrence Time of Bone Metastasis and Disease Treatment Patterns in 737 Patients with Breast Cancer Bone Metastasis: A Retrospective Study from China

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Background:Bone is the most frequent site of metastasis in breast cancer, with a significant proportion of patients experiencing bone involvement at the initial metastasis. Identifying the critical time periods for its occurrence is crucial for guiding bone metastasis screening, enabling early detection, and facilitating prompt treatment. Since bone targeted agents(BTAs) are vital component of systemic therapy for advanced breast cancer, elucidating real-world treatment patterns holds significant importance for guiding clinical practice advancements.
Methods:This single-center retrospective study analyzed patients with advanced breast cancer and bone metastasis admitted to Zhejiang Cancer Hospital between January 1, 2021, and February 28, 2023. Subgroup analyses were performed to analyze correlations between the time from diagnosis to bone metastasis and clinical factors. The skeletal-related events (SREs) were collected during the follow-up. For quantitative indicators that conformed to normal distribution, independent sample t-tests or analysis of variance (ANOVA) were employed; otherwise, non-parametric Mann-Whitney U tests or Kruskal-Wallis tests were used. Bone CT values were quantitatively assessed to evaluate the therapeutic efficacy of bone metastases.
Results:Among 737 breast cancer patients with bone metastasis, 416 (71.1%) had bone metastases at the initial metastasis, and within this group, 180 patients (43.8%) had bone as the only site of metastasis; the other 169 patients (28.9%) did not exhibit bone metastasis initially but developed it during subsequent treatment and follow-up. The time from breast cancer diagnosis to the first occurrence of bone metastasis (median, months[mos]) was significantly associated with factors such as receipt of neoadjuvant therapy (73.7mos for no neoadjuvant therapy received, 50.6mos for neoadjuvant therapy received; $p<0.001$), surgical approach (radical surgery 71.2mos, palliative surgery 24.8mos; $p=0.001$), number of postoperative pathological lymph node metastases (N0 72.5mos, N1 72.8mos, N2 60.9mos, N3 51.5mos; $p=0.001$); vascular tumor thrombus invasion (positive 48.2mos, negative 65.1mos; $p=0.001$); postoperative stage (Stage I 54.6mos, Stage II 64.1mos, Stage III 53.5mos; $p<0.001$); molecular subtype (HER2+ 48.6mos, Luminal A 77.6mos, Luminal B HER2+ 65.2mos, Luminal B HER2- 63.9mos, TNBC 43.5mos; $p<0.001$); and Ki 67 expression (73.3ms for Ki 67<15%, 55.4ms for Ki 67≥15%; $p=0.005$). Of which, the shorter time to bone metastasis were observed with higher Ki 67 values. However, no significant correlation was found between the time from breast cancer diagnosis to bone metastasis and tumor size, histological grade, or pathological type. Among these patients, 90.4% received BTA treatment, and 95.8% of them adhered to the treatment schedule. Furthermore, 35.6% of the patients changed the type of BTA during treatment. Quantitative assessment of bone lesion efficacy using CT values revealed a median pre-treatment CT value of 306.0, which increased to 445.6 post-treatment ($p<0.001$). During follow-up, 176

patients (23.9%) experienced SREs, primarily bone radiation therapy (47.4%), pathological fractures (35.8%), and bone surgery (27.8%).
Conclusion:The timing of bone metastasis after breast cancer diagnosis is significantly correlated with factors such as whether or not neoadjuvant therapy was administered, the surgical approach used, the number of metastatic lymph nodes, the postoperative staging, the molecular subtype, the expression of Ki 67, and the presence of vascular invasion by tumor thrombus. Real-world data from this center suggests that bone targeted agents for breast cancer patients with bone metastasis contribute to improving bone lesion CT values.

P3-08-15: Overall Survival and Economic Impact of Triple-Negative Breast Cancer in Brazilian Public Healthcare: A Real-World Study

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Background: Triple-negative breast cancer (TNBC) represents considerable treatment challenges, particularly in public healthcare systems within low- and middle-income countries, where access to the most advanced treatment options, such as Antibody-Drug Conjugates (ADCs), immunotherapies, and PARP inhibitors, is often limited. This study seeks to fill the knowledge gap on TNBC, analyzing the clinical, epidemiological, and economic impact of these characteristics affecting survival rates in Brazilian public healthcare system.
Methods: A real-world, retrospective cohort study was conducted at Pérola Byington Hospital in São Paulo, Brazil, involving all TNBC patients treated between 2010 and 2019. Stage-specific overall survival (OS) rates were calculated, and OS was compared among patients who received neoadjuvant or adjuvant treatment, those with and without a complete pathological response, black and non-black patients, and those treated with or without carboplatin-based neoadjuvant therapy. Cox proportional hazards regression models were fitted to estimate Hazard Ratios (HR) with 95% confidence intervals (CI). The annual costs for each stage of systemic TNBC treatment were estimated from AHCP (Authorization for High Complexity Procedures) data for the year 2023.
Results: 1.266 patients had TNBC, and 710 met the eligibility criteria for inclusion. Kaplan-Meier analyses highlight the disparities in TNBC patient outcomes across stages, with stage II patients experiencing a 47% lower mortality risk compared to stage III (HR=0.53; 95% CI 0.33 to0.85; p=0.009). Moreover, patients in the adjuvant treatment group had a reduction in the risk (HR= 0.48; 95% CI 0.34 to 0.69) compared to those in the neoadjuvant treatment. Patients achieving a complete pathological response (pCR) substantially improved OS (HR=0.21; 95% CI 0.11 to 0.43; p<0.001). (HR=0.24, 95% CI 0.13 to 0.46; p<0.001). Black patients have better survival compared to non-black patients (HR=0.58; 95% CI 0.40 to

0.86; p=0.006). The use of Carboplatin in treatment does not show a significant improvement in OS compared to treatments without Carboplatin (HR=0.96; 95% CI 0.65 to 1.43; p=0.857). The average monthly cost for systemic TNBC treatment increases with disease progression: $101.87 for Stage I and up to $314.77 for Stage IV second-line therapy. The total cumulative cost ranges from $11,918.98 for Stage I to $28,9685.24 for Stage IV treatment of TNBC.
Conclusions: This study offered a comprehensive description of the clinical and epidemiological profiles of TNBC patients within Brazil's public healthcare framework. It has demonstrated that the OS rates for TNBC patients decline as the disease progresses to advanced stages and was better for black population. Additionally, the data indicates that achieving a pCR after treatment and received adjuvant treatment is associated with a marked improvement in survival rates. Direct costs escalate substantially as patients progress to higher stages, underscoring the economic burden and the increased complexity of managing advanced TNBC.

P3-08-16: TADPOLE: A multicentre, pragmatic, phase III randomised controlled trial comparing Targeted Axillary Dissection vs axillary node clearance in patients with POsitive axillary Lymph nodes in Early breast cancer

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Background: In the UK, all patients with newly diagnosed invasive breast cancer have axillary staging with an axillary USS +/- biopsy of abnormal/equivocal nodes and currently UK NICE guidelines recommend axillary node clearance (ANC) for allpatients with biopsy proven node positive breast cancer having primary surgery, irrespective of the number of nodes involved (~20% of all patients). This highly morbid procedure leads to life-long complications in 1 in 3 patients including lymphoedema and chronic pain which dramatically impact quality of life.
ANC aims to reduce locoregional recurrence (LRR) and improve breast cancer survival but there is no evidence to support these benefits for patients with limited nodal involvement (cN0, radiologically detected disease). These patients would meet the criteria for omission of ANC based on eligibility for the ACOSOG Z0011 trial, but this approach has not been adopted in the UK due to concerns regarding false negative sentinel node biopsy (SNB) in node positive patients.
Targeted axillary dissection (TAD) which combines removal of the localised biopsy proven involved node(s) in combination with a SNB may offer an alternative to ANC, effectively addressing concerns regarding false negative rates while reducing the risk of life-changing complications
The TADPOLE study aims to determine if TAD is a clinically and cost-effective alternative to

ANC in patients with low volume node positive breast cancer having primary surgery.
Methods: TADPOLE is a multicentre pragmatic phase 3 randomised controlled trial comparing TAD and ANC in breast cancer patients with low volume nodal disease having primary surgery.
All patients with cN0 biopsy proven low volume axillary nodal disease will be eligible to participate. Excluded will be patients with >3 nodes on USS, those who have recurrent disease, previous axillary surgery or neoadjuvant systemic therapy. Participants will be randomised 2:1 to TAD or ANC. Surgical quality assurance (QA) processes will promote standardised introduction of 'primary' TAD in the UK and ensure procedure fidelity within the trial. Participants will have adjuvant therapy as per standard of care but axillary radiotherapy (ART) will be prohibited in the TAD group. Robust radiotherapy quality assurance (RTTQA) will be embedded throughout.
The co-primary end-points are:
i) Patient reported and objective lymphoedema at 12 months
ii) Single arm analysis of LRR at 5 years in the TAD cohort.
Recruitment of 390 patients in the TAD arm will be required to detect a 50% reduction in lymphoedema at 12 months with 90% power and a type 1 error of 5% and exclude an undesirable LRR of <5% in the TAD cohort at 5 years with one sided 2.5% alpha and 90% power. Inflating for multiplicity and allowing for 5% dropout and 5% crossover a total of 861 patients (574 TAD:287 ANC) will be required for the trial. 40 UK breast units will recruit to the trial.
An embedded qualitative study will optimise recruitment and a SWAT (study within a trial) will optimise the inclusion of non-English speaking participants.
Results: Consensus work with UK breast surgeons to agree how to standardise axillary surgery within the trial is underway and will underpin the surgical QA within TADPOLE. The trial will commence recruitment early 2025 and include a 9 month internal pilot phase. Recruitment is planned for 28 months with a target of 1 participant/centre/m at 40 sites.
Conclusion: TADPOLE has been designed with extensive patient and public involvement and will address the top UK breast surgery research priority identified in the James Lind Alliance Priority Setting Process. If TAD causes less lymphoedema and is oncologically safe, TADPOLE will change practice, improving outcomes for thousands of patients with node positive breast cancer each year.

P3-08-17: TBCRC 058: A Randomized Phase II Study of Enzalutamide, Enzalutamide with Mifepristone, & Treatment of Physician's Choice in Patients with Androgen Receptor-Positive Metastatic Triple-Negative or Estrogen Receptor-Low Breast Cancer (NCT06099769)

Rita Nanda, Yuan Chen, Katia Khoury, Hope S. Rugo, Erica L. Mayer, Lisa A. Carey, Michelle Melisko, Angemael Syldor, Chaya Friedman, Jennifer Savoie, Fresia Pareja, Britta Weigelt, Sarat Chandarlapaty, Nicholas Turner, Joshua Lang, Marina Sharifi, Suzanne Conzen, Tiffany Traina

Background: Triple-negative breast cancer (TNBC) refers to a heterogenous group of breast cancers that lack expression of ER, PR, and HER2. Despite recent advances with immunotherapy (IO) and antibody-drug conjugates (ADCs), TNBC remains the most aggressive subtype, characterized by a high risk of recurrence and a short overall survival in the metastatic setting. Breast tumors with low levels of ER and PR expression (1-10%) clinically behave like TNBC, and clinical management follows the TNBC treatment (tx) paradigm. We and others have identified a subset of breast tumors which are ER/PR/HER2 negative and express the androgen receptor (AR). Enzalutamide (enza), an AR-antagonist, had demonstrated activity in AR+ metastatic TNBC (Traina et al, JCO 2018). Activation of the glucocorticoid receptor (GR) has been implicated as a mechanism of resistance to AR inhibition in prostate and breast cancers (Kach et al, Sci Transl Med 2015). Advanced TNBC remains an area of high unmet need, particularly in patients who are ineligible for or progress following a checkpoint inhibitor. This randomized study will evaluate the efficacy of enzalutamide or enzalutamide plus the GR antagonist mifepristone (mif) as compared to physician's choice chemotherapy (TPC).
Methods: This is a randomized phase II trial; 201 patients (pts) will be randomized in a 1:1:1 fashion to enza, enza with mif, or TPC (carboplatin, paclitaxel, eribulin, or capecitabine). The primary endpoint (endpt) is progression free survival (PFS), and the trial is designed to test the hypothesis that PFS in the pooled enzalutamide arms is superior to TPC; there is 80% power to detect a hazard ratio (HR) of 0.70, corresponding to increase in PFS from 3.5 months (mos) with TPC to 5.0 mos with enza-based tx. Secondary endpts include comparisons of PFS among 3 arms and evaluation of response rate, clinical benefit rate, duration of response, overall survival, safety/toxicity, and patient-reported outcomes by arm. Exploratory endpts include correlation of tumor and circulating markers (AR-V7 in circulating tumor cells and circulating tumor cell DNA) with tx response. Eligible pts must have: ECOG 0-2, metastatic ER/PR low or negative, HER2 negative breast cancer (BC), measurable or evaluable disease (dz), < 2 prior lines of chemotx, any # prior endocrine txs, no prior anti-AR tx, no prior mif, concurrent CYP17 inhibitors prohibited. Pts with PD-L1+ BC must have received prior IO if not contraindicated. Tumors must have AR >/= 10%, normal organ function, no history of brain mets. As of 7/1/2024, 7 of 201 pts have begun protocol-specified tx.
This trial is supported by the TBCRC, BCRF, The TaTa Sisterhood Foundation, Pfizer/Astellas, and Corcept Therapeutics.

P3-08-18: The AmeliaTM-1 study: A phase 1b/2 study assessing the safety and efficacy of evexomostat (SDX-7320) plus a PI3K/Akt inhibitor and fulvestrant in patients with advanced HR+/Her2- breast cancer

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Clinical Rationale: Breast cancer patients with alterations in the PI3K pathway (eg, mutations in PIK3CA, Akt or loss of PTEN) have more aggressive disease and worse

outcomes relative to patients without these alterations. Agents approved or in late-stage development for treating this patient population (alpelisib, capivasertib, inavolisib) can cause on-target hyperglycemia leading to hyperinsulinemia which may limit effectiveness of these drugs by overcoming pathway inhibition and/or secondary to reduced dose-density. Restoring insulin sensitivity (which reduces systemic levels of insulin) has been shown to improve the efficacy of PI3K inhibitors in preclinical models of breast cancer.
Evexomostat is a polymer-drug conjugate of a novel small molecule methionine aminopeptidase 2 (MetAP2) inhibitor that in a xenograft model of HR+/Her2-/PIK3CA-mutant breast cancer showed synergistic anti-tumor activity with alpelisib, and in a xenograft model of HR+/Her2+ breast cancer enhanced the efficacy of capivasertib. Studies conducted in normal mice demonstrated that evexomostat significantly reduced hyperglycemia and hyperinsulinemia induced by these PI3K/Akt pathway inhibitors. Evexomostat was well-tolerated in a phase 1 monotherapy safety study in late-stage cancer patients and improved insulin resistance in patients with elevated insulin at baseline, along with improvements in other metabolic and angiogenic markers,
Study Design: This is a phase 1b/2, open-label, single-arm proof-of-concept study in postmenopausal women with HR+, HER2- metastatic breast cancer harboring alterations in the PI3K pathway who progressed following endocrine therapy plus a CDK4/6 inhibitor (www.amelia1.com; NCT05455619).
The primary objective is to determine the safety of evexomostat plus standard of care treatment (physician's choice of alpelisib or capivasertib) and fulvestrant (combined, the 'triplet therapy'), to measure the severity and number of hyperglycemic events, and to assess anti-tumor benefit of the triplet therapy.
The trial consists of a dose-escalation cohort (n=6) with evexomostat dosed at 36 mg/m2 (one dose below the phase I monotherapy MTD of 49 mg/m2) in combination with either alpelisib or capivasertib and fulvestrant dosed in accordance with their respective labels. Based on safety data from the first 6 patients in each triplet combination to complete two cycles, the safety review committee may alter the evexomostat dose for the next safety cohort of six patients.
Once the MTD of the triplet therapy is defined, additional patients will be enrolled until a total of up to 20 patients have completed at least two cycles of triplet therapy at that dose. If warranted, an additional 20 patients may be enrolled to further characterize the efficacy and safety of the triplet therapy. To date, eight patients have been enrolled.
Planned Analyses: Primary safety analysis consists of the number of patients with grade 3 or 4 hyperglycemia during the first 2 cycles of triplet therapy plus the type, frequency, and severity of treatment-emergent adverse events (TEAEs) per the NCI CTCAE, v5.0. Efficacy analyses include calculation of the ORR, consisting of complete response (CR) and partial response (PR). The number of patients alive without disease progression six months from the start of evexomostat and fulvestrant dosing will also be assessed. The CBR of CRs, PRs plus stable disease ≥24 weeks from C1D1 will be calculated.
QoL will be analyzed according to functional scores and recommendations in the EORTC scoring manual. ECOG performance status and change from baseline will be summarized.

P3-08-19: Preliminary results from PIKture-01, a First-in-Human Study of OKI-219, a mutant selective inhibitor of PI3Kα-H1047R, in mutant selected solid tumors including breast cancer.

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Background: Aberrant activation of the PI3Ka pathway contributes to tumorigenesis and is associated with resistance to anticancer therapies, making this pathway an attractive target for new therapies[1]. PI3Kα is one of the most commonly mutated oncogenes, found in approximately 13% of human cancers and 29% of breast cancer [2]. There are three predominate mutations in PI3Kα important in cancer. The H1047R mutation in the kinase domain is the most common of the three, found in 40% of HR+/HER2– breast cancers and 10-15% of HER2+ breast cancers [3]. The currently approved PI3Kα inhibitors such as alpelisib target both wild-type and mutant forms, leading to significant on-target toxicities, including hyperglycemia, rash, and diarrhea [4,5]. OKI-219, a mutant-selective PI3Kα inhibitor, has shown preclinical efficacy in PI3Kα-H1047R-mutated models, without the metabolic dysfunction associated with wild-type inhibition, supporting a potential improved therapeutic profile. We hypothesize OKI-219 may achieve greater mutant target coverage with a wider therapeutic window compared to other non-selective PI3Kα inhibitors.
Methods: PIKture-01 is a global, multi-center, first-in-human phase 1a/1b study evaluating OKI-219 as monotherapy and in combination with fulvestrant or trastuzumab in subjects with advanced solid tumors including breast cancer harboring a PI3Kα-H1047R mutation. In Phase 1a, subjects receive escalating oral doses of OKI-219 starting at 300 mg BID continuously. Phase 1b will assess OKI-219 in combination with fulvestrant in patients with HR+ breast cancer, or with trastuzumab in patients with HER2+ breast cancer. The study also includes a dose optimization phase to evaluate the optimal combination doses of OKI-219 with fulvestrant or trastuzumab.
Results: As of 30 September 2024, OKI-219 has been dosed at three dose levels as a single agent: 300 mg BID, 600 mg BID, and 900 mg BID, continuously. Across all dose levels to date, a total of ten subjects have been dosed: six subjects with HR+/HER2- breast cancer, two with HER2+ positive breast cancer, and two with colorectal cancer in the single agent dose escalation. Eight of the ten subjects remain on study. OKI-219 has been very well tolerated, with no dose-limiting toxicities, dose interruptions, or dose reductions required. The most common treatment-emergent adverse events (TEAE) that occurred in >15% of subjects were urinary tract infection, upper extremity cellulitis and pruritus. The most common treatment-related adverse events (TRAE) were grade 1 pruritus. Single-dose pharmacokinetic (PK) results of OKI-219 are consistent with predicted human exposures. At steady state, the exposures of OKI-219 exceed exposures associated with robust antitumor activity in preclinical models.
Conclusion: OKI-219 has been very well tolerated with a favorable safety profile, and only Grade 1 TRAEs observed at exposures that are consistent with preclinical activity, even at

the lowest dose level. As a single agent, OKI-219 has shown favorable PK that support pharmacologically relevant exposures, even at the lowest assessed dose levels, with a safety profile that suggests little or no inhibition of WT PI3Kα. We anticipate near completion of enrollment of the single agent portion of the study as well as the initiation of combination expansion of OKI-219 with fulvestrant by the end of the year. Data will be updated accordingly.

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P3-08-20: The PREDICT II Registry: A Prospective Study to Evaluate the Clinical Utility of a 7-Gene Predictive Biosignature on Treatment Decisions in Patients with Ductal Carcinoma In Situ

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Purpose/Objective(s): For women with ductal carcinoma in situ (DCIS) treated with breast conserving surgery (BCS), the benefit of adjuvant radiation therapy (RT) remains controversial. Since level 1 evidence supports the role of RT in reducing the risk of local recurrence, current guidelines generally recommend RT for all women having BCS even though 70-80% do not recur after BCS alone. In response to the need for prognostic and predictive tools for DCIS, a 7-gene predictive biosignature was developed to better assess risk of recurrence and RT benefit. The test provides a validated Decision Score (DS) for assessing 10-year risk of recurrence and RT benefit using individual tumor biology, as assessed by clinical and pathologic biomarkers. The primary objective of the PREDICT registries is to understand the decision impact such a tool has on treatment decisions.
Materials/Methods: This is a multicenter, prospective, observational registry for women diagnosed with DCIS. The primary endpoints are changes in treatment recommendations for surgical, radiation, and hormonal therapy. Secondary endpoints are identification of key drivers for treatment recommendations, such as age, size, grade, patient preference and biosignature status. The study includes females age 26-89 who are candidates for BCS and eligible for RT and/or systemic treatment. Subjects must not have been previously treated

for DCIS or have previous or current invasive or microinvasive breast cancer. After DCIS diagnosis, sites will send the most representative tissue block or sections to a CLIA lab for biosignature testing. Treating physicians will complete a treatment recommendation survey before and after receiving the biosignature test results. Test results, treatment recommendations, patient preferences and clinicopathologic features will be stored in a de-identified registry. Women will be followed for up to 10 years. Changes in pre- and post-test treatment recommendations will be analyzed using McNemar's test (alpha level = 0.05). Multivariate logistic regression will be used to determine odds ratios of clinicopathologic factors leading to pre- and post-test treatment recommendations. Pre-test covariates include patient age, tumor size, palpability, margin status, hormone receptor status, nuclear grade, tumor necrosis, family history of breast cancer, race, ethnicity and patient preference, as well as physician specialty (surgeons vs. radiation oncologists) and post-test covariates will also include the biosignature score. Differences in recurrence-free and overall survival will be assessed by Kaplan-Meier survival analysis using the log-rank test and/or the Cox Proportional Hazards model. The study will enroll up to 3,000 women from about 30 sites across the US. The study has been approved by WCG IRB (Tracking #20172841) and/or local IRBs at each site. ClinicalTrials.gov: NCT03448926.

P3-08-21: Trastuzumab-deruxtecan in HER2-low metastatic breast cancer patients with newly diagnosed or progressing brain metastases: The TUXEDO-4 phase II trial

Maximilian Marhold, Marta Vaz-Batista, María Gión, Manuel Ruíz-Borrego, Isabel Blancas, Cristina Morales, Cristina Saavedra, Felipe Slebe, Marta Campolier, Juliana Carvalho Santos, José Antonio Guerrero-Martínez, Miguel Sampayo-Cordero, Rupert Bartsch, Matthias Preusser

Breast cancer (BC) is the second most common cause of brain metastases (BM) among all solid malignancies, and the leading cause of leptomeningeal disease (LMD). Both BM and LMD are associated with high morbidity and mortality and treatment options are limited. While BM are commonly diagnosed late during the course of metastatic disease in patients with luminal BC, LMD is often diagnosed simultanously and no established systemic treatment option exists to date. Trastuzumab deruxtecan (T-DXd), an antibody drug conjugate combining a HER2-directed antibody with a topoisomerase I inhibitor, showed promising results in mBC patients, including HER2-positive mBC patients with active BM and patients with low HER2 expression (IHC 1+ or 2+ in the absence of ERBB2 amplification). We therefore hypothesized that T-DXd could exhibit relevant clinical activity in HER2-low mBC patients with BM with or without type II LMD. The TUXEDO-4 study is an international, multicenter, single-arm, two-stage optimal Simon's design, phase II trial (NCT06048718). A total of 27 patients (13 in stage 1, and 14 in stage 2 depending on responses in stage 1) will be included in the trial. Key inclusion criteria are: male or female adult patients with HER2-low mBC with newly diagnosed or radiological progressing BM with or without type II LMD (patients with type I LMD cannot be enrolled in the study),

Eastern Cooperative Oncology Group (ECOG) performance status 0-2, ≥1 measurable brain lesions, left ventricular ejection fraction ≥50%, and ≥1 prior line of systemic treatment in the metastatic setting. Patients will receive T-DXd 5.4 mg/kg intravenously every 21 days until unacceptable toxicity, progressive disease, death, or discontinuation for any other reason. The primary endpoint is treatment efficacy, as defined per central nervous system (CNS) objective response rate (ORR) according to Response Assessment in Neuro-Oncology (RANO)-BM criteria. Secondary endpoints are extracranial ORR, bicompartmental-clinical benefit rate, bicompartmental-disease control rate, time to response, duration of response, progression-free survival, overall survival, safety (adverse events, coagulation, and hematological monitoring) and tolerability according to the NCI-CTCAE v.5.0, quality of life, and neurologic function using the Neurologic Assessment in Neuro-Oncology (NANO) scale. Simon's two-stage design was set at a one-sided type I nominal alpha error of 5% to attain 90% power. Alternative hypothesis is CNS ORR ≥42%. Null hypothesis is CNS ORR ≤16%. The study will be stopped for futility if there are ≤2 patients (15.5%) with intracranial responses in the first stage. The primary endpoint will be met with ≥ 8 (29.6%) patients with CNS response among all patients included in the study. If positive, TUXEDO-4 could introduce T-DXd as a promising treatment for HER2-low mBC patients with newly diagnosed or progressing BM with or without type II LMD. TUXEDO-4 was activated for accrual on June 5th.

P3-08-22: Tucatinib, Trastuzumab and Capecitabine with brain and/or spinal radiotherapy (XRT) in patients with HER2+ metastatic breast cancer and leptomeningeal disease: A multi-centre phase II, single arm feasibility study ("CLIMB LMD"; NCT06016387)

Katarzyna J. Jerzak, Marie-France Savard, Mary-Jane Lim-Fat, Gregory Pond, Hany Soliman, Arjun Sahgal

Background: As patients with HER2+ metastatic breast cancer (MBC) live longer and survive to experience spread of cancer to the brain, the incidence of leptomeningeal disease (LMD) is increasing. Unfortunately, patients with HER2+ LMD have a very poor prognosis with limited treatment options. Patients with LMD were excluded from the pivotal HER2CLIMB trial, which demonstrated intra-cranial activity of tucatinib, trastuzumab and capecitabine and prolonged survival among patients with HER2+ MBC.
Methods: A multi-center phase II, single arm feasibility study with a safety run-in of 6 patients. The sample size will include 30 patients in total across participating centres in Ontario, Canada.
Intervention: In phase 1, brain and/or spinal radiotherapy (XRT) will be administered. Areas in the brain and spine will be treated as per the discretion of the treating radiation oncologist. In phase 2, patients will commence systemic therapy between 7 days and up to 21 days after completion of brain and/or spinal XRT. Tucatinib, trastuzumab (Ogivri; MYL-1401O) and capecitabine will be administered as per the HER2CLIMB protocol; however, the addition of capecitabine may be delayed and commence during Cycle 2, at the discretion

of the treating physician to allow for adequate recovery from XRT. Treatment will continue until disease progression or unacceptable toxicity.
Patients who receive XRT but do not pass through the second phase of eligibility will not be counted towards the total patient number and will be followed for survival only. If >70% of enrolled patients experience one or more grade 3+ attributable AEs or SAEs, or >25% experience one or more grade 4+ attributable AEs or SAEs, these instances will be reported to the Data and Safety Monitoring Board (DSMB) in a timely fashion and will trigger review by the DSMB. The trial will continue until the DSMB reviews AE reports and decides whether or not the trial needs to be halted or terminated.
Key inclusion criteria: 1. Men or women with HER2+ MBC. HER2+ status will be defined in accordance with ASCO-CAP 2018 guidelines, and can be diagnosed at any time prior to enrolment; 2. Evidence of LMD* in the brain and/or spine (either positive cerebral spinal fluid cytology and/or magnetic resonance imaging evidence of LMD). Measurable disease in the central nervous system is not required. *The diagnosis of LMD can occur at any time prior to enrolment; 3. Age 18+ at time of consent; 4. ECOG ≤ 2.
Key exclusion criteria: 1. Prior whole brain radiotherapy (prior stereotactic radiosurgery for parenchymal brain metastases received ≥7 days prior to consent is permitted); 2. Prior therapy specifically directed at LMD, including prior radiotherapy or systemic therapy; 3. Prior use of tucatinib at any time prior to enrollment.
Primary outcome: Overall survival (OS) from the start of XRT.
Secondary outcomes: i) Time to central nervous system (CNS) progression from the start of XRT; ii) Safety and tolerability (CTCAE v.5.0); iii) Progression free survival from the start of XRT; iv) CNS specific objective response (RANO-BM); v) Extracranial objective response (RECIST v1.1); vi) Neurologic-specific quality of life (QoL) (FACT-BR v.4); vii) Overall QoL (EORTC QLQ-C30 v.3).
Analyses: OS will be measured from start of XRT until the date of death (from any cause, assessed up to 3 years), or censored at the last known follow-up. Kaplan-Meier curves will be used to present the time to event data. In addition to the median and 95% confidence interval, estimates of OS at different time points from the Kaplan-Meier survival curve will be tabulated.
Accrual: This study opened for enrollment at the Sunnybrook Odette Cancer Centre in December 2023 and will open at the Ottawa Hospital Cancer Centre in July 2024. To-date (July 3, 2024), 4 patients have enrolled. The expected accrual period is 18 to 24 months.

P3-08-23: Using a SMART Approach to Culturally-Adapt and Remotely Deliver a Weight Loss Intervention for Latina Breast Cancer Survivors: The ¡Vida! Study Methods

Blake Langley, Eileen Rillamas-Sun, Jennifer Whitten, Yarizel Herrera, Sheryl Rothmuller, Sara Buzali, Jennifer Dearden, Ashkan Ertefaie, Chongzhi Di, Nancy Davidson, Rachel Yung, India Ornelas, Heather Greenlee

Background: Cancer is the leading cause of death in the US Hispanic/Latinx population and Latina women are 20% more likely to die from breast cancer (BC) compared to non-Latina women. An estimated 80% of US Latina women have overweight or obesity, which is a major contributor to BC incidence and recurrence. Culturally tailored, effective, and accessible weight loss interventions for Latina BC survivors are needed.
Aims: The ¡Vida! Study primary aim is to compare the effectiveness of adaptive weight loss interventions in decreasing total body weight by ≥7% at 12 months in Latinas with early-stage BC and obesity not on current chemoradiotherapy. The secondary aim will investigate baseline characteristics as moderators of the intervention effects to inform personalized strategies for weight management. Exploratory aims will examine other moderators and mediators of intervention effects, effects of the intervention on cardiometabolic biomarkers, and contextual factors that contribute to study outcomes.
Design: Participants will be recruited from NCI SEER registries in California and Washington. This study is a 4-group, 2-stage, sequential multiple assignment randomized trial (SMART) of: 1) the ¡Vida! Program, 2) ¡Vida! + Experiential Learning (EL), 3) ¡Vida! + EL + health coaching (HC), or 4) ¡Vida! + EL + HC + delivered groceries (DG). In Stage One, participants will be randomized to ¡Vida! or ¡Vida! + EL. In Stage Two, at week 8 participants who do not respond to the intervention (i.e., loss of <2% of their body weight) will be re-randomized to receive additional components. A community advisory board of project stakeholders, community-based organizations in Washington and California representing medical services, social services, and patient-advocates will provide input throughout the study process.
Intervention:

The ¡Vida! Program adapts the National Diabetes Prevention Program to Latina BC survivors using the Framework for Reporting Adaptations and Modifications. Live, remotely delivered, nutritional and physical activity (PA) educational sessions through the Fred Hutch Cancer Center Cook for Your Life website will be delivered over 12 months.
The EL component will include live, virtual, hands-on sessions delivered by lifestyle health educators (promotoras) and will focus on increasing knowledge, skills, and self-efficacy to achieve and maintain weight, diet and PA goals.
The HC component will include individualized remote sessions. Health coaches will identify patient diet and PA goals, and support weight loss self-efficacy, motivation for adopting a hypocaloric high-quality diet, and increasing moderate-to-vigorous PA.
The DG component will include a bag of supplemental fresh vegetables, whole grains, and healthy oils. DG will include ingredients used in recommended recipes and recipes prepared in the EL sessions.

Data Collected: Baseline data will be collected on participant demographics, clinical characteristics, acculturation, and taste preferences. Body weight, daily PA, and accelerometry; patient-reported food intake and diet quality, global quality of life, social support, perceived stress, and self-efficacy for healthy eating and PA; and dried blood spot biomarkers will be monitored for change between baseline and 12 months.

Conclusion: The ¡Vida! trial is on track to open in Fall 2024. The results of this adaptive, remotely delivered, and culturally tailored weight loss trial in Latina early-stage BC survivors will identify scalable, effective, personalized strategies to support weight loss and improve BC related outcomes due to obesity in this population with high cancer health disparities.

P3-08-24: Valemetostat and Trastuzumab Deruxtecan in Patients With HER2-Low, Previously Treated, Unresectable or Metastatic Breast Cancer

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Background: Human epidermal receptor (HER)2-low breast cancer, defined as either 1+ by immunohistochemistry (IHC), or IHC2+ and HER2-negative by in situ hybridization (ISH), accounts for ~ 60% of breast cancer cases and is associated with particularly poor outcomes, including decreased progression-free survival (PFS). Trastuzumab deruxtecan (T-DXd) is a HER2-directed antibody–drug conjugate (ADC) approved in more than 55 countries, including the US, for patients with HER2-low breast cancer previously treated with chemotherapy in the metastatic setting or with disease recurrence within 6 months of completing adjuvant chemotherapy. Regulatory approval was based primarily on results from the phase 3 DESTINY-Breast04 trial, where T-DXd significantly prolonged PFS compared with the physician's choice of chemotherapy (hazard ratio, 0.51 [95% confidence interval, 0.40, 0.64], $P < 0.0001$; median PFS 10.1 vs 5.4 months, respectively) in patients with HER2-low advanced breast cancer.

Valemetostat tosylate (valemetostat) is a novel, potent, and selective dual inhibitor of enhancer of zeste homolog (EZH)2 and EZH1. Valemetostat 200 mg PO QD has demonstrated clinical efficacy and favorable tolerability in multiple hematologic malignancies. EZH2 regulates gene expression, including those involved in the DNA damage response, such as DNA/RNA helicase Schlafen 11 (SLFN11). In response to DNA damage, SLFN11 binds to chromatin, causing a replication block and inducing apoptosis. Inhibition of EZH2 with valemetostat may upregulate SLFN11 and sensitize tumor cells to DNA-damaging agents, including ADCs such as T-DXd. Preclinical data suggest synergistic effects of valemetostat in combination with T-DXd and datopotamab deruxtecan.

This ongoing Master Protocol study evaluates valemetostat in combination with DXd-ADCs in solid tumors. A sub-protocol of this study evaluates the clinical activity and safety of valemetostat in combination with T-DXd in patients with HER2-low, previously treated, unresectable, or metastatic breast cancer.

Study design: This global, phase 1b, multicenter, open-label Master Protocol study (NCT06244485) will enroll ~ 70 patients per sub-protocol in the US and Japan. The breast cancer sub-protocol will enroll patients with unresectable or metastatic breast cancer, 1–2

prior lines of chemotherapy in the recurrent or metastatic setting, and adequate organ function. All patients must have HER2-low expression (IHC1+ or IHC2+/ISH-). Hormone receptor-positive disease must have progressed on endocrine therapy or the patient would no longer benefit from endocrine therapy in the investigator's opinion. Patients who have received treatment with an ADC that consists of an exatecan derivative that is a topoisomerase I inhibitor are excluded.

The study consists of two parts: dose-escalation (Part 1; ~ 30 patients) and dose-expansion (Part 2; ~ 40 patients). In Part 1, patients will receive valemetostat doses of 50–200 mg PO QD (continuous) and fixed-dose T-DXd 5.4 mg/kg IV Q3W until disease progression. The dose-limiting toxicity evaluation period will be the first treatment cycle (21 days). In Part 2, patients will receive valemetostat and T-DXd at the recommended dose for expansion identified in Part 1. The primary study endpoints are safety and tolerability in Part 1, and overall response rate (ORR), safety, and tolerability in Part 2. The secondary endpoints include ORR in Part 1, and pharmacokinetics, PFS, duration of response and overall survival in Part 1 and 2. Clinical responses will be determined by investigator assessment and defined according to Response Evaluation Criteria in Solid Tumors v1.1 criteria. The relationship between HER2 expression and clinical response will also be explored.

P3-08-25: randomized phase III study comparing the digital telemonitoring platform "CUREETY TECHCARE" to usual standard of care in patients with triple negative metastatic breast cancer initiating a first-line systemic treatment: ALTERNATIVE

Martin Babau, Florence Joly, Frederic Fiteni, Aurore Caumont-Prim, Trevor Stanbury, David Perol, Charles Parnot, Francois-Guirec Champoiseau, Adeline Poitou, Jerome Lemoonier, Francois Montestruc

Background: Triple-negative breast cancer (TNBC), accounts for 15% to 20% of all breast cancers. It is characterized by the absence of 3 receptors (estrogen/progesterone/human epidermal growth factor 2) which limits the treatment options.
Currently a therapeutic sequence for patients with mTNBC is first-line chemotherapy with or without immunotherapy, followed by sacituzumab govitecan then by successive chemotherapy lines. Considering the toxicity of these treatments there is a need to identify and monitor the signs and symptoms (S&S) of adverse events (AE). Early identification of these S&S, with appropriate management may prevent severe and fatal AEs, reduce dose delays and treatment discontinuations, thus optimizing treatment and possibly improving survival outcomes.
Cureety is a digital remote monitoring platform, specifically designed to facilitate the monitoring of S&S of treatment-specific AEs and disease progression in cancer patients. Cureety integrates the Cureety TechCare algorithm for patient clinical classification, a CE-marked medical device.
We hypothesize that patients with mTNBC may benefit from Cureety during systemic

treatments by improving the identification of S&S of potentially severe/ AEs at early stages or early progression symptoms.
Trial design: ALTERNATIVE is a French, multicentre, randomized, phase III trial comparing standard of care (SoC) with or without telemonitoring. The telemonitoring will comprise weekly AE and S&S evaluations and their analyses by Cureety. The randomization will be stratified by planned first-line treatment (immunotherapy: yes vs no) and ECOG PS (0 vs 1-2).
Eligibility criteria: Patients aged ≥18 years, with mTNBC, ECOG PS ≤2, initiating first-line systemic treatment. The patient must be able and willing to complete web-based self-report questionnaires.
Specific aims: To assess the effectiveness of SoC with digital telemonitoring compared to SoC alone, in terms of Time to definitive Health-Related Quality-of-Life score Deterioration (TUDD), Hospitalization Free Survival (HFS) and OS.
The secondary objectives will include contribution on patient's safety, treatment compliance and extent of exposure, compliance with telemonitoring, patient and medical team satisfaction with telemonitoring, global patient satisfaction with care, and socio-economic data.
Sample size: A two-sided sequential logrank test with an overall sample size of 472 subjects for 384 events achieves 90% power at a 5% two-sided significance level to detect a hazard ratio (HR) of 0.72 when the median TUDD is 4.3 months without telemonitoring and 6 months with telemonitoring. The planned study duration is 42 months (18 months of accrual and 24 months of follow up). An interim futility analysis is planned after 157 TUDD events using an alpha-spending function of O'Brien-Fleming Analog.
Statistical methods: TUDD, defined as the time from randomization to the first deterioration of ≥ 10 points out of 100 in the global health status (GHS) score (items 29 and 30 of the QLQ-C30), will be analysed using the Kaplan-Meier method. The median and Q1, Q3 event times for each arm and the corresponding 2-sided 95% confidence intervals (CIs) will be provided. A two-sided log-rank test stratified for the randomization stratification factors will be used to compare TUDD between the two study groups.
The Cox regression model, stratified for the same stratification factors, will be fitted, and the estimated HR and 2-sided 95% CIs will be provided. To preserve the alpha risk at 5% for the two analyses, the p-value will be 0.00325 at the first analysis and 0.0492 at the final analysis.
To control the overall type I error at 5%, once the superiority with TUDD is established, and the study is conclusive, HFS then OS will also be tested at 5% significance level using a hierarchical order. If the study is not conclusive for TUDD these endpoints will still be analysed but will be interpreted as exploratory.
Funding
Bpifrance and Cureety

P3-08-26: Imipramine: A Promising Therapeutic Regimen for Breast Cancer Patients

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Introduction: Cancer cells continue to replicate their DNA and survive mainly due to their unique ability to repair damaged DNA using alternate DNA repair pathways. For example, cancer cells with a deficiency in homologous recombination (HR) proteins (such as BRCA1) can repair their DNA by either relying on other highly expressed HR-related proteins (such as RAD51 or PARP1) or by using backup DNA repair mechanisms such as ALT-NHEJ. Alterations in DNA repair pathways commonly occur during breast cancer (BC) progression. For example, TNBCs have dysfunctional BRCA1/2 but express high levels of RAD51. Further, ER+BC employs ALT- NHEJ, HR, or BER to repair their DNA. The objective of this study was to identify FDA-approved non-cancer drug/s capable of inhibiting DNA repair in BC cells, thereby inhibiting their growth, with potential clinical benefits for BC patients.

Methods: BC cells were treated with vehicle and FDA-approved drugs for 72 hours and were subjected to cell-titer Glo assay. The anti-tumor effect of imipramine alone and in combination with Olaparib and tamoxifen was validated using orthotopic xenograft mouse models. The effect of imipramine on DNA repair was determined by immunofluorescence using antibody against 53BP1, and functional DNA repair assays. Based on these results, a window of opportunity clinical trial was conducted to test the efficacy of imipramine in early-stage breast cancer patients. After having a breast core needle biopsy, 15 eligible patients with stage I-III breast cancer were enrolled in the trial and were treated with imipramine at a target dose of 200 mg PO daily for an average of 28 days. Patients were evaluated on day 7, day 14, day 21, and at the end of treatment for toxicity. The primary endpoint for the trial was the absolute change in the Ki67. IHC using an antibody against Ki67 was performed on core biopsy (pre-treatment) and tumor tissue after imipramine treatment. The secondary objectives of this trial were to further define the toxicity profile of imipramine.

Results: Imipramine treatment significantly reduced the viability of TNBC and ER+ BC cells. Further, imipramine treatment inhibited the migration and invasion of BC cells. Systemic delivery of imipramine suppressed the growth of BC. Importantly, imipramine blocked the DNA repair capacity of BC cells by inhibiting the expression of DNA repair proteins including FOXM1 and RAD51. Notably, imipramine treatment improved the efficacy of Olapraib in TNBC and sensitized the tamoxifen response in endocrine-resistant ER+ BC cells. The clinical trial on 15 patients treated with imipramine showed a marked reduction in Ki67-positive cells in post-surgical tumor tissues compared to core needle biopsy tissues. Toxicity was mild with only grade 1 and 2 toxicities that included some instances of dizziness and nausea. There was no dose reduction, interruption, or treatment discontinuation.

Discussion: Our preclinical and clinical studies showed that imipramine can block DNA

repair in both TNBC and HR+ breast cancer. Short-term treatment with imipramine can effectively decrease Ki67 in breast cancer patients. Future clinical trials will involve combining imipramine with other regimens such as Olaparib for TNBC patients and elacestrant/CDK4/6 inhibitor for therapy-resistant ER+ breast cancer patients.

P3-08-27: Novel Combination Immune Therapy for Metastatic Breast Cancers leveraging weaknesses in DNA damage response in p53 Mutant cancer

Ashley Guo, Priyanka Rajan, Mohammed Alruwaili, Joseph Barbi, Scott Abrams, Thomas Melendy, Christos Fountzilas, Andrei Bakin

Metastatic breast cancer (MBC) is a life-threatening disease with lowest 5-year survival rates in patients with metastatic triple-negative breast cancer (mTNBC). Most MBCs carry mutant p53 that drives cancer progression and metastasis in part by promoting the build-up of myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs), major immune suppressive cells in the immune environment. At present, there are no effective treatment options for p53-mutant BC, while existing chemotherapy-based treatments exhibit low selectivity for p53mut status and high frequency of adverse side effects. We developed a novel therapeutic strategy for selective damaging p53-mutant tumors addressing the limitations mentioned above. Our novel strategy for p53mut cancers uses two drugs, one acting as an inducer (TAS102) and the other an amplifier (PARP inhibitor, PARPi), to achieve selective damage to p53mut cancers. Thymidine nucleotide analogue TAS102 does not block DNA synthesis but activates post-replicative repair leading to DNA breaks, while PARPi blocks repair of these DNA lesions. The two-drug strategy showed high efficacy, without adverse effects, in preclinical cancer models. Our first-in-human phase I study with the two-drug TAS102-PARPi regimen for colon cancer (NCT04511039, PI: Fountzilas) did not identify major signs of toxicity and the preliminary efficacy is promising. The dose-expansion part of the study is actively accruing patients with one patient already attaining a partial radiographic response. The present work investigates the impact of the two-drug TAS102-PARPi regimen on the immune landscape in mice implanted with mTNBC tumor cells. We observed that TAS102-PARPi reduced tumor growth and metastases to the lungs and liver. The two-drug regimen reduced levels of MDSCs and TAMs and increased levels of lymphocytes, indicating that the regimen may positively cooperate with immunotherapy directed to enhance anti-tumor activity of T cells. Depletion of CD8+ T cells markedly reduced anti-tumor activity of the TAS102-PARPi regimen. Immunophenotyping showed that the two-drug therapy increased tumor infiltration by CD8+ T cells expressing immune-checkpoint receptors PD1 and LAG3. Testing the two-drug therapy in combination with antibodies blocking the inhibitory receptors anti-PD1 or anti-LAG3 showed a dramatic reduction in tumor growth and metastases. Together these results demonstrate that the two-drug TAS102-PARPi regimen can be combined with immune checkpoint blockade therapy for effective treatment of MBCs including mTNBCs.

P3-08-28: VRTX531: A Novel Best-in-Class USP1 Inhibitor for the Treatment of Triple-Negative Breast Cancer

Rishi Rahangdale, Prashant Bhavar, Uday Surampudi, Partha Sarma, Murali Bokka, Anuj Kshirsagar, Appaji Mandhare

TNBC accounts for about 10-15% of all breast cancers and tends to have a poorer prognosis compared to other breast cancer subtypes and is more commonly seen in younger women under 40, who are Black, or who have a BRCA1 mutation. TNBC, especially those with BRCA1 mutations, tend to be more sensitive to DNA-damaging chemotherapies, such as platinum-based agents, due to their inability to repair DNA effectively. Tumors with BRCA mutations have shown vulnerability to PARP inhibitors (PARPi), targeting compensatory DNA repair pathway that is more active in BRCA-mutant tumors, effectively leading to synthetic lethality, i.e. the simultaneous loss of BRCA function and PARP inhibition leads to cancer cell death. However, only 20–40% of the patients benefit from PARPi and are alive 5-years after diagnosis.
Ubiquitin specific protease 1 (USP1) belongs to a group of deubiquitinating enzymes (DUB) and is a key regulator in the DNA damage repair (DDR) pathway. A deficiency in USP1 results in compromised genomic stability, making it a potential therapeutic target in tumors that exhibit defects in the DNA damage repair mechanism.
VRTX531 is a potent, selective, and allosteric oral inhibitor of USP1, specifically designed to target HRD Tumors. Treatment with VRTX531 resulted in decreased viability in cell lines with BRCA1/2 mutations and HRD+ tumors. USP1 inhibitors are anticipated to induce cell death via a pathway distinct from PARPi, and thus have the potential to overcome resistance to PARPi. The synergistic effect of VRTX531 combined with PARPi was demonstrated in cell-based assays and further validated in mice bearing TNBC tumors cells. In TNBC model of MDA-MB-436, treatment with PARPi alone led to a delayed onset of response with statistically significant variability between subjects, whereas the combination of VRTX531 and PARPi led to rapid onset of response, followed by complete tumor regression (TGI > 98%), across all subjects with near zero variability. Even after the treatment ended, the combination continued to show a durable response, whereas animal treated with PARPi alone experienced early relapse. USP1 inhibitors have the potential to overcome resistance to chemo and PARPi by targeting compensatory mechanisms that allow HR-deficient cancer cells to survive.
USP1 inhibitor VRTX531, exhibited best-in-class pharmacokinetics, with exemplary safety profile, that supports its further development.

P3-08-29: Early Clinical Experience with TTX-MC138, a First-in-Class Therapeutic Candidate for Metastatic Breast Cancer

Zdravka Medarova, Neil Robertson, Subrata Ghosh, Hsiao-Ying Wey, Mariane LeFur, Onofrio Catalano, Ciprian Catana, Peter Caravan, Susan Duggan, Andreas Varkaris

For the past 20 years our research has specifically focused on developing potential therapies targeting specific properties of tumor cells that have metastasized. Although the mechanisms and triggers of metastasis are not completely understood, cancer cells exhibit the ability to break out of the primary tumor mass, travel through the circulation, and colonize a new vital organ in the process of metastasis. Of therapeutic significance, these metastatic cells are genetically and phenotypically distinct from the majority of the cells in the tumor mass serving to limit the effectiveness of those therapies whose focus is the primary tumor itself. In our earlier discovery efforts, miRNA-10b was identified as a master regulator of the viability of metastatic tumor cells. miRNA-10b not only promotes the capacity of tumor cells to migrate and invade surrounding tissue (become metastatic) but, most notably, serves as a powerful master regulator of the viability of these cells and their capacity to survive outside of the primary tumor microenvironment. Detailed cellular and preclinical mechanistic studies confirmed the existence of a miR-10b-triggered pathway that regulates the viability and proliferation of tumor cells only after they have acquired the ability to metastasize. This knowledge allowed us to develop a therapeutic strategy based on miR-10b inhibition. The specific inhibition of miR-10b was achieved using inhibitory oligonucleotides (locked nucleic acid, LNA-based antagomirs) delivered to metastatic sites by aminated dextran-coated iron oxide nanoparticles (termed TTX-MC138). Our research demonstrated that TTX-MC138 could cause complete and persistent regression of metastases in cancer models with no evidence of systemic toxicity. On the path to clinical development of TTX-MC138, we conducted critical, exploratory IND enabling studies in rats, dogs, and non-human primates with TTX-MC138. Ultimately, the information generated resulted in FDA authorization under IND163800 allowing for initiation of an ongoing microdosing Phase 0 clinical trial in patients with advanced metastatic cancer. The strategy of conducting an initial Phase 0 study was not only to assist in the identification of susceptible tumor types (or patients) but also to support future clinical efforts by providing proof of concept and quantification of delivery to clinical metastases. The Phase 0 clinical trial involves a single injection of a microdose of Cu-64 labeled TTX-MC138 which allows for direct visualization in cancer patients via PET-MR imaging, with a primary endpoint of confirming its localization (%ID/cc) to the metastatic lesions. We have now obtained initial clinical data on drug PK and accumulation in clinical metastases to the lungs, bone, and liver, as well as drug stability, metabolism, and pharmacodynamic activity. We have demonstrated TTX-MC138 uptake by bone, lung, and liver metastases, as well as tumor to blood ratios reflective of selective retention of the drug by tumor tissue. We have established that the drug has a long circulation time of 20 hrs in humans. Remarkably, even at a 100 microgram microdose, the drug showed robust PD activity with a 70% inhibition of the miR-10b target in blood over the full time course of the study. The process leading to the implementation of TTX-MC138 in the clinic is critically dependent on the innate tropism of the TTX delivery platform to tumors and represents a first step towards developing effective nucleic-acid based therapeutics against cancer.

P3-08-30: Development of humanized anti-FABP4 monoclonal antibodies for potential treatment of breast cancer

Bing Li

Breast cancer is the most common cancer in women diagnosed in the U.S. and worldwide. Obesity increases breast cancer risk without clear underlying molecular mechanisms. Our studies demonstrate that circulating adipose fatty acid binding protein (A-FABP, or FABP4) links obesity-induced dysregulated lipid metabolism and breast cancer risk, thus potentially offering a new target for breast cancer treatment. We immunized FABP4 knockout mice with recombinant human FABP4 and screened hybridoma clones with specific binding to FABP4. The potential effects of antibodies on breast cancer cells in vitro were evaluated using migration, invasion, and limiting dilution assays. Tumor progression in vivo was evaluated in various types of tumorigenesis models including C57BL/6 mice, Balb/c mice, and SCID mice. We identified over 30 clones which specifically bound to FABP4. One clone, named 12G2, was shown to significantly inhibit mammary tumor growth. After further confirming the therapeutic efficacy of the chimeric 12G2 monoclonal antibody consisting of mouse variable regions and human IgG1 constant regions, 16 humanized 12G2 monoclonal antibody variants were generated by grafting its complementary determining regions to selected human germline sequences. Humanized V9 monoclonal antibody showed consistent results in inhibiting mammary tumor growth and metastasis by affecting tumor cell mitochondrial metabolism. Thus, our data suggest that targeting FABP4 with humanized monoclonal antibodies represents a novel strategy for the treatment of breast cancer and possibly other obesity-associated diseases.

P3-09-01: Time to postmastectomy radiotherapy in the setting of immediate implant-based breast reconstruction: does it impact on adjuvant treatment delay compared with mastectomy alone?

Lilian Paz, Jorge Biazús

Background: Immediate breast reconstruction (IBR) integrates with the surgical treatment of breast cancer, improves quality of life, psychological health and self-esteem. However, IBR following mastectomy has a higher rate of surgical complications compared to mastectomy without reconstruction, and also constitutes an independent risk factor for reoperation, which may result in delays regarding the administration of adjuvant therapies. The ideal timeframe in which to administer postmastectomy radiotherapy (PMRT) following surgery has not been well-established. The scope of this study was to evaluate whether IBR with implants has an impact on the time to start radiotherapy.
METHODS: The present retrospective study reviewed 1,898 medical records of women with breast cancer submitted to mastectomy between January 2018 and December 2022 at the Aristides Maltez Hospital, a reference institution for cancer care in the state of Bahia, Brazil. Of these, 506 participants classified between stages I-III who underwent PMRT without adjuvant chemotherapy were included for analysis. The study sample was divided into two

groups: women who underwent mastectomy only without reconstruction (n=416, 82.2%) and women who had undergone to mastectomy followed by IBR with subpectoral implant placement (n=90, 17.8%). The patients' demographic, oncological and surgical data were collected. Kaplan-Meier analysis with the log-rank test was used to estimate the interval from the last oncological surgical treatment until the time of the first administration of radiotherapy. Participants were additionally categorized into three groups according to the time since the onset of PMRT (≤8 weeks, >8 and <16 weeks, ≥16 weeks). Multivariable logistic regression analysis was used to explore the clinic-pathological variables hypothesized to be associated with time to start radiotherapy.
Results: Most of the studied individuals were black women (n=460, 90.9%) with stage III breast cancer (n=304, 68.5%) who were treated with neoadjuvant chemotherapy (447, 88.3%). The average time of follow-up was 102.6 ±29.8 days. The median age was 49 (39-53) years old. The participants who underwent IBR were younger at surgery, more often a pre-menopausal status, fewer comorbidities, lower body mass index, no history of smoking and a higher proportion of I-II clinical stage. Kaplan-Meier analysis revealed that the IBR group started radiotherapy earlier compared to those who underwent mastectomy alone [95.84 (95% CI: 89.66, 102.03) versus 104.09 (95% CI: 101.25, 106.93) days; log-rank p=0.04)]. The proportion of women who started radiotherapy after 16 weeks was higher in the group that did not undergo breast reconstruction [164 (39.4%) versus 22 (24.4%), p=0.006]. Interestingly, mastectomy without IBR (OR=1.99; 95% CI: 1.21, 3.27; p=0.007) and hormone receptor-positive breast cancer (OR=1.71; 95% CI: 1.19, 2.48; p=0.004) were associated with a greater chance of starting radiotherapy after 16 weeks; age was not significantly associated with delayed onset of radiotherapy under regression analysis.
Conclusion: The present real-world study found that patients underwent postmastectomy IBR in the absence of adjuvant chemotherapy started radiotherapy sooner than in the mastectomy without breast reconstruction group. In addition, mastectomy without IBR and hormone receptor-positive breast cancer were predictive factors for the delayed initiation of PMRT. We hypothesize that IBR could be considered a marker of higher socioeconomic status, which may explain the disparity in access to cancer treatment, even among patients treated at the same reference institution.

P3-09-03: Cost effectiveness analysis of approaches to axillary management in the setting of contralateral prophylactic mastectomy

Christopher Vetter, Judy C. Boughey, Jeffrey E. Johnson

Background: Occult malignancy (OM) found in contralateral prophylactic mastectomy (CPM) specimens presents challenges for axillary staging as post-mastectomy sentinel lymph node (SLN) surgery is not validated. No consensus exists on the optimal strategy for evaluating the axilla in this setting. Approaches include preoperative breast magnetic resonance imaging (MRI), selective SLN surgery determined by risk of OM, routinely performed SLN surgery, routinely omitted SLN surgery and selective SLN mapping for delayed SLN surgery using superparamagnetic iron oxide. Herein we evaluate the cost

effectiveness of these strategies.
Methods: A decision tree representing the possible choices and outcomes for axillary staging in the setting of OM in CPM was constructed. A literature review was conducted to determine event probabilities. For selective SLN mapping, a threshold of 7% preoperative risk of OM was based on previously identified risk factors. Cost data for surgical, anesthetic, pathology, and facility fees were obtained from FAIR Health. Quality-Adjusted Life Years (QALYs) were calculated using previously published values for the rate of lymphedema following axillary surgery, the associated health-related quality of life decrease, and expected survival following bilateral mastectomy. A cost-utility analysis was conducted to determine expected costs, impact on QALYs, and incremental cost effectiveness ratios (ICER) for each of the possible approaches relative to the historical standard, mastectomy followed by axillary dissection if occult malignancy is identified. A sensitivity analysis was performed to identify the risk threshold at which SLN mapping becomes cost effective.
Results: The pooled rate of OM in the contralateral breast for women diagnosed with unilateral breast cancer was 1.89%. Costs ranged from $12,728 for mastectomy alone to $15,295 for routine preoperative MRI. Minor differences in QALYs were noted, ranging from 34.006 for preoperative MRI to 34.027 for preoperative risk assessment and SLN mapping, due to the low incidence of OM and low need for axillary dissection. Cost per QALY ranged from $374.04 for mastectomy alone to $449.77 for preoperative MRI. Preoperative MRI was the only strategy that was not cost effective relative to historical standard, yielding increased cost with decreased QALYs. The most cost-effective strategies included mastectomy with no additional axillary surgery, mastectomy with selective SLN mapping, and mastectomy with postoperative SLN surgery. On sensitivity analysis, risk assessment with selective SLN mapping remained cost effective across the expected levels of risk.
Conclusions: Different strategies for axillary management for CPM yield minor differences in QALYs. Routine MRI is the least cost-effective strategy, resulting in highest cost and lowest QALY. Selective SLN surgery resulted in lower QALYs than no initial axillary staging or selective SLN mapping with no significant improvement in cost. Selective SLN mapping allows for axillary staging with an improvement in QALYs and lower cost and should be considered the optimal strategy for axillary staging in women undergoing CPM.

P3-09-04: Does additional margin excision during LumiSystem pegulicianine fluorescence-guided lumpectomy surgery affect patient breast satisfaction?

Abigail E Daly, Kyle J Anderman, Manna Chang, Kelly K Hunt, E Shelley Hwang, Irene L Wapnir, Peter W Blumencranz, David Carr, Kate Smith, Brian Schlossberg, Jorge Ferrer, Barbara L Smith

Background: Achieving microscopically negative breast cancer lumpectomy margins remains challenging. Trials of the LumiSystem for real time margin assessment in breast cancer lumpectomy surgery showed removal of additional tumor and reduction of 2nd surgeries compared with standard lumpectomy surgery. We asked if the additional margin

tissue removed with this system negatively impacted patient satisfaction with breast appearance.
Methods: Stage 0-3 breast cancer patients were randomized 3:1 to pegulicianine LumiSystem-assisted vs. standard lumpectomy surgery in a multicenter trial. Patients received an IV injection of pegulicianine 2-6 hours before surgery. After the standard lumpectomy was completed, additional LumiSystem-guided cavity margins were excised at sites of positive pegulicianine fluorescence in the lumpectomy cavity walls in device arm patients using a hand-held imaging device and patient-calibrated cancer detection software. Patient, tumor, and lumpectomy data was collected. BREAST-Q surveys with a scale of 0-100 and Patient Preference Information (PPI) surveys with a 1-5 scale were given at pre-op and post-op time points assessing breast satisfaction and preferences.
Results: Post-operative breast satisfaction surveys were completed 6-12 months after surgery by 63 patients (75% of participants), 14 in the control arm and 49 in the device arm. Among patients with postoperative satisfaction surveys, LumiSystem device readings prompted excision of additional margin tissue in 25 (51%) device arm patients, with median volume of 10.5cc. There was no significant difference in mean post-operative breast satisfaction scores between device and control arm patients (78.2 out of 100 for control arm patients vs. 79.8 out of 100 for device arm patients, $p = 0.359$). A total of 58 study patients (69% of participants) had both pre- and post-operative breast satisfaction scores available for comparison. Both the control and device arm patients exhibited an increase in breast satisfaction from the pre-operative timepoint to the post-operative timepoint. There was no significant difference in the change in breast satisfaction from pre-operative to post-operative time points between device and control arms (mean increase of 14.4 points for control arm patients vs. 10.8 points for device arm patients, $p = 0.649$). A total of 51 patients (61% of all participants) completed post-operative PPI questions weighing preference for optimizing cosmetic outcome versus avoiding a second surgery. The median PPI score in both study arms was 5, indicating a strong preference for avoiding a second surgery over optimizing cosmetic outcome. Overall, 70.6% of surveyed participants strongly preferred or preferred avoiding a second surgery, 17.6% had no preference, 11.8% preferred to optimize cosmetic outcomes and no patient strongly preferred to optimize cosmetic outcomes. Univariate and multivariate binomial logistic regression analyses were performed to identify predictors of decreased breast satisfaction after surgery. Excision of additional LumiSystem-guided margin specimens and total tissue volume excised were not significant predictors of decreased breast satisfaction. BMI, larger breast size, age > 50, use of neoadjuvant therapy, and oncoplastic lumpectomy closure also failed to correlate with post-operative breast satisfaction.
Conclusions: There was no decrease in breast satisfaction following use of the LumiSystem for intraoperative margin assessment in breast cancer lumpectomy surgery, despite excision of additional margin tissue. The majority of patients valued avoidance of a second surgery over maximization of cosmetic outcome.

P3-09-05: ROBOTIC VS. CONVENTIONAL NIPPLE-SPARING MASTECTOMY IN BREAST CANCER: AN UPDATED SYSTEMATIC REVIEW AND META-ANALYSIS

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Introduction: With the growing concern to improve cosmetic results and quality of life without compromising oncological outcomes, nipple-sparing mastectomy (NSM) has gained prominence, especially with novel techniques such as robotic-assisted, which allows for the absence of cosmetic scars and improves patient satisfaction.

Objective: Our aim is to compare conventional (C-NSM) and robotic-assisted NSM (R-NSM) in terms of clinical outcomes, such as intraoperative parameters and complication rates, as well as evaluating recurrence rates in both approaches METHODS: In accordance with the PRISMA Guidelines, the MEDLINE, Embase, and Cochrane databases were searched for studies comparing R-NSM versus C-NSM in patients with early-stage breast cancer, without evidence of lymph node metastasis or invasion of the nipple, skin, or chest wall. The main outcomes were mean operation time, postoperative hospital stay, overall and specific incidence of complications (nipple or areolar necrosis, seroma, hematoma, poor wound healing), blood loss and recurrence. Statistical analysis was performed using RStudio Version 4.4.0. Heterogeneity was assessed with I^2 statistics.

Results: We included 1810 patients from 9 studies comparing R-NSM versus C-NSM, with 569 (31,4%) of them undergoing robotic surgery, with a mean age of 45 years. Compared with the C-NSM group, the R-NSM group had longer surgical time (MD 51.69; 95%CI (20.47-82.92); $p < 0,01$; $I^2 = 91\%$) and longer postoperative hospital stay (MD 1.09; 95% CI (0.48-1.7); $p < 0.01$; $I^2 = 83\%$), although R-NSM was associated with less bleeding (MD -36.55; 95% CI (-65.38–7.72); $p = 0.01$; $I^2 = 88\%$). There was no statistical difference between the groups regarding recurrence (OR 0.77; 95% CI (0.28-2.11); $p = 0.612$; $I^2 = 36\%$), overall complications (OR 0.88; 95% CI (0.65-1.19); $p = 0.407$; $I^2 = 25\%$) and specific complications such as nipple or areolar necrosis (OR 0.64; 95% CI (0.35-1.19); $p = 0.159$; $I^2 = 39\%$), seroma (OR 1.16; 95% CI (0.53-2.53); $p = 0.708$; $I^2 = 0\%$), hematoma (OR 1,32; 95% CI (0.71-2.45); $p= 0,385$; $I^2=0\%$) or poor wound heal (OR 0.55; 95% CI (0.18-1.72); $p = 0.305$; $I^2 = 38\%$).

Conclusion: Both techniques do not differ in terms of recurrence and complications, however the robotic approach is associated with a longer surgical time and postoperative hospital stay. Although there is a trend towards improved aesthetic results and less bleeding in R-NSM, considering the costs, it should be indicated with caution.

P3-09-06: Predictors of axillary lymph node pathological complete response in clinical T4 breast cancer after neoadjuvant chemotherapy: results from a reference center in Brazil

Lilian Paz, Sabrina Fontana, Jorge Biazús

Background: Breast cancer of any size with direct extension to the chest wall and/or to skin (skin nodules or ulcerations) is defined as T4. In general, locally advanced breast cancer has a poor prognosis and increased likelihood of axillary lymph nodal disease. Upfront neoadjuvant systemic therapy is the standard care for clinical T4 (cT4) breast cancer and contributes to the downstaging of breast and axillary tumors. Current evidence has found that in patients with clinically node-positive breast cancer converted to node-negative with neoadjuvant chemotherapy, the false negative rate for sentinel lymph node biopsy (SLNB) performance is less than 10% when 3 or more sentinel lymph nodes are sampled. However, studies that evaluated the identification and false negative rate for SLNB following neoadjuvant chemotherapy included few cT4 tumors. Therefore, using SLNB in this setting is controversial and lacks established consensus. The prevailing standard approach today is still axillary lymph node dissection (ALND), especially for inflammatory carcinoma. Therefore, the objective of this study was to identify the factors predicting the nodal pathologic complete response (pCR) of patients with cT4 breast cancer who underwent neoadjuvant chemotherapy.
Methods: Women aged 18 or over diagnosed with cT4, cN0-N3, non-metastatic breast cancer who had received neoadjuvant chemotherapy followed by mastectomy with ALND and radiotherapy between 2018 and 2022 at Aristides Maltez Hospital in Bahia, Brazil, were eligible for the study. Demographics, clinical, tumor characteristics, and pathologic data were all collected through retrospective medical record review. For analysis, the resulting population was divided into two groups: one with node pCR; and another who had residual axillary nodal disease. Univariate and multivariate logistic regression analysis were used to explore the clinic-pathological variables hypothesized to be associated with nodal pCR.
RESULTS: The study included 202 patients with cT4, cN0-N3 breast cancer treated with neoadjuvant chemotherapy. Most were black women (162, 83.1%) with a median age of 51 years old and clinically node-positive breast cancer (174, 86.1%). Of all cases, 102 (55.4%) had residual axillary nodal disease and 90 (44.6%) had axillary nodal pCR. The proportion of patients with pCR in the breast and axilla was 22.4%. In univariate regression analysis, predictive factors of nodal pCR included breast pCR (OR=5.94; 95% IC: 3.09, 11.90), hormone receptor-positive status (OR=0.50; 95% IC: 0.28, 0.89), triple-negative subtype (OR=2.30; 95% IC: 1.21, 4.47), Ki-67 rate (OR=1.02; 95% IC: 1.01, 1.03), angiolymphatic invasion (OR=3.26; 95% IC: 1.46, 8.05), and pathologic invasion of the skin (OR=3.47; 95% IC: 1.33, 10.84). On multivariable analysis, the breast pCR and triple negative subtype remained strongly associated with nodal pCR, and clinically node-negative (cN0) was identified as a predictive factor as well.
Conclusion: For patients with cT4 cN0-N3, non-metastatic breast cancer undergoing neoadjuvant chemotherapy, we found that breast pCR, triple negative subtype, and clinically node-negative can be considered predictors of nodal pCR. While there was no consensus

regarding the axillary treatment approach for cT4 breast cancer, the predictive clinical and pathologic characteristics studied here can assist multidisciplinary teams in the decision to surgical de-escalation.

P3-09-07: Prospective cohort study of oncological outcomes following partial breast reconstruction with chest wall perforator flaps to facilitate breast conservation in breast cancer.

Pankaj Roy, Zita Jessop, Akriti Nanda, Bethany Elder, Sarun Thongvitokomarn

Purpose: The oncological safety of oncoplastic breast surgery lacks evidence. This prospective cohort study was undertaken to assess the long-term clinical and oncological outcomes of partial breast reconstruction (PBR) with lateral chest wall perforator flaps (LCWPF).

Participants: Patients diagnosed with DCIS or breast cancer undergoing breast conservation surgery (BCS) with PBR with LCWPF were included in the study. A prospective database has been maintained since 2011. The hospital electronic records were interrogated for women who have completed a minimum of 5 years follow-up and oncological outcomes were compared to published literature.

Findings: 105 patients underwent PBR with CWPFs between 2011 and 2018 with an average follow-up of 101 months (8.4 years, ranging 6.3 – 12.6 years). 65% were ER positive and Her-2 negative, 18.5% were triple negative whilst 16.5% were Her-2 positive. 15% were node positive at diagnosis and 50% had node positive disease after formal nodal assessment. The majority were T2 tumors, but 25% of these had either associated DCIS or multifocality which meant that larger volumes were excised than T status implies. 16.6% had neoadjuvant chemotherapy. 74% underwent cancer resection and PBR as one operation whilst 26% underwent PBR as two-stage approach. The median tumor size on pre-op imaging was 30.7 mm for one-stage approach and 39.9 mm for two-stage approach (p value=0.003). The re-operation rate for close margins was 10% with 4% requiring mastectomy. The local recurrence rate was 3.2%, contralateral breast cancer 2.1%, distant recurrence rate 12.3% which compares favorably to published literature. The disease-free survival was 83.2%, distant disease-free survival (DDFS) 88.4%, overall survival (OS) 88.4% and disease specific survival (DSS) 91.6% at 8.4 years median follow-up. All patients were followed up with clinical review for at least 5 years and >80% had very good to excellent and stable aesthetic outcome despite radiotherapy.

Conclusion: Our study establishes the oncological safety of this approach. To our knowledge, this is the first cohort study to publish evidence on long term oncological outcomes after BCS with CWPF. This approach can avoid mastectomy for relatively large or multifocal tumors and results in stable aesthetic results despite radiotherapy.

P3-09-08: Cryoablation in the Treatment of Early Breast Cancer - FIRST (FreezIng bReaST cancer in Brazil): A Before-and-After Study

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Background: Image-guided tumor ablation is a non-surgical, minimally invasive therapy available for the local treatment of carcinomas, offering an alternative to surgery. However, studies evaluating this therapy in early breast cancer have reported variable success rates, raising the question of whether omitting surgery is a viable option. Objective: The primary objective of this study is to evaluate the efficacy of cryoablation for the local treatment of early breast cancer. Secondary objectives include analyzing the negative predictive value of magnetic resonance imaging (MRI) and evaluating the necessary size of the ice ball relative to the largest tumor size to achieve complete tumor ablation. Methods: This is a multicenter, non-randomized, single-arm, before-and-after clinical trial. Inclusion criteria are patients with unifocal invasive breast carcinoma, tumors ≤ 2.5 cm, lesions visualized by ultrasound, and surgery indicated as the first treatment option. Exclusion criteria include ductal carcinoma in situ, multifocal or multicentric tumors, clinical axillary involvement, lesion-to-skin distance less than 5 mm, presence of distant metastases, and neoadjuvant treatment. All patients will undergo local cryoablation treatment, followed by conventional surgical treatment 14 to 28 days later. Imaging exams (mammography, ultrasound, and breast MRI) will be performed before and after cryoablation. The efficacy of cryoablation will be evaluated based on the success rate, defined as the absence of malignant neoplastic cells, both invasive and in situ, in the surgical specimen. If the expected success rate of cryoablation is like the 92% rate presented in the ACOSOG Z1072 study for patients without multifocal disease, this study will require at least 32 patients to determine if the technique is satisfactory (success rate greater than 70%), with a statistical power of 95% and a significance level of 5%. The study was approved by the local Ethics Committee and registered at Clinical Trials (NCT05398497). Results: A total of 47 patients were included in the study. However, 6 patients were excluded due to screening failures, presenting with suspicious enhancement on MRI greater than 2.5 cm or multicentric lesions, and 1 patient withdrew at the time of the ablation procedure. Therefore, 40 patients were evaluated.The mean age of the patients was 61.5 years, and the mean tumor size was 1.2 cm (0.5 – 2.5 cm). All participants underwent two cycles of freezing and thawing, using either a protocol of 6 minutes of freezing and 4 minutes of thawing or 8 minutes of freezing and 8 minutes of thawing, with a single cryoprobe connected to an argon and helium gas system. The complete ablation rate was 92.5%, while the ablation rate, considering only the absence of invasive lesions, was 100%. For tumors smaller than 1 cm, the success rate was 100%. Considering all cases that underwent MRI and showed focal lesions with enhancement up to 2 cm, the complete ablation rate was also 100%. The negative predictive value of breast MRI

was 95%. The average diameter of the ice ball observed on ultrasound was 3.9 times the size of the breast lesion. Among the 3 cases of residual lesion, 3 cases of residual ductal carcinoma in situ (DCIS) were observed, with an average size of 1.3 mm. Among the cases of residual lesion, 2 patients had not undergone MRI due to contraindication from having a cardiac pacemaker, and the other 1 showed enhancement on MRI of 2.2 cm. Conclusions: This study highlights cryoablation as a promising therapy and a viable alternative to surgery for patients with early-stage breast cancer. With a high rate of complete ablation, the technique proves effective for tumors up to 2 cm. MRI is crucial in planning the ablation, enhancing the success rate. The advancement of minimally invasive therapies reinforces cryoablation as a significant and effective option for treating early-stage breast cancer.

P3-09-09: Axillary staging techniques and oncologic outcomes for breast cancer patients with high clinical nodal burden undergoing neoadjuvant systemic therapy: A cancer registry study

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Background: Evidence for de-escalated axillary surgical staging after neoadjuvant systemic therapy (NAST) mainly exists for breast cancer patients with cN1 disease but not for patients with higher clinical nodal burden (cN2/3). We aimed to evaluate the role of axillary lymph node dissection (ALND) vs. targeted approached like sentinel lymph node biopsy (SLNB) or targeted axillary dissection (TAD) for patients with cN2/3 breast cancer undergoing NAST in a real-world setting.

Methods: We identified patients with cN2/3 breast cancer undergoing NAST diagnosed between 2009 and 2022 within the Baden-Württemberg cancer registry (BWCR), Germany. Invasive disease-free survival (iDFS) was assessed using Kaplan-Meier statistics and multivariate Cox regression models (adjusted for age, ALND vs. targeted approach, cN stage, cT stage, ypN stage, use of radiation therapy, and tumor biology).

Results: A total of 261 patients with a median follow-up of 24.9 months were identified: 69% (180 of 261) with cN2 stage and 31% (81 of 261) with cN3 stage. Median patient age was 59.6 years. cT stage was cT1-2 in 58.5% (152) of patients, cT3-4 in 41.6% (108). Tumor biologic subtype was Luminal in 49.4% (124) of patients, HER2 positive in 32.7% (82), and triple-negative in 17.9% (45). ypN stage was ypN0 in 44.4% (108), ypN1 in 19.8% (48), ypN2 in 22.6% (55), and ypN3 in 13.2% (32).
Use of ALND vs. a targeted approach as primary surgery was 88.1% (230 of 261) vs. 11.9% (31 of 261) overall; it was 35.4% (81) vs. 22.6% (7) in patients with cT3-4 stage, and 48.7% (112) vs. 48.4% (15) in patients aged over 60years, respectively. ypN stage was ypN0-1 in 48.7% (134) following initial ALND vs. 81.5% (22) after an initial targeted approach and ypN2-3 in 38.0% (82) vs. 18.5% (5). Use of radiotherapy was 84.3% (194) vs. 74.2% (23). Of the 31 patients undergoing a primary targeted approach, 12.9% (4) underwent

secondary completion ALND.
Median iDFS was 61 months in the ALND group and not reached in the targeted approach group (>60% without event). Multivariate Cox regression analysis revealed no significant influence for the use of a targeted axillary approach as primary surgery on iDFS: HR 0.64 (95% CI 0.23 to 1.80) for targeted approach vs. ALND; cT4d (HR 3.27, 95% CI 1.57 to 6.80) and ypN2-3 (HR 1.90, 95% CI 1.16 to 3.10) were significantly associated with worse iDFS.

Conclusion: These data suggest that a targeted approach, such as SLNB or TAD , may not have a disadvantage (i.e. shortening of the disease-free survival) compared to ALND as the first axillary surgery for patients with high clinical nodal burden. Complete response in the axilla (ypN0) for patients with cN2/3 was frequent at 44% and only 13% of SLNB patients underwent a secondary completion ALND. Thus, targeted approaches may be considered as a primary surgical intervention for patients with high clinical nodal burden (i.e. cN2/3) undergoing NAST. Larger studies with longer follow-up are welcome to fully inform this discussion. This study also demonstrates the advantage of real-world data collected through cancer registries, which allow the analysis of small patients groups that are difficult to consider in a randomized trial.

P3-09-11: Effect of Removed Lymph Nodes Number on Survival in Node-negative Early Breast Cancer: A Multicenter Retrospective Study (CSOBO YOUNG-03)

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Background: Although Sentinel lymph node biopsy (SLNB) is the standard treatment for clinically node-negative early breast cancer patients, axillary lymph node dissection (ALND), which always removes more than ten lymph nodes, is still widely used. Moreover, in SLNB, although the increased number of sentinel lymph nodes (SLNs) reduces false-negative rates, the effect of extensive lymph node removal on regional immune function and prognosis is unclear. For patients with no lymph node metastasis post-ALND (pN0), the impact of such removal on local immune barriers and prognosis remains uncertain. This study explores the association between the number of lymph nodes removed and survival in pN0 early breast cancer patients, aiming to clarify the effects of extensive lymph node removal on regional immunity and long-term outcomes.
Method: This study included 31,278 breast cancer patients diagnosed between 2013 and 2020 who underwent SLNB/ALND in 12 hospitals in China. Patients with lymph node metastases and neoadjuvant treatment were excluded. Propensity scores were utilized to match confounding variables between patients with SLNB and ALND within each subtype of breast cancer. Univariate, multivariate, and Cox proportional hazard models were implemented to evaluate the effect of SLNB/ALND on recurrence-free survival (RFS).

Results: In the univariate analysis, patients undergoing ALND had a worse RFS compared with those receiving SLN (HR=1.64, 95% CI: 1.37-1.97, P<0.001); after adjustment for confound factors, the tendency is still observed (HR=1.45, P=0.055). After the propensity score matching (1:2), 17,350 breast cancer patients were included. The SLNB group (n=6886) showed a significant difference in RFS (HR=1.31, 95% CI: 1.08-1.59, P=0.007) compared to the ALND group (n=3,556). For different subtypes, patients in the SLNB group showed statistically significant differences from those in the ALND group with triple-negative breast cancers (TNBC) (HR=1.76, 95% CI: 1.81-2.63, P=0.006) and a similar trend in the Luminal subtype (HR=1.21, 95% CI: 0.94-1.56, P=0.141), but no difference in the HER2+ subtype (HR=0.99, 95% CI: 0.60-1.63, P=0.962). The observed significant survival benefit in the SLNB group is mainly contributed by the TNBC group.
We further analyzed the number of lymph nodes removed on survival in the SNLB and ALND groups, respectively. In the SLNB group, a significant difference in RFS was shown for patients who had SLN<3 (HR=1.52, 95% CI: 1.14-2.02, P=0.004) and SLN≥5 (HR=1.33, 95% CI: 0.98-1.81, P=0.063) when compared to SLN between 3 and 5. Likewise, in the TNBC subtype, SLN between 3 and 5 showed significantly better RFS (HR=5.96, 95% CI: 2.27-15.6, P<0.001) compared to SLN≥5, while no significant differences were observed in the luminal and HER2-positive subtypes. In the ALND group, across all subtypes, compared with the group with ≤15 lymph nodes removed, no RFS compromise was observed for those removing more than 15 lymph nodes.
Conclusion: In pN0 breast cancer patients, SLNB is associated with better RFS compared to ALND, especially in the TNBC subtype. In SLNB, removing 3-4 SLNs may provide optimal outcomes, whereas removing more than 5 does not confer additional benefit and may negatively impact prognosis; in ALND, removing more than 15 nodes does not further improve RFS. Our findings imply that surgical removal of lymph nodes may disrupt the lymphatic immune barrier, particularly affecting TNBC patients. Further research is needed to illustrate our clinical findings and to optimize axilla surgical strategies.

P3-09-12: Impact of Extracapsular Extension on Recurrence in Sentinel Lymph Node Positive Breast Cancer Patients: A Retrospective Cohort Study

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Background: After the ACOSOG Z0011 trial, sentinel lymph node (SLN) biopsy became the standard for staging early breast cancer, demonstrating that in select patients with limited lymph node involvement, SLN biopsy alone was sufficient. However, this trial excluded patients with extracapsular extension (ECE), leaving its impact on recurrence rates unclear. This study evaluates the impact of ECE on recurrence rates in SLN-positive breast cancer patients and compares recurrence rates in patients with ECE based on the type of axillary surgery.

Methods: We conducted a cohort study of 1,579 SLN-positive breast cancer patients treated at Seoul National University Hospital from 2009 and 2018. Included were patients with invasive breast cancer, T1 or T2 tumor stage, and positive pathology results in both frozen and permanent SLN biopsy. Radiation status and type of surgery (breast-conserving or mastectomy) were not considered. Those who received neoadjuvant chemotherapy were excluded. Patients were divided into two groups based on the presence (N=266) or absence (N=1,313) of ECE. Recurrence rates were analyzed by evaluating disease free survival (DFS) using Kaplan-Meier and Cox regression models.

Results: The presence of ECE was associated with significant differences in patient characteristics. ECE-positive patients were older (51.8 vs 50.2 years, p=0.023) and more likely to undergo SLNB+ALND (82.0% vs 65.7%, p<0.001) compared to ECE-negative patients. They also had larger tumors and more advanced nodal disease (both p<0.001).
To reduce selection bias, we performed propensity score matching (PSM) with a 1:2 matching ratio. After PSM to account for pathologic T stage, number of positive SLNs, type of axillary surgery, and molecular subtype, the comparison between ECE-positive (N=252) and ECE-negative (N=441) patients showed no significant differences in DFS (log-rank p = 0.96). Using the same PSM data for multivariate Cox regression analysis, adjusted for tumor size, nodal involvement, and molecular subtype, there was no significant difference in DFS between ECE-positive and ECE-negative groups (HR = 0.936, 95% CI: 0.609-1.437, p = 0.762).
Among the 266 ECE-positive patients, we compared DFS between those who underwent SLNB alone and those who received SLNB followed by ALND. The addition of ALND did not significantly improve DFS among ECE-positive patients (log-rank p=0.33).

Conclusion: Our analysis revealed that the presence of ECE in SLN-positive breast cancer patients did not significantly affect recurrence rates. Furthermore, among ECE-positive patients, additional ALND after SLNB did not significantly improve DFS. These findings suggest that while ECE may indicate more advanced disease characteristics, it does not independently predict recurrence when other factors are adjusted for. Consequently, in certain cases, it may be feasible to omit ALND in ECE-positive patients with positive SLN. These results highlight the need for prospective studies to confirm these results and investigate potential therapeutic strategies.

P3-09-13: Applicability of SOUND trial in the practice of breast-conserving surgery at tertiary-care hospital in Thailand

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Background: The SOUND trial investigated omitting SLNB in EBC, reporting low recurrence rates and suggesting a shift towards minimal axillary treatment. Despite a trend towards

less radiation therapy (RT), the majority of SOUND participants received whole breast radiation. Current de-escalating treatments for HER2-positive and escalating treatment in high-risk ER+/HER2- cancers still depend on nodal status. Our study targets patients eligible for the SOUND protocol to assess the applicability of our patient's impact of skipping SLNB on treatment decisions.

Methods: Patients with cT1N0M0 breast cancer meeting SOUND trial criteria underwent breast-conserving surgery from 2012 to 2023 at a Thai tertiary single institute and were reviewed. Chi-square and Wilcoxon rank-sum tests were compared to clinicopathologic characteristics, with significance set at $P < 0.05$. Study endpoints included the applicability of the SOUND trial and the impact on adjuvant treatment by studying APBI percentages per ASTRO criteria, eligibility for APT trial, adjuvant CDK4/6i based on monarchE and NATALEE trials, and recurrence rates.

Results: In a study of 355 patients, the average age was 55, with 53.26% being premenopausal and 70.14% over 50. The most common cancer subtype was IDC at 89.86%. Most patients did not have lymphovascular invasion (83.38%), had pT1c (52.68%), and pN0 (89.55%), with a median tumor size of 1.5 cm. Nodal positivity was observed in 9.6% without pN2. Grades 1 and 2 were predominant at 82.11%. For subtypes, 87.32% were ER+, and 62.04% had Ki-67 ≥ 20. Hormone therapy was used in 86.97% of patients, covering 98.08% of those ER+. Chemotherapy (CMT) was given to 46.46%, which was 87.88% of patients with nodal positive and 50.61% of premenopausal patients. Among ER+/HER2- patients, 35.76% received CMT. Patients under 40 and below 65 received CMT at higher rates (66.67% and 51.8%, respectively). Node-positive patients had more positive LVI, higher Ki-67, and larger tumor size (pT1c-2). Trastuzumab was given to 43.9% of HER2-positive patients. RT was provided to 95.69%, mainly as WBRT (88.29%). Locoregional recurrence was 2.27%. According to ASTRO criteria, 33.24% were "suitable" and 61.69% were "suitable/cautionary" for APBI. RT omission was possible for 4.35%, and 8.73% qualified for the APT trial. Eligibility for adjuvant CDK4/6i per monarchE and NATALEE was at 1.78% and 14.95%, respectively. This cohort essentially differed from the SOUND study by being younger, more premenopausal, with higher T stages, more ER-negative, less HER2-positive, higher Ki67 ≥ 20, fewer pN1 cases, receiving more CMT but less trastuzumab. Recurrence, distant metastasis, contralateral breast cancer, and mortality rates were comparable after a median follow-up of 5.02 years.

Conclusion: The omission of axillary surgery according to the SOUND trial criteria might be acceptable to Thai patients despite a different population background. This is essential because of less axillary disease and more frequent adjuvant CMT use in Thai patients. However, the decision to deescalate axillary treatment should be cautious, particularly in patients with larger tumors, LVI positivity, high Ki-67 levels, and lack of genomic data due to the potential impact of nodal status on CMT. SOUND's effect on RT is minimal as the

widespread use of WBRT continues. The importance of long-term monitoring for axillary recurrence is highlighted. Caution is advised for HER2+ patients considering APT trials. The adoption of abemaciclib had a minor effect, whereas ribociclib use in node-negative cases could be influenced, although this remains low. If nodal status is needed, a second operation for SLNB is viable. Further research is necessary to understand the effects of omitting SLNB on costs, patient satisfaction, arm morbidity, and lymphedema compared to traditional SLN surgery.

P3-09-14: SHIELD study: Reoperation Rate in Breast-Conserving Surgery using Confocal Histolog® Scanner for Intraoperative Margin Assessment

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Objective: Immediate and accurate methods for the intraoperative assessment of surgical margins during breast conserving surgery (BCS) are expected to reduce the proportion of patients undergoing a re-operation due to cancer-positive margins identified post surgery. Previous studies suggest that the Histolog® Scanner is a promising tool to address this need by providing immediate microscopic images of ex-vivo specimens. The SHIELD study was conducted to prospectively quantify the reduction of re-operation rate of BCS when the Histolog Scanner is used intraoperatively to evaluate the margins.

Materials & Methods: Patients undergoing BCS due to invasive breast cancer and/or DCIS prior to any adjuvant treatment were enrolled in the study. Surgical excision specimens were imaged with the Histolog® Scanner during surgery immediately followed by margin assessment in the acquired Histolog® images by the surgeon. In addition, standard assessment techniques (visual inspection, palpation, ultrasonography and radiography) were performed at the discretion of the surgeon. Positive margin(s) identified in the Histolog images trigger the excision of additional tissue recuts during the same surgery. Subsequent re-operation rate was compared to historical data from the observational Polarhis study (Sandor MF et al. The Breast 2022, 66:118 - NCT05946759) performed at the same certified breast center by the same surgeons. Positive margin detection rates using Histolog® Scanner were compared to final pathology assessments.

Results: A total of 54 patients were screened, from which 2 did not fulfill the final inclusion and exclusion criteria and 2 did not adhere to the imaging protocol. Mean age was 63.56 years and tumor type distribution was 32% of pure invasive cancer(s), 18% of pure DCIS and 50% of invasive cancer(s) with associated DCIS. Rate of re-operation achieved using the Histolog® Scanner is 10% (5/50) while the historical rate from Polarhis study was 30% (12/40) corresponding to a 67% ($p = 0.016$) reduction of reoperation rates when using Histolog Scanner for intraoperative margin assessment. Among these five reoperations two patients had their positive margins correctly detected with the Histolog® Scanner but the corresponding intraoperative recuts were not sufficient to achieve cancer-negative status

and the protocol did not include the Histolog Scanner assessment of the recuts. 17/21 and 4/21 positive margins were intraoperatively identified respectively with the Histolog Scanner and with standard techniques used at the center. All positive margins detected using standard techniques were also identified using the Histolog® Scanner. Sensitivity of 80.9% and specificity 99.5% were achieved with the use of the Histolog® Scanner while the combined values of the standard techniques used at the center provided 17.4% and 97.3% respectively. Overall time of Histolog® Scanner margin assessment including specimen processing, imaging and analysis was 13.8 (+/- 5.5) min per patient.

Conclusion: Study results present that the use of the Histolog® Scanner for the intraoperative margin assessment during breast conserving surgery is providing a significant and substantial 2/3 reduction in the re-operation rate, with sustainable sensitivity and specificity for positive margin detection when performed by surgeons during BCS. Further studies are warranted to confirm these findings in a multicentric approach.

P3-09-15: Is it possible to recruit and randomise participants to an implant-based breast reconstruction trial in the UK? Results from the Best-BRA Study

Kirsty Roberts, Clare Clement, Nicola Mills, Shelley Potter

Background: Implant-based breast reconstruction (IBBR) is a rapidly evolving technique and evidence to support best practice is lacking. Randomised controlled trials (RCTs) in breast reconstruction are needed but are challenging due to patient and surgeon preference. Feasibility work prior to a full-scale trial is therefore essential.
The Best-BRA (Is subpectoral or pre-pectoral implant placement Best in immediate BReAst reconstruction?) pilot RCT aimed to determine whether it would be possible to recruit and randomise women to a study comparing two different approaches to implant-based breast reconstruction in the UK.

Methods: Best-BRA was a pragmatic, multicentre external pilot RCT in which women >18 who elected to undergo immediate IBBR for breast cancer or risk reduction and were considered eligible for both pre and subpectoral techniques by their operating surgeon were randomised 1:1 to either technique. Surgeons were permitted to perform the procedure according to their standard practice (e.g. patient/implant selection; use/type of mesh). The primary outcomes related to the feasibility of a future large-scale trial and included the number of participants who were recruited and consented to be randomised. A QuinteT Recruitment Intervention (QRI) was embedded to explore anticipated recruitment challenges and provide practical support for sites and the study team. The QRI involved two phases. In Phase 1, the sources of recruitment difficulties were investigated by: mapping eligibility and recruitment pathways; recording recruitment discussions between clinicians and patients; semi-structured interviews with the study team and site

staff; observing investigator meetings; reviewing screening logs and study documentation. Phase 2 involved supportive and responsive feedback to the study team and site staff and the implementation of recruitment intervention strategies informed by Phase 1. Phases 1 and 2 were undertaken in an iterative and cyclical manner.

Results: Best-BRA opened on 19/7/21 after a 20-month delay in set-up due to COVID-19. The average time to site opening was 310 days (range 71-557). A total of 12 UK sites were opened and 11 participants randomised over a 19-month period. The trial was closed in January 2023 due to poor recruitment. A future large-scale trial comparing pre and subpectoral IBBR in the UK was not considered feasible.
Screening logs revealed that only 22% participants screened were considered suitable for both IBBR techniques and, of these potentially eligible participants, only 41% were approached to take part in Best-BRA. Qualitative data from the QRI indicated both the lower-than-expected number of eligible patients and acceptability rates were due to a rapid adoption of pre-pectoral IBBR in the UK leading to a loss of equipoise. This change in practice was due to surgeons' perception that pre-pectoral reconstruction was a 'better' technique for patients. It was perceived as quicker and easier to perform and with improved cosmetic outcomes and less post-operative pain. The lack of high-quality evidence to support this change was acknowledged but surgeons felt the limited evidence that prepectoral IBBR was 'no worse', coupled with their personal experience of benefit was sufficient to change practice. This was coupled with a move away from IBBR techniques more generally due to high complication rates and poor long-term outcomes. These factors were further exacerbated by COVID-19-related delays and pressures and were beyond addressing by the QRI.

Conclusions: A large-scale RCT comparing pre and subpectoral IBBR is not feasible in the UK due to the widespread adoption of prepectoral reconstruction despite a lack of high-quality evidence. Prepectoral IBBR needs to be robustly evaluated and well-designed, large-scale studies such as iPREPARE will be essential support best practice.

P3-09-16: SerpinE2 mediated cell competition during breast cancer metastasis to the liver

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Background: Patients with breast cancer liver metastasis (BCLM) have a 5-year survival rate of 8%, highlighting an urgent need for novel therapeutics to target liver-specific treatment for BCLM. The liver is a cellularly dense organ, and it remains unclear how metastatic cancer cells (MCC) colonize and expand in the liver. Cell competition is a

developmentally conserved process that explains how cells use fitness-sensing mechanisms leading to survival and expansion of one cell population, and the induction of cell death through apoptosis in the other. This process is critical to organogenesis and tumorigenesis. We previously showed that MCC are able to create space during metastatic colonization of the liver by causing apoptosis in neighboring hepatocytes (HEP), which make up 80% of liver mass. Through a biological and bioinformatic screen, we found that MCC expresses SerpinE2, a serine protease inhibitor. SerpinE2 has been shown to correlate with increased metastatic potential but the mechanistic cause is still unknown. In this study, we tested the hypothesis that MCC engage in SerpinE2-mediated cell competition with HEP to create space and expand within the liver by eliminating neighboring HEP through apoptosis resulting in tumor expansion.

Methods: We develop new in-vitro and mouse lineage-traced models to model cell competition between MCC and HEP using syngeneic mouse breast cancer cells and hepatocytes. These models directly capture cell-cell interaction between MCC and HEP so we can assess for apoptotic and extrusion events, known modes of cell competition, through immunostaining and live imaging. We used hydrodynamic tail vein injections (HDTVI) to engraft mice livers with MCC. Prior literature suggests that the developing mammary gland informs both cell competition and metastasis, so we performed a bioinformatics screen to identify signals contributing to cell competition between MCC and HEP. We generated a new single-cell RNA-seq dataset incorporating information from the developing mammary gland and breast cancer cells. Through this, we identified a target gene, SerpinE2, enriched in both breast cancer and the developing mammary gland. We validated that SerpinE2 is more highly expressed in MCC compared to HEP. We used CRISPR-Cas9 technology to generate a SerpinE2 knock-out (KO) 4T1 cell line and lentiviral gene editing to restore the full length SerpinE2 gene in SerpinE2 KO MCC to generate a genetic rescue line. We then used our previously established in vitro and in vivo models to evaluate the functional role of SerpinE2 in cell competition and metastatic colonization in the liver.

Results/Discussion: Using our in vitro coculture models we demonstrated that wild-type (WT) MCC induce apoptosis in neighboring HEP through cell competition. MCC-induced apoptosis in neighboring HEP is 7.62-fold higher compared to apoptosis in neighboring MCC. KO of SerpinE2 in MCC did not affect their viability or growth rates in monocultures. In coculture with HEP, SerpinE2 KO resulted in increased apoptosis in MCC by 9.04-fold compared to WT MCC that neighbor HEP. There was a 4.75-fold decrease in HEP apoptosis compared to HEP in coculture with WT MCC. We then used our genetic rescue of SerpinE2 in MCC and cocultured them with HEP. SerpinE2 KO MCC with SerpinE2 restored increased apoptosis in HEP by 5.89-fold while decreasing MCC apoptosis by 4.10-fold when compared to cocultures with SerpinE2 KO MCC, similar to WT MCC. HDTVI of SerpinE2 KO MCC in mice showed a 10-fold decrease in micrometastasis in livers compared to those injected with WT MCC. These data suggest SerpinE2 regulates competition between MCC and HEP. Future work involves understanding the mechanistic regulation of metastatic colonization by SerpinE2 in the liver. These findings may suggest a novel therapy for BCLM patients that reduces MCC fitness or restores HEP fitness during liver colonization by MCC.

P3-09-17: Trastuzumab deruxtecan (T-DXd) in combination with capecitabine or capivasertib in patients with HER2-low metastatic breast cancer: a Phase 1b, multicenter, open-label study (DESTINY-Breast08)

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Background: T-DXd is approved in over 55 countries for patients with HER2-low (immunohistochemistry [IHC] 1+, IHC 2+ / negative results on in situ hybridization), unresectable/metastatic breast cancer (mBC) who received prior chemotherapy in the metastatic setting or developed disease recurrence during or within 6 months of completing adjuvant chemotherapy. DESTINY-Breast08 explored the safety, tolerability, and antitumor activity of T-DXd combinations in HER2-low mBC (NCT04556773). Results reported here are from the final data cutoff (DCO) of the dose-expansion phase (Part 2) of DESTINY-Breast08, for T-DXd + capecitabine (CAPE) and T-DXd + capivasertib (CAPI).

Methods: Eligible patients had locally confirmed HER2-low mBC with measurable disease per RECIST 1.1. In the T-DXd + CAPE arm, patients with hormone receptor–negative (HR–) or HR-positive (HR+) disease were eligible. In the T-DXd + CAPI arm, patients with HR– disease were eligible. Across both arms, patients with HR– mBC were allowed ≤1 prior line of chemotherapy. In the T-DXd + CAPE arm, patients with HR+ mBC were allowed ≤1 prior line of endocrine therapy ± a targeted therapy for mBC, with no prior chemotherapy in the metastatic setting. Patients received T-DXd 5.4 mg/kg intravenously (IV) every 3 weeks (Q3W) + CAPE 750 mg/m^2 orally (PO) twice daily (BID) on Days 1–14 Q3W, or 5.4 mg/kg T-DXd IV Q3W + CAPI 400 mg PO BID every week on Days 1–4 within a 21-day treatment cycle. Primary objectives were safety and tolerability; key secondary endpoints were objective response rate (ORR) and progression-free survival (PFS) by investigator per RECIST 1.1, overall survival (OS), and duration of response (DOR).

Results: As of August 16, 2023, 20 patients in the T-DXd + CAPE arm and 40 patients in the T-DXd + CAPI arm had received study treatment. Median age was 57.5 and 56.0 years, respectively. In the T-DXd + CAPE arm, 30.0% (n=6/20) of patients had HR– mBC, and 70.0% (n=14/20) of patients had HR+ mBC. In the T-DXd + CAPI arm, 32.5% (n=13/40) of patients had centrally confirmed AKT/PTEN/PIK3CA alterations detected by ctDNA assays. In patients with HR– disease, 83.3% (n=5/6) and 45.0% (n=18/40) received first-line chemotherapy for mBC in the T-DXd + CAPE and T-DXd + CAPI arms, respectively. In patients with HR+ disease in the T-DXd + CAPE arm, 64.3% (n=9/14) had received first-line hormonal therapy ± a targeted therapy for mBC. Median actual treatment duration was 11.1 months for T-DXd and 7.0 months for CAPE in the T-DXd + CAPE arm, and 6.2 months for T-DXd and 5.5 months for CAPI in the T-DXd + CAPI arm. Grade ≥3 adverse events (AEs) occurred in 55.0% (n=11/20) and 67.5% (n=27/40) of patients treated with T-DXd + CAPE and T-DXd + CAPI, respectively. Serious AEs were reported in two patients (10.0%) in the T-DXd + CAPE arm, and 13 patients (32.5%) in the T-DXd + CAPI arm. Three (15.0%) adjudicated drug-related interstitial lung disease / pneumonitis events were reported in the

T-DXd + CAPE arm (Grade 2, n=2; Grade 5, n=1) and eight (20.0%) in the T-DXd + CAPI arm (Grade 1, n=1; Grade 2, n=7). Confirmed ORR was 60.0% for both T-DXd + CAPE and T-DXd + CAPI. At final DCO, median DOR was not reached for T-DXd + CAPE but was 7.1 months for T-DXd + CAPI. Median PFS was 13.4 months and 9.0 months for T-DXd + CAPE and T-DXd + CAPI, respectively. Median follow-up duration was 15.2 months and 8.6 months for T-DXd + CAPE and T-DXd + CAPI, respectively. OS was not mature at final DCO for either arm; the survival rate at 12 months was 78.0% (95% confidence interval [CI] 51.5, 91.1) for T-DXd + CAPE and 92.0% (95% CI 77.2, 97.4) for T-DXd + CAPI.

Conclusion: Safety profiles for T-DXd + CAPE and T-DXd + CAPI were generally consistent with the known safety profile of each agent, and both combinations demonstrated antitumor activity; further research is warranted.

P3-09-18: Comparative analysis of protein and gene expression of biomarkers and therapeutic targets in patients with metastatic hormone-receptor positive breast cancer of no-special type versus invasive lobular breast cancer

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Background: Invasive lobular breast cancer (ILC) represents 15% of all breast cancer (BC). As of today, patients with metastatic ILC are treated similarly to patients with metastatic invasive BC of no-special type (IBC-NST), mainly due to the lack of dedicated research. In this study, we compared the expression levels of standard histopathological markers (estrogen receptor (ER), human epidermal growth factor receptor 2 (HER2), stromal tumor-infiltrating lymphocytes (sTIL)) as well as gene expression levels of targets for antibody-drug conjugates (ADC) and immune checkpoint inhibitors (ICI) in 503 metastatic samples from 22 patients with metastatic ILC versus IBC-NST obtained in the context of our post-mortem tissue donation program UPTIDER (NCT04531696).
Methods: In this study, we considered histopathological data from 22 UPTIDER patients who were diagnosed with primary HR+/HER2- BC (5 ILC, 14 IBC-NST, 2 mixed IBC-NST/ILC, 1 ILC with medical history of contralateral IBC-NST) and analyzed the markers according to the histology of the metastases (IBC-NST=303, ILC (originating from both primary ILC and mixed IBC-NST/ILC)=200). sTIL were scored according to international guidelines. ER expression was scored according to the Allred scoring system, with a cut-off of 1% for positivity in line with the ASCO/CAP 2020 guidelines. HER2 was scored according to the ASCO/CAP 2023 guidelines. Expression levels of 72 ADC and 23 ICI targets from established drugs or drugs under active clinical development were retrieved from complementary bulk RNA sequencing data. Associations between gene expression

(outcome) and histological subtype (co-variate of interest: ILC vs IBC-NST) was assessed by linear mixed quantile regression with random effect on patient ID.
Results: Although all but one patients had a primary ER+ tumor, 23% of patients with IBC-NST metastases and 40% of patients with ILC metastases displayed at least one ER- metastasis. A median percentage of 0% ER- IBC-NST metastases (range: 0-92.3%) and 0% ER- ILC metastases (range 0-59.1%) was observed per patient. sTIL levels were significantly lower in ILC (0.3% (range: 0-13.3%)) versus IBC-NST (median: 1.7% (range: 0-25%))) metastases (p<.001). A median of 63.6% HER2-low (range: 0-88.6%) and 48.69% (17.4-80%) metastases was observed in the 7 patients with IBC-NST and 3 patients with ILC analyzed so far. Regarding ADC targets, mRNA expression levels of FGFR2, CDH3, FN1, F3 and PTK7 were higher in IBC-NST metastases and expression levels of GUCY2C, ITGAV and VTCN1 were higher in ILC metastases (p<.05). There was no evidence of differences in mRNA expression levels regarding HER2 and HER3, while TROP2 levels were non-significantly higher in IBC-NST versus ILC metastases (p= .12). Regarding ICI targets, mRNA expression levels of HVEM were higher in IBC-NST metastases (p=.04). CD137 and TIM3 levels were non-significantly higher in IBC-NST metastases (p=.07 and .08, respectively). No differences were observed for PD-1 and PD-L1.
Conclusions: This work reports on the differences in the expression of clinical biomarkers and therapeutic targets of interest between patients with IBC-NST and ILC metastases. This further emphasizes the notion to consider, at least for some markers, ILC as a distinct BC entity.

P3-09-19: Tumor genomics in young patients with metastatic breast cancer

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Background: Breast cancer (BC) patients diagnosed at young ages (≤40y) often present with more aggressive tumors than older patients, highlighting the need to define differences in tumor biology by age. Limited data exists on tumor genomics in young metastatic BC (MBC) patients.

Methods: The Ending Metastatic Breast Cancer for Everyone (EMBRACE) study is a prospective cohort enrolling women with MBC who receive care at Dana-Farber Cancer Institute. Women in EMBRACE with targeted sequencing conducted via Oncopanel for at least one tumor sample (collected at metastatic or primary diagnosis), were included. Tumors were classified by molecular subtype [HR+/HER2- (classified as luminal A- or B-like, with luminal-B like defined by primary tumor grade=3 or metastatic tumor progesterone receptor staining <10%), HR+/HER2+, HR-/HER2+, and HR-/HER2-]. After filtering for germline SNPs, oncogenic and likely oncogenic variants were selected. Tumor

mutational burden (TMB) and variant frequencies were compared by age at MBC diagnosis (≤40y, >40-55y, >55y) using Fisher's exact test, overall and within tumor molecular subtypes. Odds of individual gene mutations within age groups were compared using multivariable logistic regression models, adjusting for molecular subtype, histology, race, TMB, de novo v. recurrent MBC, and primary v. metastatic sample type. Kaplan-Meier curves, stratified by age group, assessed overall survival (OS, measured from MBC diagnosis) by genetic mutation status. Multivariable Cox regression analysis, adjusting for MBC diagnosis age, tumor factors, and race, was used to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for OS by gene mutation.

Results: Tumor samples from 2,363 patients (1,594 metastatic, 769 primary) tested between July 2013-December 2020 were included. Median age at MBC diagnosis was 54 years (range: 18-91y). Median disease-free interval was 2.4 years (range: 0-33y), and younger women more likely to have de novo MBC (≤40y: 37% v. >55y: 23%, p<0.001). High TMB (≥10) was more common in older women with recurrent MBC (>55y: 28% v. ≤40y: 13%, p<0.001), though TMB did not differ by age for those with de novo MBC. After adjusting for tumor factors and race, compared to those >55y, patients ≤40y at MBC diagnosis were two to three times more likely to harbor mutations in BRCA1 [Odds ratio (OR) (95% CI)=3.11 (1.36-7.10), p<0.001], BRCA2 [OR (95% CI)=2.46 (1.15-5.27), p<0.001], and ERBB2 [OR (95% CI)=2.68 (1.46-4.90), p<0.001]. Mutations in GATA3 and TP53 were also more frequent among younger patients [OR (95% CI) GATA3 =1.68 (1.06-2.67), p<0.05; TP53=1.83 (1.22-2.73), p<0.01]. Mutations in CDH1 and PIK3CA were more common in older individuals [OR (95% CI) CDH1=0.07 (0.02-0.30), p<0.001, PIK3CA=0.45 (0.30-0.68), p<0.001]. Differences in OS by age were not statistically significant for de novo MBC patients, though OS was worse for recurrent MBC patients ≤40y v. >55y [median OS (95% CI)=2.8 (2.4-3.4y) v. 3.6 (3.3-3.9y), p=0.04]. In multivariable Cox models, mutations in TP53, MYC, and AKT1 were associated with shorter OS [HR (95% CI) TP53=2.05 (1.79-2.34), MYC=1.26 (1.01-1.56), AKT1=1.42 (1.10-1.84)], while mutations in GATA3, ERBB2, and MAP3K1 were associated with longer OS [HR (95% CI) GATA3=0.83 (0.69-1.00), ERBB2=0.53 (0.41-0.69), MAP3K1=0.75 (0.57-0.99)]. TMB was not associated with OS. Mutation-specific associations with survival did not differ by age group at diagnosis.

Conclusions: In EMBRACE, differences in mutational frequency of several genes were observed by age at MBC diagnosis among patients with recurrent MBC, most notably for HR+/HER2- patients. Lower OS among younger recurrent MBC patients may be driven by these differences, particularly by high frequency of TP53 mutations in this age group. Further investigation of these genes is warranted to elucidate pathways leading to metastasis and to improve survival for young MBC patients.

P3-09-20: Re-evaluation of Human Epidermal Growth Factor Receptor 2 (HER2) Immunohistochemistry (IHC) 0 or 1+ in Metastatic Breast Cancer (mBC) Samples to Characterize the Proportion of HER2-ultralow (IHC 0 With Membrane Staining)

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Background: The efficacy of fam-trastuzumab deruxtecan-nxki (T-DXd) in patients with HER2-low (IHC 1+ or IHC 2+/ISH negative) mBC was established by results of DESTINY-Breast04 and substantiated in DESTINY-Breast06 (HR+/HER2-low), which explored T-DXd efficacy in HER2-ultralow (IHC 0 with ≤10% cells with faint, incomplete membrane staining) as a secondary endpoint. Published evidence on the prevalence and characterization of HER2-ultralow in patients with mBC is limited. In this study, whole slide images (WSI) originally scored HER2 IHC 0 (with or without membrane staining) and 1+ were rescored to generate insights into identification of HER2-ultralow and scoring concordance.

Methods: WSI from 2020 or later, originally scored as HER2 IHC 0 (n = 400) or IHC 1+ (n = 200) stained by VENTANA HER2 4B5 (Roche), were manually rescored by 3 pathologists according to 2023 American Society of Clinical Oncology-College of American Pathologists (ASCO-CAP) guidelines. No training for ultralow was provided. Following a washout period of 2-10 weeks, each pathologist performed a second reading; readings were blinded and WSI were randomized. The review time and the percentage of cells with incomplete, faint membrane staining were recorded. WSI were rescored as either IHC 0 without membrane staining, HER2-ultralow, or IHC 1+/2+. The primary objective was to estimate the proportion of HER2 IHC 0 WSI rescored as HER2-ultralow. Secondary objectives included evaluating inter- and intraobserver concordance and observer concordance with the original scores. Concordance analyses were described by overall percentage agreement (OPA) and Cohen Kappa. Consensus was defined as a score that either 2/3 or 3/3 pathologists agreed on.

Results: Rescoring of the 600 WSI assessed, 2/3 pathologists showed consensus in 250 (41.7%) and 3/3 showed consensus in 336 (56.0%); 255 WSI were classified as IHC 0 per consensus rescore, with 129 (21.5%) showing no membrane staining and 126 (21.0%) with membrane staining. Of the 400 WSI originally scored IHC 0, 263 (65.8%) were rescored as HER2-ultralow (113, 28.3%) or IHC 1+ (150, 37.5%); 125 (31.3%) were scored as IHC 0 without membrane staining. Of the 200 WSI originally scored IHC 1+, 4 (2.0%), 13 (6.5%), 175 (87.5%), and 6 (3.0%) were rescored as IHC 0 without membrane staining, HER2-ultralow, IHC 1+, and IHC 2+, respectively.
Concordance

Concordance between pathologists varied from substantial (OPA [95% CI], 86.5% [83.5%-89.1%]; Cohen Kappa [95% CI], 0.774 [0.729-0.818]) to moderate (OPA [95% CI], 61.2% [57.1%-65.1%]; Cohen Kappa [95% CI], 0.427 [0.378-0.476]). Average interobserver concordance (average OPA) was 69.9%. Intraobserver concordance was high (OPA [95% CI] values ranged from 88.2% [85.3%-90.6%] to 84.7% [81.5%-87.5%]; Cohen Kappa [95% CI] values ranged from 0.805 [0.763-0.846] to 0.765 [0.722-0.807]). Agreement between pathologists' consensus scores and original scores was moderate (OPA [95% CI], 70.8% [67.0%-74.4%]; Cohen Kappa [95% CI], 0.439 [0.376-0.502]).
Review Time
Median review time in minutes (min) was shorter for WSI rescored as HER2 IHC 0 without membrane staining (5.7 min) than WSI with any staining up to 10% (HER2-ultralow [7.5 min] or IHC 1+ [7.0 min]).

Conclusions: While 28.3% of IHC 0 WSI were rescored as HER2-ultralow, 65.8% could be reclassified with a clinically actionable level of expression (HER2-ultralow or IHC 1+). The discrepancies in scoring and a trend to extended review times for HER2-ultralow and IHC 1+ highlight the need for training, digital tools for decision support, and increased awareness of the available therapies for HER2-ultralow and IHC 1+ cases, to motivate pathologists to improve consistency in scoring for optimal patient care and treatment selection.

P3-09-21: Phase IB/II Trial of Alpelisib with iNOS Inhibitor and Nab-paclitaxel in Patients with HER2 negative Metastatic or Locally Advanced Metaplastic Breast Cancer

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Introduction: Metaplastic breast cancer (MpBC) comprises only about 1.2% of all breast cancer cases, but this rare form of triple negative breast cancer is extremely aggressive and chemo-refractory, with median overall survival of only 3-8 months in the metastatic setting. Inducible nitric oxide synthase (iNOS) is critical for triple negative (TNBC) and MpBC because of nitric oxide's important role in many oncogenic pathways, such as PIK3CA/AKT (often mutated in MpBc), RAS/ERK, HIF1α, and NF-κB. Through combined blockade of iNOS and PIK3CA, with L-NMMA and alpelsib, along with nab-paclitaxel chemotherapy, we aimed to improve outcomes for patients with metastatic or locally advanced MpBC. We report here the results from the Phase IB portion of this trial.

Methods: Patients with metastatic or locally advanced, unresectable triple negative MpBC received nab-paclitaxel 260 mg/m2 every 3 weeks, and L-NMMA 20 mg/kg (given on days 1-5 of each cycle) every 3 weeks. Varying doses of alpelisib were given, starting at dose

level 0 (250 mg on days 1-21), and eventually reduced to dose level -1 (250 mg on days 1-10). Co-primary objectives were as follows: 1) to define the recommended phase II dose (RP2D) of alpelisib in combination with nab-paclitaxel and L-NMMA, and 2) to determine the objective response rate (ORR) of this combination therapy in MpBC.

Results: Nine female patients were enrolled on this trial, with median age of 58 years. Four patients (44.4%) carried a PIK3CA mutation. Three patients received dose level 0, and 6 patients received dose level -1. With only 2 dose-limiting toxicities (DLT's) at dose level -1, this was determined to be the recommended phase 2 dose, planned for the remainder of the trial. DLT's at dose level 0 included grade 3 rash and mucositis. At dose level -1, two patients experienced grade 3 rash. The ORR was 44.4%, and Clinical Benefit Rate (CBR) was 77.8% (1 CR, 3 PR's, 3 SD's). Two responders were able to stay on treatment for >6 months without progression. One stopped due to grade 3 neuropathy from nab-paclitaxel, and the other stopped because she wanted to return home. Only 22.2% (2 patients) experienced progressive disease after 2 cycles of therapy. Reponses were especially robust in the 4 patients with PIK3CA mutation – one experienced CR, two had PR, and one had SD.

Conclusion: In this highly refractory group of patients, this novel, rationally designed therapy has shown remarkable efficacy, not previously demonstrated in MpBC, including some sustained responses, particularly in those subjects with PIK3CA mutations. Equally as important, the regimen was also found to be safe and tolerable, as we begin the multi-center phase II portion of this trial.

P3-09-22: Phenomenal: Efficacy and safety of liposomal irinotecan in patients with HER2-negative breast cancer and brain metastases

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Background: Liposomal irinotecan (nal-IRI) is a novel formulation of irinotecan, a topoisomerase 1 inhibitor encapsulated in a liposome drug delivery system that has shown promising activity in patients (pts) with brain metastases (BMs). Phenomenal is a non-randomized phase IIa study evaluating the efficacy and safety of nal-IRI as single agent in HER2-negative (HER2[-]) breast cancer pts with previously untreated, stable or progressive BMs following local treatment.

Methods: Phenomenal (NCT03328884) is an open-label, single-arm, multicenter, Simon two-stage phase IIa trial. Pts aged >18 years with pretreated HER2[-] breast cancer with previously untreated, stable, or progressive BMs were enrolled. Pts received intravenous nal-IRI at 60 mg/m2 (if based on irinotecan hydrochloride trihydrate [salt base]) or 50 mg/m2 (if based on irinotecan anhydrous [free-base]) on day 1 of a 14-day cycle until disease progression, unacceptable toxicity, or consent withdrawal. Primary endpoint was intracranial objective response rate (IC-ORR) according to RANO-BM in pts with progressive BMs. It was locally confirmed at the next tumor assessment (after 6 weeks) or with a ≥65% volumetric reduction of IC lesions by blinded independent central review. Secondary endpoints included IC, extracranial (EC) and overall (IC + EC) ORR, progression-free survival (PFS), overall survival (OS), duration of response) and clinical benefit rate at 12 weeks according to RECIST v.1.1; and safety and tolerability as per NCI-CTCAE v.4.0. Primary analysis estimated the IC-ORR (H0≤5.0%; H1: IC-ORR ≥15.0%); 95% CI and p-value were calculated with the method of Jung and Koyama. Sample size was planned to attain an 80% power at a nominal α level of 0.05.

Results: Between July 2017 and April 2024, 56 pts were allocated at 16 sites in Spain. Median age was 52 (range 32-83) years. A total of 3 and 51 pts had previously untreated and progressive BMs, respectively. Two patients had stable BMs. Twenty-seven pts (48.2%) had HR[+]/HER2[-] tumors and 29 pts (51.8%) had triple-negative breast cancer. The median number of previous lines of therapy for advanced disease was 3 (range, 1-8). At data cutoff (May 15th, 2024), with a median follow-up of 5.7 months (mo) (range, 0.4-56.5), 2 pts (3.6%) remained on therapy. The primary endpoint of the study was met with 10 pts out of 51 (19.6%; 95% CI 11.1-28.9) with progressive BMs achieving a confirmed IC-ORR ($p<0.001$). IC-ORR in all pts was 19.6% (11/56 pts; 95% CI, 10.2–32.4). EC-ORR in pts with measurable extracranial disease was 2.7% (1/37 pts; 95% CI, 0.0-14.2) and overall (IC + EC) ORR in all pts was 5.4% (3/56 pts; 95% CI, 1.1–14.9). Median PFS was 1.5 mo (95% CI, 1.4-2.8). Median OS was 6.4 mo (95% CI, 4.3-10.8). The most common treatment emergent adverse events of any grade (G) were fatigue (55.4%; 7.1% G≥3) and headache (44.6%; 1.8% G≥3). There were no treatment-related deaths and no new safety issues were reported.

Conclusions: Although the primary endpoint was achieved, nal-IRI showed limited antitumor activity in HER2[-] breast cancer pts with progressive BMs. These results do not support further evaluation of this treatment in this patient population.

P3-09-23: Investigating differences in the composition of circulating tumor cells (CTCs) clusters in invasive lobular and ductal carcinoma to decipher lobular breast cancer metastasis

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Background: Invasive lobular carcinoma (ILC) has distinctive clinical and genomic features compared to invasive ductal carcinoma (IDC); however, for patients (pts) with ILC treatment is selected according to the same guidelines as IDC. Better characterization of ILC and development of specific approaches for ILC pts is an unmet need. Liquid biopsy (LB) is a useful tool to achieve this goal. Our group showed that, compared to IDC, ILC has specific circulating tumor DNA (ctDNA) alterations and higher CTC count. Another study reported higher detection of CTC clusters (CTC-CL), considered the main seed of metastasis, in ILC. This finding is paradoxical since cell-cell adhesion (a key feature of CTC-CL) is impaired in ILC. Interestingly, an association between CTC-CL counts and ctDNA CDH1 alterations (a hallmark of ILC) was reported, regardless of histology. To investigate whether different mechanisms are responsible for CTC clustering in ILC and IDC, in this study we characterized CTC-CL according to the breast cancer (BC) histotype.

Methods: Blood samples were collected from 351 pts with stage IV BC before starting a new line of therapy at Northwestern University (Chicago, IL) between 2016 and 2021 (NU16B06 trial). Blood samples were processed with the CellSearch system for CTC and CTC-CL enumeration by a single expert operator. CTC-CL were defined as groups of ≥2 CTCs , or ≥1 CTC clustered with ≥2 white blood cells (WBCs). The number and size of CTC-CL, and the presence of WBCs in CTC-CL (heterotypic) were compared between ILC and IDC. Also, the association between CTC-CL and overall survival (OS) was tested. To further explore mechanisms of CTC-CL formations, we assessed potential differences in the association between CTC-CL presence and ctDNA alterations in ILC vs IDC. For ctDNA analysis, matched plasma samples were analyzed using Guardant360 and tested for the 10 most altered genes and CDH1.

Results: Of the 351 pts included, 255 (73%) had IDC while 45 (13%) had ILC. Overall, CTC-CL were identified in 45 (13%) pts and only in those with ≥ 5 CTCs. The presence of CTC-CL was significantly higher among ILC pts (27% vs 11% in IDC, p=0.004) but the total number of clustered CTCs was significantly lower in ILC than IDC (median 4.5 vs 9.5, p=0.039), suggesting a smaller size of CTC-CL in ILC. Indeed, the median and maximum number of CTCs per CTC-CL was numerically lower in ILC than IDC. Heterotypic CTC-CL were identified in 6 (50%) and 10 (36%) of ILC and IDC pts, respectively. Among these pts, there

was a trend for a higher median (0 vs 2, p=0.37) and maximum number of WBCs (2.5 vs 3.5, p=0.26) in CTC-CL in ILC than IDC. Overall, the presence of >3 CTC-CL was associated with shorter OS (6 vs 21 months, p=0.001). A matched plasma sample for ctDNA analysis was available for 129 IDC and 19 ILC pts. CDH1 alterations were detected in 2 IDC and 1 ILC pts and associated with CTC-CL (p=0.015) only in IDC. No significant association between ctDNA alterations and CTC-CL was observed in ILC possibly due to the small sample size; further analysis is ongoing.

Conclusion: ILC is characterized by a higher number of CTC-CL than IDC. CTC-CL in ILC appear to be different from IDC, being smaller but more frequently associated with WBCs. This suggests a possible different biology for CTC-CL formation in ILC related to the impaired cell-cell adhesion and a specific role played by the CTC-CL microenvironment. Indeed, the interaction with immune cells in ILC may promote the survival in the bloodstream of smaller CTC-CL thus enhancing metastatic efficacy. Further studies including a larger number of pts are needed to validate and better elucidate these findings. The study of CTC-CL could shed light on the distinct pattern of metastatic spread of ILC, potentially offering therapeutic opportunities, and serving as a useful prognostic factor.

P3-09-24: PLEURAL T CELLS FROM PATIENTS WITH METASTATIC INVASIVE LOBULAR CARCINOMA PRODUCE EFFECTOR CYTOKINES AND MOUNT ANTI-TUMOR IMMUNITY

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Breast cancer (BC) metastatic to the pleura is uniformly fatal with a median survival of six months and quality of life that is diminished by dyspnea, pain and discomfort. There are currently no curative treatments once malignant pleural effusions (MPE) have occurred. Despite significant clinical progress in immuno-oncology, there has been almost no change in survival or quality of life for patients with MPE. We have shown that BC MPE are characterized by a distinct and complex pleural secretome that varies little between breast cancer subtypes. Our studies on freshly isolated pleural T cells (PIT) from breast cancer MPE of all subtypes indicate that these cells are quiescent rather than exhausted, and poised to mount potent anti-autologous tumor effector responses, but locally suppressed by the pleural environment. To date, there are no reports on cytokine secretion and immune checkpoint expression in pleural T cells isolated from patients with invasive lobular carcinoma (ILC) metastatic to the pleura. In this abstract we show that freshly isolated pleural T cells from patients with ILC secrete T-cell effector cytokines (IL-2 or IFNγ), are not exhausted (lack or have low expression of PD1, TIM3, LAG3, TIGIT, CTLA4) and a minority express CD137/4-1BB activation/signal transduction molecule, critical to anti-tumor effectors. Consistent with our findings in BC grouped by HR status, the 8 most prevalent cytokines in the pleural secretome (CXCL10, CCL2, sIL-6Rα, IL-6, CXCL1, TGFβ, CCL11, IL-

10) are indistinguishable between ILC and IDC. However, ILC cases clustered together in hierarchical clustering across 40 measured cytokines, due chiefly to a paucity of G-CSF, VEGF, IL-7, and an excess of IL-4. The data suggest that ILC PIT are immunologically competent when removed from their suppressive environment and can be used to generate an adoptive cellular therapeutic.

P3-09-25: Fam-trastuzumab Deruxtecan-nxki Versus Chemotherapy as Post-Chemotherapy Treatment in Patients with HER2-Low Metastatic Breast Cancer: An Analysis Of Real-World Data from the IntegraConnect PrecisionQ De-Identified Database

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Background: The HER2-targeting antibody-drug conjugate fam-trastuzumab deruxtecan-nxki (T-DXd) is indicated for the treatment of metastatic HER2-low breast cancer. This real-world study sought to characterize the use of T-DXd in the post-chemotherapy setting and compare the outcomes between patients treated with T-DXd versus subsequent chemotherapy after chemotherapy failure. Methods: This was a retrospective, observational analysis of data from patients in the IntegraConnect PrecisionQ real-world de-identified database, enriched with information obtained by curation. The study included adults (age ≥18 years) with HER2-low metastatic breast cancer (mBC) who initiated systemic chemotherapy after mBC diagnosis and subsequently initiated a later line of therapy (LOT; chemotherapy or T-DXd) between June 1, 2022 and February 29, 2024. The index date was the date of subsequent treatment initiation, with follow-up through May 20, 2024. The primary outcome was overall survival (OS); also included were patient demographics, clinical characteristics, and the type of post-chemotherapy treatments used. Data are presented using descriptive statistics. Results: Of the 347 patients with HER2-low mBC and post-chemotherapy treatment included in the analysis, most were female (99.4%) and white (75.8%). The distribution of Eastern Cooperative Oncology Group score was as follows: ECOG 0: 33.2%; ECOG 1: 50.2%; ECOG 2: 13.8%; ECOG 3: 2.5%; ECOG 4: 0.3%. Most patients were on metastatic LOT2 (38.3%), followed by LOT4+ (35.2%) and LOT3 (26.5%). Baseline demographics were generally similar between patients treated with T-DXd versus chemotherapy in the post-chemotherapy setting. Overall, 155 patients received T-DXd and 192 received chemotherapy as the post-chemotherapy LOT. Over a median follow-up (IQR) of 9.2 (4.4, 15.0) months in the T-DXd group and 7.9 (4.5, 12.9) months in the chemotherapy group, a total of 47 (30.3%) patients and 84 (43.8%) patients died, respectively. T-DXd was the second most common post-chemotherapy treatment (27.3%) and was used more frequently in the LOT4+ setting than the LOT2 setting (37.6% versus 19.6%, respectively). Across all post-chemotherapy LOTs, the median OS was not reached (NR) (14.3, NR) in the T-DXd group and was 13.6 (11.3, 17.2) months in the chemotherapy group. At 6 months, the probability (95% confidence interval) of survival was 84.1% (76.8, 89.3) and 76% (69,

81.7) in the T-DXd and chemotherapy groups, respectively. At 12 months, these values were 69% (59.4, 76.7) and 54.1% (45.2, 62.1), respectively, and at 21 months, they were 53.9% (42.5, 64) and 31.2% (21.2, 41.7). Conclusion: Patients with HER2-low mBC treated with T-DXd after failing chemotherapy have higher survival probability than those treated with chemotherapy. These data support the use of T-DXd in the second- and later-line metastatic settings. Limitations of this analysis include a limited follow-up time and those inherent in observational, real-world studies. Future research will build on these insights to help inform treatment choice in this setting.

P3-09-26: Efficacy of trastuzumab deruxtecan (T-DXd) in patients with metastatic lobular breast cancer with or without HER2 mutations: the MSKCC experience

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Background: In patients with HER2-low metastatic breast cancer (MBC), T-DXd improves outcomes compared to chemotherapy. Metastatic invasive lobular carcinoma (mILC) is associated with lower rates of HER2 amplification compared to invasive ductal carcinoma, but higher rates of HER2 (ERBB2) mutations, which are found in 14-15% of mILC. The SUMMIT trial demonstrated meaningful activity of neratinib + fulvestrant + trastuzumab in patients with HR+, HER2 non-amplified, HER2-mutant MBC. In DESTINY-PanTumor01, patients with solid tumors with HER2 mutations, 20% of whom had MBC, received T-DXd; median progression-free survival (PFS) was 5.4 months (95%CI 2.7-7.1). The activity of T-DXd in HER2-low is not well described by histology, nor for HER2-mutant ILC.
Methods: This single-center retrospective cohort study identified patients with HER2-negative mILC treated with T-DXd from 2018-2024 at Memorial Sloan Kettering Cancer Center (MSKCC). We abstracted patient demographics, clinicopathologic characteristics, T-DXd treatment history, and dates of disease progression (defined as radiographic or clinical progression leading to treatment change) from medical records. Presence or absence of HER2 or PIK3CA/AKT/PTEN mutations was determined using the MSK-IMPACT next generation sequencing platform from tissue samples taken prior to initiation of T-DXd. Median time to progression was computed using the Kaplan-Meier method.
Results: Among 41 patients, 34 (83%) had HR+ disease and 7 (17%) had TNBC. The median number of prior therapies in the metastatic setting was 5 (range 2-21); median number of prior chemotherapies was 1 (range 0-6). Among HR+ patients, the number of median prior endocrine therapies was 3 (range 1-10), 31/34 (91%) had prior CDK4/6 inhibitor, and median duration of CDK4/6 inhibitor was 11 months (range 1-72). Across all metastatic site biopsies, the highest degree of HER2 expression per IHC was 0 in 3 patients (7%), 1+ in 23 patients (56%), and 2+ in 14 patients (34%). 10 (24%) patients had HER2 mutations and 18 (44%) had PIK3CA/AKT/PTEN mutations. Median time to progression (TTP) in the total cohort was 7.2 months (95%CI 4.6-13.1). In patients with HER2 mutations, TTP was not reached (95%CI 6.6-NR) vs. 6.4 months (95%CI 4.0-12.4) in patients without HER2

mutations (p=0.09). In patients with PIK3CA/AKT/PTEN mutations, TTP was 4.6 months (95%CI 3.6-8.7) vs. 9.2 (95%CI 6.6-NR) in patients without pathway mutations (p=0.01). There were no significant differences for HR+ vs. TNBC or HER2 IHC 0/1+ vs. 2+. 27 (66%) patients discontinued T-DXd for disease progression, 3 (7%) for toxicity, and T-DXd treatment was ongoing in 11 (27%). Median overall survival in the total cohort was 18.2 months (95%CI 15.8-NR). 1 patient with HER2 mutation and HER2 IHC 0 on all biopsies in the early-stage and metastatic setting has been on T-DXd for 32 months; treatment is ongoing. Additional data on prior neratinib use in this cohort will be presented at the meeting.
Conclusions: In patients with mILC and HER2 mutation, there was a trend toward longer TTP with T-DXd. Genomic data could aid in prognostication in patients with mILC treated with T-DXd. These findings warrant confirmation in larger cohorts.

P3-09-27: Identification of glucocorticoid receptor dependence in metastasis of invasive lobular carcinoma using an in vivo xenograft MIND model

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Invasive lobular carcinoma (ILC) originates in the milk-producing glands of the breast and exhibits a nearly 100% five-year survival rate when confined to local invasion of nearby tissues. However, distant metastatic spread significantly reduces the five-year survival rate to approximately 22%, highlighting the unmet clinical need for added treatment options. Advancements in clinical management of ILC may benefit from a comprehensive understanding of metastatic tumor biology. Estrogen receptor (ER)-positive ILC relies on ER for growth and proliferation, yet little is known about the biological factors that drive metastatic proclivity and how the interplay with the body's stress response hormone, glucocorticoid receptor (GR), mitigates this cascade.
Previously, it was shown that ligand activation of tumor GR could impede ER transcriptional activity through displacement of both wild-type and mutant ER, leading to reduced activation of ER-mediated pro-proliferation gene expression (Tonsing-Carter et al., 2019). It has also been shown that activation of tumor cell GR in ILC cell lines has a similar anti-proliferative effect on ER-mediated gene expression (Porter et al, 2023). While ILC growth at the primary site has been well studied, there is limited data characterizing the establishment of the pre-metastatic niche by circulating tumor cells and elucidation of tumor biology at metastatic sites in the context of tumor cell GR.
Circulating tumor cells are highly prevalent in lobular carcinoma making it a good model for studying their molecular characteristics using the established in vivo xenograft MIND model (Behbod et al. 2009). We hypothesize that the prevalence of circulating tumor cells correlates with tumor cell GR status and may drive metastasis to secondary organs such as bone and brain. Utilizing in vivo models to collect ILC circulating tumor cells (SUM44, MM134, BCK4) at 60, 90, 120, 160 days will help us better understand the role of tumor cell

GR in the timing of the metastatic cascade and establishment of the pre-metastatic niche. Here, we show the presence of tumor cells within the vascular regions (blood) of intact femurs using highly specialized microscopic and tissue clearing techniques in our in vivo xenograft MIND model. Ongoing studies include the combination of circulating tumor cell collection and bone marrow extraction, to quantify the prevalence of tumor cells in high versus low tumor cell GR states among different ILC cell lines. Future studies include the use of GR modulators in xenograft MIND model to mitigate the establishment of metastatic tumors.

P3-09-28: Long-term follow-up on survival outcomes in patients developing ipsilateral breast tumor recurrences.

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Background: As more patients are treated with breast conserving surgery, there are increasing cases of patients experiencing the development of ipsilateral breast tumor recurrences (IBTRs). In this study, we aimed to investigate factors affecting survival outcomes in patients with IBTR. We placed special interest in addressing the relative importance of IBTR tumor characteristics in predicting survival outcomes in comparison with the characteristics of the initial breast cancer.
Methods: This is a retrospective study that reviewed data of the patients who were treated at Seoul National University Hospital between January 2000 and March 2022 for their IBTRs. The study included only cases of invasive breast cancer recurrence, excluding cases of ductal carcinoma in situ IBTR. To identify the factors influencing distant metastasis-free survival (DMFS), we performed multivariate Cox proportional hazards regression analysis.
Results: A total of 235 patients with IBTR were identified. The median follow-up period is 141.7 months and 60 patients developed distant metastasis during the follow-up.
Survival analysis associated with both initial and IBTR tumor characteristics revealed distinct patterns of prognostic impact showing the features of IBTR tumors showing more significant associations with DMFS compared to the features of the initial tumors. Compared to T1 stage, T2 stage IBTR showed a two-fold increase in the risk of developing distant metastasis (95% CI, 1.03-3.87), while T3 or T4 stage tumors had a 4.2-fold higher risk(95% CI, 2.16-8.13). Triple-negative breast cancer (TNBC) IBTR demonstrated a 2.2-fold higher risk compared to HR+/HER2- subtypes (95% CI, 1.16-4.29). The presence of lymphovascular invasion in IBTR was associated with a 2.6-fold increase in risk(95% CI, 1.44-4.63). Notably, node-positive recurrence tumors did not show significant differences in DMFS compared to node-negative or non-dissected cases. In contrast, when examining the impact of initial tumor characteristics on DMFS, only lymphovascular invasion showed significant prognostic importance, with a 1.9-fold increased risk (95% CI, 1.12-3.34). The T stage, N stage, and subtype of the initial tumors did not significantly affect DMFS after the development of IBTR.
Conclusion: In patients with IBTR, the characteristics of the recurrent tumor demonstrated

more significant prognostic impact on DMFS compared to those of the initial tumors. These findings suggest that the biological features of the recurrent tumor, rather than those of the initial tumor, can be the primary basis for determining the risk of subsequent distant metastasis and guiding treatment decisions for patients with IBTR.

P3-09-29: A Novel BBB-permeable Agent for Breast Cancer Brain Metastases

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Metastatic breast cancer is the second leading cause of cancer-related deaths among American women. The most common sites of breast cancer metastases are the bones, lungs, brain, and liver. Among the different sites, breast cancer brain metastasis (BCBM) constitutes approximately 10-30% of metastatic breast cancer and is associated with worst dismal prognoses, with a median survival time of 7.9 months. The poor outcomes are attributed to the limited understanding of the driving factors of BCBM and the scarcity of drugs that can cross the blood-brain barrier (BBB) and blood-tumor barrier (BTB). This underscores the critical need to develop new therapies for BCBM. Our lab identified truncated glioma-associated oncogene homolog 1 (tGLI1) as an alternatively spliced, gain-of-function, tumor-specific variant of GLI1. Since its discovery, tGLI1 has been reported to promote breast cancer stem cells (CSC) and breast cancer preferential metastasis to the brain. Additionally, we recently identified the FDA-approved antifungal ketoconazole to selectively target tGLI1-positive breast cancer cells in vitro. Ketoconazole was also found to prevent the preferential metastasis of tGLI1-positive breast cancer to the brain and suppress the progression of established tGLI1-positive BCBM in vivo (Doheny et al., Cancers 14:4256, 2022). Ketoconazole is a potent inhibitor of the cytochrome P450 enzyme CYP3A4 and has been associated with liver damage, adrenal insufficiency, and drug interactions. To mitigate this issue with potential toxicities, we modified the chemical moieties of ketoconazole and generated novel derivatives. We screened the compounds using isogenic brain-tropic breast cancer cell lines engineered to stably express the vector, GLI1, or tGLI1 and identified the novel derivative WF-229A to selectively target tGLI1-positive breast cancer stem cells. Foremost, our data shows that WF-229A lost the ability to inhibit CYP3A4 enzymatic activity in vitro. Additionally, WF-229A showed no toxic effects on normal brain cells (astrocytes) or the liver cells (HepG2). In efforts to evaluate the pharmacological properties of WF-229A, we conducted an in vivo maximum tolerated dose (MTD) study. Our results demonstrated that, unlike ketoconazole, increasing doses of WF-229A did not elevate serum alanine transaminase (ALT) levels, indicating no liver toxicity. Additionally, increasing doses of WF-229A did not cause an increase in serum Adrenocorticotropic hormone (ACTH) levels suggesting, no adrenal insufficiency, unlike ketoconazole. Furthermore, our experimental mouse metastasis study using intracardiac inoculation demonstrated that systemic administration of WF-229A suppressed the progression of tGLI1-positive BCBM. WF-229A can penetrates the BBB in mice. For further

characterization of WF-229A, we conducted a cellular thermal shift assay (CETSA) and found that WF-229A directly binds to tGLI1 protein, but not GLI1. Additional mechanistic studies showed that WF-229A suppresses the CD44+/CD24- stem cell population, hampers migration, and induces apoptosis in tGLI1-positive BCBM cells. Our study establishes the rationale to further characterize the pharmacological properties of WF-229A for fine-tuned targeting of BCBM, and to examine WF-229A's ability to offer additional therapeutic benefits including the prevention of BCBM in vivo.

P3-09-30: Sequential ADC Treatments in Metastatic Breast Cancer

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Background: The clinical activity of antibody-drug conjugates (ADCs) when used sequentially remains uncertain. Given the increasing utilization of ADCs in both HER2-positive and HER2-negative metastatic breast cancer, our aim was to assess the efficacy of sequential ADC treatments in metastatic breast cancer patients across subtypes.
Methods: We conducted a retrospective analysis using data from the Dallas Metastatic Breast Cancer Study, which encompasses all metastatic breast cancer patients treated at multiple hospitals within a single academic medical center. Clinical data were gathered through retrospective chart reviews. Progression-free survival (PFS) was defined from the initiation of ADC treatment to disease progression or death.
Results: At our institution, 156 metastatic breast cancer patients were treated with ADCs between March 21, 2013 and March 29, 2024. Among these, 35 patients received sequential ADC treatments. In our analysis, we included 32 patients who switched to a second ADC after disease progression on their first ADC. Patients who switched ADCs due to side effects were excluded from our analysis. The median age at metastatic diagnosis was 52 years (range: 34-75 years). The cohort consisted of 21 patients with HER2-positive breast cancer and 13 patients with HER2-negative breast cancer. Of the patients with HER2-negative disease, six patients had hormone receptor positive disease and 12 had HER2-low disease. Overall survival for the entire cohort was 17 months (range: 6-78 months).
In the HER2-positive subgroup, 15 patients initially treated with trastuzumab emtansine (T-DM1) had a median PFS of 4 months (95% CI: 0.44-7.55). Subsequent treatment with trastuzumab deruxtecan (T-DXd) resulted in a median PFS of 8 months (95% CI: 3.41-12.58). Four patients initially treated with T-DXd had a median PFS of 4.5 months (95% CI: 0-26.67) and subsequent treatment with T-DM1 resulted in a median PFS of 4 months (95% CI: 0-11.98).
In the HER2-negative subgroup, eight patients initially treated with sacituzumab govitecan (SG) had a median PFS of 7.5 months (95% CI: 3.84-11.15). Subsequent T-DXd treatment resulted in a median PFS of 4.5 months (95% CI: 2.01-6.98). Looking closer specifically within the HER2-low subset, the median PFS of SG remained at 7.5 months (95% CI: 3.31-11.68), while subsequent PFS on T-DXd was slightly higher at 5 months (95% CI: 1.65-8.34). Five patients initially treated with T-DXd had a median PFS of 5 months (95% CI: 0-11.00) and subsequent treatment with SG resulted in a median PFS of 2 months (95% CI: 0-14.70).

Conclusions: Our findings indicate that in the HER2-positive metastatic setting, treatment with T-DXd, despite sequential use after disease progression on T-DM1, results in a longer PFS compared to the initial T-DM1 treatment. This suggests that T-DXd retains its efficacy even after disease progression on T-DM1 despite both treatments targeting the same HER2 antigen. Conversely, in the HER2-negative metastatic setting, the sequential use of SG and T-DXd, which share the same payload, resulted in a lower PFS compared to initial treatment with SG or T-DXd. These data suggest and contribute to growing evidence that payload resistance may reduce clinical benefit across subtypes instead of antigen resistance. Future studies into novel ADC development should focus on using different payloads.

P3-10-01: Interval Improvement in Overall Survival of Patients with De Novo Metastatic Breast Cancer. Real World Data from a Resource-Restricted Country

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Introduction: Despite the advances attained in early detection of breast cancer, 5% or more of newly diagnosed breast cancer in developed countries, and much more (10-30%) in resource-restricted countries, like ours, present with distant metastatic disease at the time of diagnosis (de novo MBC). The recent introduction of many new anti-breast cancer drugs has significantly improved patients' outcomes. These drugs include the cyclin-dependent kinases 4/6 (CDK4/6) inhibitors which has revolutionized the management of advanced hormone receptor (HR)-positive/HER2-negative MBC. In addition to targeting HR, drugs that are directed at downstream elements of the molecular pathways have been developed to overcome endocrine resistance. Many anti-HER2 drugs, including antibody-drug conjugates (ADCs) are in use to treat HER2-positive MBC. In this study, we aim to review the overall survival of breast cancer patients diagnosed with de novo metastatic disease treated at our institution across two intervals to identify if the prognosis resulted from the introduction of new therapeutic intervention has improved the outcome of patients with de novo MBC.
Methods: This is a retrospective cohort study analyzing patients' records from 2011 to 2022. All consecutive patients whose first presentation was with pathologically-confirmed diagnosis of MBC (de novo MBC) were enrolled. Patients were divided into two cohorts; those diagnosed and treated prior to 2017 and those diagnosed and treated in 2017 and beyond. To allow for longer follow-up, patients diagnosed in the last two years were not enrolled. Data was collected from a well-structured hospital-based cancer registry. In year 2017, many of the new endocrine treatments including CDK4/6 inhibitors and new anti-HER2 drugs were introduced at the center. Immunotherapy, specifically pembrolizumab and PARP inhibitors were not formulary drugs during the study period.
Results: During the study period, a total of 1,512 patients with de novo MBC were

diagnosed, treated, and followed up at King Hussein Cancer Center; 641 (42.4%) were treated before 2017, while 871 (57.6%) others were treated in 2017 or after. Median age at diagnosis was 51 (26-89) years, and except for 16, all were female. Majority (n=1,286, 85.1%) had invasive ductal carcinoma (IDC), 168 (11.1%) invasive lobular carcinoma (ILC), while 58 (3.8%) had other subtypes. Hormone-receptors (HR) were positive in 1,248 (82.5%), HER2 positive in 417 (27.6%), while 109 (7.2%) others had triple-negative (TN) disease. Patients treated prior to 2017 had a median overall survival (OS) of 33.8 months (95% CI, 30.3-36.1) while it was unreached (95% CI, 40.0-NA) in patients treated in 2017 or after, P <0.001. Difference in median OS was more prominent in patients with HER2-positive disease; 29.7 months (95% CI, 22.9-33.8) in patients treated prior to 2017 while it was unreached (95% CI, 37.9-NA) in those tread more recently, P<0.001. Additionally, patients with HR-positive had better OS if treated recently where the median OS was not reached (95% CI, NA-NA) compared to 36.7 months (95% CI, 34.1-NA) for those treated prior to 2017, P<0.001. However, across the study period, patients with TN disease had no improvement in median OS; 19.7 (95% CI 16.9-29) for those treated in recent years, compared to 29.2 months (95% CI, 18.4-36.1), in patients treated before 2017, P=0.065. Conclusions: Our study clearly showed remarkable and significant improvement in median OS of patients with de novo MBC and such improvement was more notable in patients with HER2-positive disease and in those with HR-positive/HER2-negative. No improvement observed, during the study period, in patients with TN disease which may be a reflection of late introduction of immunotherapy.

P3-10-02: Real-world study of Abemaciclib in Patients with Hormone Receptor-Positive/Human Epidermal Growth Factor Receptor 2-Negative Breast Cancer: a Multi-Institutional Prospective Study

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Background: Abemaciclib has been approved in combination with endocrine therapy in adult patients with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, early breast cancer (BC) at high risk of recurrence and locally advanced or metastatic BC. We aimed to assess real-world toxicity and efficacy data of patients with BC who received treatment with abemaciclib.
Methods: This was a prospective collection of clinicopathological, toxicity and outcome data from patients with early- or advanced- stage HR-positive/HER2-negative BC who received treatment with abemaciclib in combination with endocrine therapy. Treatment combinations of abemaciclib with any endocrine therapy were accepted. Patients needed to have received at least two months of treatment with abemaciclib. The primary end point was toxicity rate in all patients of the study, both with early and advanced BC.

Results: From June/2021 to May/2024, 227 women received abemaciclib/endocrine combination therapy; median age was 56 years, and 152 (70.4%) patients were postmenopausal. Hormonal therapies given in combination with abemaciclib in advanced BC were fulvestrant (0.7%), aromatase inhibitors (88.6%) and tamoxifen (10.7%). Abemaciclib was administered as adjuvant treatment in 157 (69.1%) patients. At the time of the data cutoff (May 2024), 4 (2.5%) patients had completed the 2-year treatment period, and 133 (84.7%) patients remained in the 2-year treatment period. The most common adverse events (AEs) were diarrhea (46.5%), fatigue (18.5%), ALT/AST increase (7.0%) and arthralgia (6.4%). Grade 3 diarrhea was reported in 8 (5.1%) patients; no Grade 4 diarrhea was observed. Diarrhea was observed at a median of 6 days from treatment initiation (range 1 to 270 days). No thrombotic event was reported. AEs led to dose modification and treatment discontinuation in 42 (26.8%) and 8 (5.1%) patients, respectively. There was no difference in modification/discontinuation rates between older patients (>65 years) and the remaining patients. Patients who received abemacilib as adjuvant treatment had similar toxicity rates with patients with advanced BC. The median 1-year and 2-year disease-free survival rate for patients who received adjuvant treatment with abemaciclib was 100% and 98.7%, respectively. Almost half (47.1%, 33/70) of the patients were diagnosed with de novo metastatic disease. In patients with advanced cancer (70, 30.8%), a median of one site of metastasis was reported at diagnosis of advanced disease (most commonly bones, 54,3%). With a median follow up of 11.8 months, 14 patients had disease progression; median progression-free survival (PFS) was not reached yet and 12-month PFS rate was 79.3%.
Conclusions: This study provides prospectively collected real-world data on abemaciclib in combination with endocrine treatment, confirming the safety and efficacy in an unselected patient population. These results are consistent with the registration trials and no new safety concerns were reported.
Clinical trial registration: ClinicalTrials.gov NCT04985058

P3-10-03: Real-world first-line immunotherapy use and overall survival rates for triple-negative breast cancer: analyses from a 2018-2020 US population-based cohort

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Background: Combination immunotherapy (IO) and chemotherapy (CT) for triple-negative breast cancer (TNBC) improves overall survival (OS) over CT alone in both the neoadjuvant and metastatic setting. Large-scale real-world analysis of IO +/- CT for TNBC has been limited to date. We present prescribing patterns and OS of initial chemo-immunotherapy treatments in a large US population-based cohort of TNBC patients.

Methods: Patients with TNBC diagnosed from 2018-2020 were identified in the National Cancer Database (NCDB), a US-wide oncology outcomes database. Those with undocumented staging and stage 0 disease were excluded. The primary outcome, OS, was evaluated by initial treatment: chemotherapy/IO (CT/IO), chemotherapy/no IO (CT), IO/no chemotherapy (IO), or no chemotherapy/no IO (NT), using cox proportional hazards models and Kaplan-Meier methods. The secondary objective was real world practice patterns. Categorical variables were compared between the groups using a Chi-square test. Age was compared using a Kruskal-Wallis test.

Results: 58,128 new TNBC diagnoses were identified in NCDB between 2018-2020, with 6.7% de novo metastatic. IO use steadily increased over time (2018 = 19.5%, 2019 = 31.0%, 2020 = 49.5%). Academic research programs most commonly used IO.

Most patients received CT (73.3%, n = 32,858), with 5.6% (n=3276) receiving CT/IO, 0.1% (n=66) receiving IO, and 20.5% (n=11,926) NT following initial diagnosis. CT +/- IO use was associated with younger age, whereas IO alone or NT was more common in older patients (median age: CT/IO = 55 years [y], CT = 58y, IO = 71y, NT = 72y; p< 0.001). Treatment with CT/IO or CT was most common among private insurers (57.6% and 53.3%, respectively) whereas Medicare was the most common payor for IO or NT (59.1% and 64.1%, respectively; p<0.001). Proportionally, stage I TNBC received less treatment (CT/IO 13.3%, CT 34.1%, IO 18.2%, NT 55.3%; p< 0.001) and single agent IO treatment was more common in stage IV TNBC (CT/IO 24.3%, CT 4.9%, IO 53.0%, NT 8.4%; p< 0.001). Pathologic complete response (pCR) rates after neoadjuvant chemotherapy (NACT; n = 27,900) differed by treatment group, with pCR most common following CT (CT/IO 27.6%, CT 29.6%, IO 16.7%, NT 1.8%; p<0.001). OS was similar among treatment groups in the full TNBC population, with median survival for NT at 36 months.

Race differed significantly between treatment (p<0.001). Among White patients with TNBC (70.9%), 72.1% received CT/IO and 70.5% received CT. However, among Black patients with TNBC (11.9%), 19.4% received CT/IO and 23.6% received CT. OS was highest in the CT group, whereas the IO only group had the worst survival outcome – even worse than NT (hazard ratio (HR) CT 0.52, IO 2.22, NT 1.17; ref CT/IO, p< 0.001 each HR). Nonetheless, Black race was associated with worse OS than White race and the addition of IO to CT did not fully mitigate the OS racial gap (HR Black CT/IO 1.38, White CT 0.52, Black CT 0.65; ref White CT/IO, p< 0.001 each HR).

Conclusions: This large NCDB cohort analysis of newly diagnosed TNBC cases revealed practice patterns for initial TNBC treatment varies widely. Furthermore, this analysis captured new TNBC diagnoses from 2018 to 2020 and describes significant off-label use of IO+/- CT in both the NACT (Keynote-522 FDA approval 2021) and metastatic (ImPassion130 FDA accelerated approval 2019) settings with some relation to the payor. In addition, single agent IO use was higher than expected for stage IV and resulted in worse OS, again raising concerns of off-label use of the tumor agnostic IO approvals (tumor mutation

burden-high, microsatellite instability). Finally, racial discrepancies of use and efficacy of IO was present. Further studies and interventions are needed to address TNBC treatment disparities.

P3-10-04: Outcomes following less-than-standard therapy in operable breast cancer patients

Alan Celik, Tobias Berg, Maj-Britt Jensen, Ann Knoop, Bent Ejlertsen

BackgroundThe primary purpose of this study was to evaluate the use of less-than-standard surgical therapy in early-stage operable breast cancer nationwide in Denmark. A secondary purpose was to evaluate the use primary endocrine therapy (PET).
MethodsWe assembled a nationwide, population-based cohort of early-stage operable breast cancer patients registered between 2000 and 2020 in Denmark using the Danish Breast Cancer Group Database, National Patient Registry, and National Pathology Registry. Patients were divided into two groups: those who underwent up-front surgery within 182 days of diagnosis and those who did not, with the latter group further subdivided by use of primary endocrine therapy versus not. We performed Cox regression analyses to assess all-cause mortality risk, adjusting for tumor size, age, comorbidity, estrogen receptor status and year of diagnosis.
ResultsWe identified 84,487 patients with early-stage operable breast cancer. Of these 5,916 patients received less-than-standard therapy, and hereof 2,576 patients received PET. The number of patients for whom surgery was omitted increased from 226 (6.3%) in 2000 to 433 (11.8%) in 2020 and while none of the patients diagnosed in 2000 received PET 296 (8.1%) patients diagnosed in 2020 did. Less-than-standard therapy was associated with a significant higher rate of all-cause mortality.
ConclusionFrom 2000 to 2020 there was a significant increase in both omittance of surgery and the use of PET among breast cancer patients. Omitted surgery both with or without PET was associated with increased mortality, even after adjusting for tumor size, age, comorbidity, estrogen receptor status and year of diagnosis.

P3-10-05: Real-World Efficacy of Fam-Trastuzumab Deruxtecan in HER2-Low Metastatic Breast Cancer

Sarah Blocker, Qamar Khan, Priyanka Sharma

Background: Fam-trastuzumab deruxtecan (T-DXd) is recommended after 1-2 prior lines of chemotherapy for HER2-low metastatic breast cancer (MBC). In the Destiny Breast-04 (DB-04) clinical trial, median progression free survival (PFS) with T-DXd was 9.9 months in the overall population and 10.1 months in the HR+ cohort. The purpose of this study was to evaluate the real-world (RW) safety and efficacy of T-DXd in patients with HER2-low MBC.
Methods:
This RW analysis includes female patients with HER2-low MBC who received at least 1 dose

of T-DXd from June 1, 2022, to May 14, 2024, at a single institution. Efficacy and toxicity related to T-DXd were extracted from medical charts. The primary endpoint was RW investigator assessed PFS (rwPFS). Hormone receptor (HR)-positive was defined as estrogen or progesterone receptor expression >0%.

Results: Ninety-one patients were included with a median age of 61 years (range 33-84). Seventy-two (79%) patients had HR-positive disease, and 19 (21%) had HR-negative disease. HER2 immunohistochemistry (IHC) was 1+ in 76% of patients and was 2+[PS1] /ISH- in 24%. Ninety-one percent had visceral disease, and 4.4% had bone-only disease. Prior to T-DXd initiation, patients had received zero (11%), one (42%), two (24%), or > three (23%) lines of cytotoxic chemotherapy. Eleven percent of patients had received prior sacituzumab govitecan. After a median follow-up of 16.2 months, the median rwPFS was 6.8 months (95% CI, 5.4 to 8.2)[QK2] . Among HR+ and HR- subgroups, the rwPFS was 6.4 months (95% CI, 5.4 to 8.7) and 6.1 months (95% CI, 3.6 to 8.8), respectively. Dose reductions and discontinuation due to toxicity occurred in 29 (32%) and 9 (10%) patients, respectively. Investigator assessed pneumonitis associated with T-DXd was reported in 12 (13%) patients on therapy.

Conclusions: This RW analysis shows that T-DXd is prescribed to a more heavily pre-treated population. The rwPFS of 6.8 months in patients with HER2-low breast cancer treated with T-DXd is lower than expected and may be due to the more pre-treated population.

P3-10-06: Missed opportunities: understanding the factors contributing to non-receipt of neoadjuvant chemotherapy in breast cancer patients

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Neoadjuvant chemotherapy (NACT) for breast cancer (BC) offers benefits such as tumor downstaging, enabling breast-conserving surgery, and customization of adjuvant therapies. However, some eligible patients do not receive it. Identifying the reasons behind this is crucial for refining patient care and improving outcomes.
We conducted a retrospective study of women who received care in a BC referral center. Eligible patients were those with stage III HR+/HER2- BC or stage II-III triple-negative or HER2-overexpressed BC, which are accepted indications for NACT. Independent samples T-test, X2, and Fisher's exact tests were used to evaluate associations between variables, employing logistic regressions to calculate odds ratios (OR) when appropriate.
Among 255 patients, 105 (41%) did not receive NACT. Women who did not receive NACT were more likely to be >65 years (16% vs. 7%, p=0.015), have stage III (61% vs. 40%, p=0.001), HR+/HER2- (42% vs. 14%, p<0.001), or low grade (63% vs. 43%, p=0.002) disease, had more out of site surgeries (62% vs. 34%), and underwent more axillary

dissections (58% vs. 40%, p=0.021). The reasons for not receiving NACT were initial care in another center (53%), upstaging following surgery (29%), patient decision (7%), discordant immunophenotypes between biopsy and surgical specimen (5%), and relative contraindication due to comorbidities or advanced age (5%). Predictive factors for upstaging following surgery included grade 3 disease in the initial biopsy (OR 12.3) and HER2-negative status (OR 10.6).
In our study, 41% of eligible patients did not receive NACT, mainly due to out of site surgeries, highlighting the need to educate providers about the benefits of NACT. In addition, some patients missed NACT due to upstaging following surgery, stressing the need for thorough preoperative staging, particularly with high-risk characteristics like grade 3 and HER2-negative disease.

P3-10-07: 10y overall survival (OS) in patients (pts) with breast cancer (BC) at a comprehensive cancer center in Colombia

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In Colombia, approximately 17,000 new cases are diagnosed each year with an incidence of 50 cases/100 hab/year [1]. Access to screening [2], integration of curative with adjuvant therapies [3-6], and new systemic treatments [7,8,9] in ABC have been shown to improve survival outcomes. However, the impact on long-term overall survival has not been reported in the Colombian population.

Methods: Pts with BC, complete relevant clinical information and long term follow up between January 2013 to December 2023 at The Comprehensive Cancer Center Clinica de Occidente in Cali-Colombia, were retrospectively identified. We collected relevant variables from electronic medical records. Our primary objective was to determine the 10 years OS by stage and important clinical variables. We performed Kaplan Meier, and cox regression analysis. The study was approved by the institutional ethics committee.

Results: We identified 831pts with complete clinical information and adequate follow-up. Demographic characteristics are described in table 1.
De novo metastatic disease was found in 10% of pts. This was higher when health insurance was exclusively subsidized by the state (15%) vs. mixed insurance (9%) (private + public), OR 1.7 (95% CI 1.0-2.9), P=0.01.
Table 2 and Figure 1 describes the 10y OS by stage, with 88% in stage I vs. 23% in stage IV. Multivariate analysis showed that density of lymph node involvement, metastasis at diagnosis, poor PS, and advanced age were significantly associated with increased risk of death. However, living in rural areas or small cities, type of health insurance, history of DM2, hypertension, smoking, family history of breast cancer, or HER2-positive subtype did not significantly affect survival.

The 10y OS was 62% in non-Luminal A subtypes (figure 2), 44% in T4 tumors, 41% in patients with Age^{3}80y, 32% in N3 lymph node involvement and only 5% in patients with PS ECOG^{3}2 (figure 3).

Conclusions: We report the longest follow up for overall survival in a cohort of pts with BC from an comprehensive cancer center in Colombia and confirm that the prognosis in survival has improved, especially for luminal tumors, but it is still poor if it is detected late in a locally advanced or metastatic stage (approximately 27% of all cases) or with deteriorated functional status. Therefore, increasing early detection and the inclusion of new systemic therapies are necessary to improve the probability of survival in higher-risk subgroups.

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P3-10-08: Elacestrant real-world progression-free survival (rwPFS) of adult patients with ER+/HER2-, advanced breast cancer: a retrospective analysis using insurance claims in the United States

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Background: Endocrine therapy (ET) + CDK4/6i is the standard-of-care (SOC) for 1st-line treatment of ER+/HER2- advanced or metastatic breast cancer (mBC). In current practice, sequential endocrine monotherapy or combination therapies are used in the 2nd-line post ET+CDK4/6i as recommended by guidelines. Combination regimens in ≥2nd-line mBC can be associated with significant toxicities and high discontinuation rates. ESR1 mutations represent a type of acquired resistance that eventually emerges in up to 50% of patients during ET for ER+/HER2- mBC. The EMERALD trial (NCT03778931) demonstrated median PFS of 3.8 months for elacestrant vs 1.9 months for SOC ET (HR = 0.55; 95% CI, 0.39-0.77; P = 0.0005) with manageable safety in patients with ER+/HER2- mBC and ESR1-mutated tumors previously treated with ET+CDK4/6i (Bidard, 2022). Elacestrant is the first and only oral SERD approved targeting ESR1-mutated tumors. Sufficient time has passed since approval in January 2023 to characterize the real-world use of elacestrant in the current

treatment landscape. This study aims to describe the rwPFS of patients who received elacestrant after Food and Drug Administration (FDA) approval in the United States.
Methods: Adults with newly diagnosed ER+/HER2- mBC who initiated elacestrant in a real-world setting between January 2023 and December 2023 were identified in the Komodo Research Database (KRD+). Demographic information was described at the time of first administration of elacestrant (index date); metastatic sites were identified during the 6-month baseline period prior to the index date; and prior treatments received in the mBC setting were described among patients with sufficient health plan enrollment between the first observed mBC diagnosis and the index date. rwPFS, defined as time from index date until the earliest outcome (start of the next line of therapy or death), was assessed using time-to-event and Kaplan-Meier methods. Patients were censored at the first occurrence of the following: discontinuation of all therapy, end of data availability, or end of health plan enrollment. Elacestrant is indicated for the treatment of postmenopausal women or adult men with ER+/HER2- ESR1-mutated advanced or mBC with disease progression following at least one line of ET. ESR1-mutation status was not available in the database at the time of analysis.
Results: A total of 212 patients treated with elacestrant with sufficient health plan enrollment were evaluated (median age was 63 years, 97% were female). Prior to initiating elacestrant, 89% of patients received prior treatment with a CDK4/6i, 58% were treated with only 1-2 prior lines of ET in the metastatic setting, and 62% had visceral metastases. The median follow-up time was 5.2 months. In the overall population analysis, median rwPFS (95% CI) was 6.8 months (5.4-not reached [NR]). Median rwPFS for patients with 1-2 lines of prior ET in mBC was 8 months (4.9-NR), and median rwPFS for patients with visceral metastases was 6.1 months (4.7-NR). Updated results and additional information will be presented based on longer follow-up.
Conclusion: This retrospective observational study expands our understanding of elacestrant's activity in ER+/HER2- mBC patients with prior exposure to ET in a real-world setting.

P3-10-09: Prognostic implications of HER2 changes after neoadjuvant chemotherapy: A real world data of matched breast cancers with the inclusion of HER2-Low category

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Objectives: The aim of this study was to evaluate the changes in HER2 status after neoadjuvant chemotherapy (NAC) and the implications of HER2 changes for clinical outcomes, including the HER2-low category.
Methods: This retrospective cohort study included female patients over 18 years of age diagnosed with non-metastatic breast cancer undergoing NAC from 2011 to 2023. Patients who did not achieve complete pathological responses were evaluated for changes in

immunohistochemistry (IHC) before and after NAC. HER2 IHC was re-evaluated with consensus according to the current ASCO/CAP guidelines. Tumors were categorized into HER2-negative (IHC 0), HER2-low (IHC1+ or IHC2+/ISH-), and HER2-positive (IHC3+ or IHC2+/ISH+) subgroups. Quantitative and qualitative factors related to changes in IHC were assessed. he prognosis of these patients were assessed by examining OS and DFS.. The study received approval from the research ethics committee (CAAE 80127724.1.0000.5463).
Results: We included 369 patients, most of whom (215/58.3%) did not change their IHC profile. Baseline statuses were HER2-negative (24/6.5%), HER2-low (256/69.4%), and HER2-positive (89/24.1%). Tumors with HR+ were observed in 227 patients (61.5%). HER2-positive tumors exhibited more changes in IHC (63/63.0%, p<0.0001) compared to HER2-negative (5/20.8%) and HER2-low tumors (92/35.9%). Significant differences were found in the changes: all HER2-negative tumors changed to HER2-low; HER2-positive tumors changed to HER2-low in 30 cases (43.7%) and to HER2-negative in 26 cases (41.2%). Most HER2-low tumors (51/55.4%) remained HER2-low or changed to HER2-negative (26/29.2%). The presence of HR+ was significantly associated with greater changes to HER2-negative (125/55.0%) and HER2-low (83/36.5%). We did not observe a significant difference in the number of deaths, loco-regional recurrences, and distant metastases when evaluating patients according to HER-2 status. Initially, HER-2 low patients had more unfavorable outcomes, and after NAC, those who converted to HER-2 negative had worse outcomes. Overall Survival (OS) was significantly worse in HER-2 low patients, both pre-NAC and post-NAC, with median OS of 51.6 months and 53.6 months, respectively. Disease-Free Survival (DFS) was significantly longer only in patients initially classified as HER-2 low, with a median DFS of 50.9 months.
Conclusions: Our findings indicate that changes in IHC profiles post-NAC are common, particularly among HER2-positive and HER2-low tumors. Significant alterations in HER2 status were observed, with many HER2-positive tumors shifting to HER2-low or HER2-negative post-NAC. Additionally, the presence of hormone receptors (HR+) is associated with a higher likelihood of conversion to HER2-negative. These changes are clinically relevant, as they correlate with increased mortality rates, underscoring the importance of re-evaluating HER2 profiles post-NAC as potential prognostic indicators in breast cancer patients.

P3-10-10: Sequencing PIK3CA and AKT Inhibitors in Hormone Receptor-Positive, HER2-Negative Metastatic Breast Cancer: A Retrospective Analysis

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Background: PI3K/AKT/PTEN signaling pathway alterations are present in approximately half of patients with hormone receptor positive (HR+), HER2 negative breast cancer (BC). The PI3K alpha-selective inhibitor (PI3Ki) alpelisib in combination with fulvestrant is

approved for patients with PIK3CA-mutated HR+ advanced breast cancer, and the AKT inhibitor (AKTi) capivasertib in combination with fulvestrant is approved for patients with advanced/metastatic HR+/HER2- breast cancer (MBC) with alterations in PIK3CA, AKT1, or PTEN. Patients with PIK3CA mutant MBC are now candidates for both therapies, but there is minimal data to guide sequencing of these agents. Here we report our single-institution experience.

Methods: All patients with HR+/HER2- MBC treated at the Mass General Cancer Center who received AKTi (either capivasertib or an experimental AKTi in a clinical trial/TAKTIC) were included. Patients were divided into three categories: those who received AKTi only (control), those who received PI3Ki first and then AKTi (PI3K > AKT) and those who received AKTi and later PI3Ki (AKT > PI3K). Patients could have intervening therapies between sequential PI3Ki/AKTi. Clinical information was abstracted by chart review. Progression-free survival (PFS) was evaluated as time from start of treatment to disease progression or death from any cause. Time on treatment was defined as the start of treatment to discontinuation. 95% confidence intervals were calculated for all mean and median estimates.

Results: 56 patients were identified who received AKTi with/without PI3Ki for advanced/metastatic HR+/HER2- breast cancer from August 2019-June 2024. The median age at start of first PI3Ki/AKTi was 61.5 years. 42 patients (76.4%) experienced disease progression on their first AKTi/PI3Ki. 49 patients (89.1%) had known alterations in PIK3CA/AKT/PTEN pathway, and six patients (10.9%) had no known alterations and were treated on trial. Mutations of PIK3CA were present in 30 patients (54.5%), AKT in 8 patients (14.5%), and PTEN in 8 patients (14.5%). 39 patients (69.6%) received AKTi only, 10 patients (17.9%) were in the PI3K > AKT group, and 4 (7.1%) received AKT > PI3K, and the remaining 3 patients (5.4%) received 3 or more AKTi/PI3Ki. Overall median time on first treatment was 5.8 months (95% CI 4.6-11.0 months) and median time on second treatment was 5.4 months (95% CI 2.0-NR). Median progression-free survival (PFS) for patients who received AKTi alone was 5.5 months (95% CI 3.7-9.2 months). For patients who received PI3Ki before AKTi, the median PFS on second treatment (AKTi) was 4.6 months (95% CI 2.0-NR). An analysis of genomic predictors of response is in process and a multi-institution follow-up analysis is planned. Further updates to be presented at the meeting.

Conclusions: In this single-institution study, we report median treatment times for patients treated with PI3Ki prior to AKTi and AKTi alone. Patients may still benefit from receiving second-line AKTi after PI3Ki. Biomarker data is needed to drive personalized selection of sequential use of agents targeting the same pathway.

P3-10-11: Breast Cancer Patient Navigation Program Saves Lives: Brazilian Experience in a Real-World Setting in the Public Health System

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Background: In 2012, the Brazilian government issued Law No. 12.732/12 from the Ministry of Health, the "60-Day Law". This law mandates that cancer treatment for public health system patients must start within 60 days of definitive diagnosis. The average treatment time is up to 31 days in private health with 18% of cases diagnosed in stages III and IV, while in the public system, it averages 93 days, sometimes reaching 180 days with 40% of cases in these advanced stages.
Aim: To evaluate whether a Breast Cancer (BC) Patient Navigation Program (PNP) contributes to increasing compliance with the "60-Day Law". Consequently, to assess if complying with this Brazilian federal law helps reduce BC mortality in public health system patients.
Methods: We conducted a longitudinal observational and retrospective study with women over 18 years diagnosed with BC. Data were collected from medical records via the PNP. The sample was selected based on exploratory evaluation of records from the Rio Imagem Diagnostic Center and Heloneida Studart State Hospital in Rio de Janeiro, from 2017 to 2022. Active case follow-up involved cross-referencing medical records and contact via phone or text messages. The Kaplan-Meier method estimated the 5-year specific survival probability. The independent effect of survival variables was identified using univariate and multivariate Cox proportional hazards models (Hazard Ratio – HR), with a significance level of α=0.05.
Results: Out of 1,022 women diagnosed with BC, 840 were eligible after excluding those lost to follow-up, who refused treatment, died of unrelated causes, or received palliative care. 79 patients died from BC. The 5-year specific survival rate was 92.6%. Survival was higher for patients treated initially with surgery (97.9%, $p < 0.0001$), histological grade 1 (94.5%, $p = 0.002$), Luminal biological profiles (98.4%, $p < 0.0001$), and treatment within 60 days (95.3%, $p = 0.005$). Stratifying 5-year mortality risk by advanced stage revealed higher mortality among women who were not treated within 60 days as per the law (HR=2.00[1.23; 3.24]).
Conclusion: Failure to comply with the “60-Day law” doubled the risk of mortality from BC. Patients starting treatment within 60 days had higher survival rates compared to those who did not. In Brazil, the PNP could be an opportunity to properly implement existing legislation, potentially significantly impacting BC control.

P3-10-12: Retrospective analysis of adverse events and outcomes of patients receiving neoadjuvant pembrolizumab for triple negative breast cancer in Australia.

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Introduction and objectives:Neoadjuvant pembrolizumab combined with chemotherapy has become the standard of care since 2022, for patients with triple negative breast cancer (TNBC) in Australia, following the KEYNOTE-522 trial, which identified significantly higher pathological complete response (pCR) compared to placebo, acknowledging the serious immune-related adverse events (irAEs) (1). A secondary analysis of this trial included patients enrolled in Asia and found similar rates of grade 3-4 irAEs in the pembrolizumab and placebo groups (3). Reports of adverse outcomes are often overshadowed by the significant benefit of pembrolizumab in TNBC, a subtype of breast cancer with significant risk of recurrence and mortality (2). Our objective was to collect real-world data relating to the use of neoadjuvant pembrolizumab in patients with TNBC, to identify the pattern of adverse events and outcomes.

Methods: Retrospective data was collected across three tertiary hospitals in Melbourne, Australia, for patients diagnosed with TNBC, who received neoadjuvant pembrolizumab; baseline demographics, medical background, primary tumour details, pembrolizumab and chemotherapy regimen, serious adverse events and their management, breast surgery and pathological response.

Results: There were 54 female patients who received neoadjuvant pembrolizumab; median age 52 years and median follow up of 11 months. 4 patients had a history of autoimmune disease and 2 had immunosuppressant therapy during or within 2 years prior to treatment with pembrolizumab. At primary diagnosis, 69% had nodal involvement.
At least one irAE occurred in 54% of patients; thyroiditis (22%), adrenal insufficiency (20%), colitis (7%), hepatitis (6%) rash of at least grade 3 severity (4%) and pneumonitis (4%). Other less frequent irAEs include autoimmune haemolytic anaemia, transverse myelitis, arthralgia and type 1 diabetes mellitus. Of those with irAEs, 45% were hospitalised, 1 patient required intensive care and 69% have ongoing sequelae. The median time from commencement of pembrolizumab to irAE onset was 84 days. An interruption in pembrolizumab dose occurred in 23 patients, with 70% of these discontinuing therapy early. 41 patients underwent surgical intervention, and 40 had lymph node dissection. pCR was achieved in 37%, while 60% of those who achieved pCR experienced at least one irAE.

Conclusion: There are significant immune related adverse events associated with the use of neoadjuvant pembrolizumab, a notable proportion of whom required hospitalisation, which poses an important and concerning burden for patients and the healthcare system. These

real-world outcomes will continue to be monitored. Further work should also look at survival outcomes and quality of life, to better inform shared-decision making.
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P3-10-13: A Comprehensive Analysis of Dysregulation in the PTEN/PI3K/AKT Pathway in Breast Cancer Among the Chinese Population

Ziang Li, Meizhen Hu, Fei Pang, Bo Peng, Aodi Wang, Huanwen Wu

Background: Breast cancer (BC) has the highest incidence rate among cancers in females in China. The PTEN/PI3K/AKT pathway regulates key biological processes, including apoptosis, metabolism, cell proliferation and growth in BC. This is the first comprehensive study to explore the association between these pathway gene alterations and clinical outcomes in a large cohort of Chinese BC patients.

Methods: We retrospectively reviewed 1018 Chinese BC patients with clinical information from OrigiMed Chinese Real-World Database between 2016 and 2023. Tumor tissue samples and paired peripheral blood leukocytes were analyzed using an OrigiMed 450 gene next-generation sequencing panel. Chi-square test and Fisher's exact test assessed differences in these genetic alterations across different subgroups and to examine the co-occurrence of alterations between these genes and other genes.

Results: A total of 38.5%, 5.3% and 8.4% of Chinese BC patients exhibited alterations in PIK3CA, AKT1 and PTEN genes, respectively, which is similar to the Caucasian-dominated MSK-IMPACT cohort (PIK3CA: 38.5% vs. 35.2%, P =0.102; AKT1: 5.3% vs. 5.3%, P =0.995; PTEN : 8.4% vs. 7.0%, P =0.232). Subgroup analysis revealed higher PTEN alteration in stage IV patients compared to stage I-III (12.7% vs. 6.4%, P =0.001). Furthermore, HR+/HER2- BC patients exhibited a significantly higher incidence of PIK3CA (45.2% vs. 30.8%, P <0.001) and AKT1 (7.7% vs. 2.5%, P <0.001) gene alterations, compared to other subtypes. The overall incidence of alterations in the PTEN/PI3K/AKT pathway was 50.9%, which was higher among HR+/HER2- BC patients compared to other subtypes (57.0% vs. 43.9%, P <0.001). PIK3CA alterations were associated with lower AKT1 alterations (3.1% vs. 6.7%, P =0.011). ERBB2 alterations were associated with lower AKT1 (0.4% vs. 6.9%, P<0.001) and PTEN (4.4% vs. 9.7%, P =0.009) alterations. TP53 alterations were associated

with higher PTEN/PI3K/AKT pathway alterations (55.4% vs. 45.9%, P =0.002). No significant differences in PIK3CA (25.6% vs. 21.4%), AKT1 (7.0% vs. 9.5%), and PTEN (14.0% vs. 23.8%) gene alterations were observed between primary and metastatic lesions in the same patients (N=41, P >0.05). No significant differences were found in the alterations of PIK3CA (38.7% vs. 41.1%), AKT1 (5.8% vs. 5.4%), and PTEN (10.4% vs. 7.6%) between samples taken before and after treatments such as chemo-immunotherapy and targeted therapy (N=840, P >0.05). Survival analysis (median follow-up: 32.7 months) showed PTEN alterations correlated with higher mortality risk (HR 2.779, 95% CI 1.083-7.132, P =0.034), while PIK3CA alterations were associated with reduced mortality risk (HR 0.338, 95% CI 0.141-0.820, P =0.016), with overall survival (OS) as the outcome.

Conclusion: This study highlights the significant role of PIK3CA, AKT1 and PTEN gene alterations in Chinese BC patients. In Chinese BC patients, alterations in the PTEN/PI3K/AKT signaling pathway are common, and the frequencies of PIK3CA, AKT1 and PTEN gene are similar to that observed in Western countries. The gene alterations in this pathway are associated with clinicopathological features as well as prognosis, such as disease stage and BC subtypes, but not with primary/metastatic status or treatment. PIK3CA alterations were a benign predictor of survival, whereas PTEN alterations were a negative predictor. These findings suggest that genetic profiling of the PTEN/PI3K/AKT pathway could guide treatment strategies and prognosis prediction in Chinese BC patients. Future studies should focus on validating these findings in prospective cohorts and exploring targeted therapies based on these genetic alterations.

P3-10-14: Tolerability of First-Line (1L) Treatment (tx) With Ribociclib (RIB) for Metastatic Breast Cancer (MBC) Using 2 Large US Data Sources

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Background: In addition to PFS and OS benefits, 1L RIB + endocrine therapy (ET) demonstrated a tolerable and manageable safety profile across all phase 3 MONALEESA (ML) randomized controlled trials (RCTs). RIB + ET is recommended by the National Comprehensive Cancer Network® (NCCN®) as NCCN category 1 preferred CDK4/6 inhibitor for 1L tx of HR+/HER2− MBC with no visceral crisis in postmenopausal patients (pts) or premenopausal pts with ovarian ablation/suppression.a In the real world (RW) setting, observed tolerability for RIB can reassure clinicians and complement RCTs. Here, we describe RW adverse events (AEs) of interest with RIB (as available) in 2 large US datasets: administrative healthcare claims and enriched electronic health records, both notably including pts ≥65 y of age.
Methods: Two independent, observational, retrospective cohort analyses were conducted in the nationwide Flatiron Health EHR-derived deidentified database (EHR) and the deidentified Komodo Research Database (KRD; commercially insured, managed-Medicare

[excluding fee for service] and Medicaid-insured administrative claims). The study cohorts consisted of adult pts with HR+/HER2− MBC who initiated 1L RIB + ET between Mar 2017 and Aug 2022 (EHR) and between Feb 2018 and Dec 2022 (KRD), with follow-up up to Nov 2022 (EHR) and June 2023 (KRD). New onset of medical conditions of interest, selected from AEs reported in 1L RIB + ET RCTs, were summarized. Cardiovascular (CV) conditions, as reported in claims data, were only available in KRD. All pts, including those aged ≥65 y, are described. Variables are based on clinician documentation in enriched EHR and diagnosis codes in KRD. Some measures are not included for both datasets due to limitations of the databases.
Results: A total of 373 (EHR) and 350 (KRD) pts who received 1L RIB + ET (of whom 5.9% and 9.4%, respectively, self-identified as Black/African American) were included. In EHR, the mean age was 62.6 (SD ± 12.6) y, 183 pts (49.1%) were aged ≥65 y, and 65 pts (17.4%) were ≥75 y. In KRD, the mean age was 56.5 (SD ± 10.5) y, 57 pts (16.3%) were aged ≥65 y, and 16 pts (4.6%) were ≥75 y. The majority of pts received an aromatase inhibitor as ET partner (76.1%, EHR; 79.7%, KRD). Furthermore, the data suggest an increase in the use of RIB in the 1L in RW clinical practices in 2022; as observed in KRD more pts initiated 1L RIB + ET in 2022 (40.6%), the remainder started 1L RIB between 2018 and 2021.
New onset any-grade AEs in pts treated with 1L RIB + ET were as follows: neutropenia in 53.4% (EHR) and 21.3% (KRD) of pts, elevated liver enzymes in 9.0% (EHR) and 4.6% (KRD) of pts, and QTc prolongation in 4.0% (EHR) and 2.0% (KRD) of pts. Evidence of newly diagnosed or suspected CV medical conditions (cardiomyopathy, CHF, CAD, cardiac arrhythmias, cardiac conduction abnormalities, hypertension, IHD, hypercholesterolemia, and pericardial disease), available only in KRD, was relatively low. Incidence of AEs for pts aged ≥65 y was consistent with the overall cohort.
Conclusions: In this retrospective cohort study, data from 2 large US databases reaffirm the safety of 1L RIB seen across the ML trials, with no new RW safety signals observed among medical conditions of interest. In the RW setting, 1L RIB + ET is consistently well tolerated for pts with HR+/HER2− MBC, including older pts.
a Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.2.2024. © National Comprehensive Cancer Network, Inc. 2024. All rights reserved. Accessed June 7, 2024. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

P3-10-15: Prognostic impact of progesterone receptor status in patients with breast cancer and with isolated locoregional recurrence: A retrospective cohort study based on the Japanese Breast Cancer Registry

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Aims: Clinical evidence for treatment strategies for breast cancer (BC) patients with isolated locoregional recurrence (ILRR) is insufficient due to the various recurrence patterns and treatment options for individual patient with ILRR. Furthermore, the clinical impact of progesterone receptor (PR) status of ILRR tumor in patients with estrogen receptor (ER)-positive/humane epidermal growth factor receptor 2 (HER2)-negative ILRR has not been well investigated. This study aimed to evaluate the prognostic impact of PR status of ILRR tumor in patients with ER-positive/HER2-negative ILRR.
Methods: Using data from the Japanese National Clinical Database from 2004 to 2015, we enrolled breast cancer patients with ER-positive/HER2-netative ILRR. We divided enrolled patients into three groups according to their PR status of primary and recurrent tumors; patients with primary PR-positive/recurrent PR-positive tumors (pPR+/rPR+), patients with primary PR-positive/recurrent PR-negative (pPR+/rPR-) tumors, and primary PR-negative/recurrent PR-negative (pRP-/rPR-) tumors. Based on the registry, we classified the recurrent sites into the following three categories; ipsilateral breast tumor recurrence (IBTR), axillary lymph node (ALN), and chest wall (CW)/skin/regional node (RN). Overall survival (OS) and breast cancer-specific survival (BCSS) after ILRR diagnosis among the 3 groups were estimated using the Kaplan-Meier method, and survival estimates were compared using the log-rank test. Multivariable Cox proportional-hazards model from patients' age, primary surgical procedure, primary tumor size, primary lymph node positivity, disease free interval, recurrence site, PR status, use of post-recurrence treatments (surgery, chemo, endocrine, radiation) were constructed to identify the prognostic factors on OS and BCSS after ILRR.

Results: Among 1625 patients enrolled, 1180 patients (72.6%) had pPR+/rPR+ tumors (Group1), 306 (18.8%) had pPR+/rPR- tumors (Group2), and 139 (8.6%) had pPR-/rPR-tumors (Group3), respectively. The median follow-up time after ILRR diagnosis was 1254 days. During the follow-up period, 129 patients died from breast cancer and 19 patients died from causes other than breast cancer. The distribution of recurrence sites was as follows: group 1: IBTR 47%, ALN 28%, CW/skin/RN 35%; group 2: IBTR 38%, ALN 38%, CW/skin/RN 42%; group 3: IBTR 34%, ALN 31%, CW/skin/RN 48%. There were no differences in treatment for ILRR among the 3 groups, with surgical resection, chemotherapy, endocrine therapy, and radiotherapy given to approximately 80%, 30%, 80%, and 30%, respectively. Regarding OS, patients in Group2 and patients in Group3 had significant worse 5-year OS after ILRR than patients in Group1 (72.5% vs. 88.3%, P<0.0001; 74.7% vs 88.3%, P<0.0001, respectively). Multivariable analysis revealed that the factors associated with worse OS after ILRR diagnosis were lymph node metastasis at primary BC diagnosis (HR 2.37; 95% CI 1.63-3.44; P<0.001), disease-free interval (DFI) shorter than 24 months (HR 2.04; 95% CI 1.41-2.94; P<0.001), pPR(+)/rPR(-) tumor (HR 1.77; 95% CI 1.21-2.61; P=0.004) and pPR(-)/rPR(-) tumor (HR 2.08; 95% CI 1.25-3.44; P=0.005) as compared with pPR(+)/rPR(+), and chemotherapy administered for ILRR (HR 1.77; 95% CI 1.24-2.54; P=0.002). Those undergoing resection of ILRR tumor had longer OS after ILRR diagnosis (HR 0.35; 95% 0.24-0.52; P<0.001) compare with those who did not. Regarding BCSS, patients in Group2 and patients in Group3 had significant worse 5-year BCSS after ILRR

than patients in Group1 (74.4% vs. 89.9%, P<0.0001; 77.2% vs 89.9%, P<0.0001, respectively). Multivariable analysis revealed that factors associated with worse BCSS after ILRR diagnosis were similar to those for OS. As with OS, resection of ILRR tumor was significantly associated with favorable BCSS after ILRR diagnosis.

Conclusions: The results from this nationwide database study demonstrated that PR-negativity of ER-positive/HER2-negative ILRR is one of the poor prognostic factors after ILRR diagnosis regardless of the PR status of the primary BC.

P3-10-16: Phase I/II of Personalized Neoantigen Peptide-Based Vaccine (PNeoVCA) in Combination with Pembrolizumab in Patients with Early-Stage Triple-Negative Breast Cancer with Residual Disease after Neoadjuvant Chemotherapy

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Background: While immune checkpoint inhibitor (ICI) has significantly improved outcomes in patients with early-stage triple-negative breast cancer (TNBC), patients with residual disease after pembrolizumab-based neoadjuvant chemotherapy continue to have particularly poor outcomes. Patients with larger amounts of residual disease with residual cancer burden (RCB)-III and RCB-II had extremely poor outcomes, with 72.5% and 25.5% of patients developing recurrence within the first 3 years, respectively. Recent breakthroughs in identifying personal neoantigens via a comprehensive analysis of cancer sequencing data have brought increased attention to neoantigen cancer vaccines. Personalized cancer vaccines targeting neoantigens have shown early promising results in melanoma. While neoantigens have recently been investigated in some cancer types, the current neoantigen prediction algorithms have often focused on the MHC class-I subtype, single nucleotide mutations (SNM), small insertions, and deletions (INDEL). We recently developed an informatics workflow, REAL-neo, for the identification, quality control (QC), and prioritization of both class-I and class-II human leukocyte antigen (HLA) bound neoantigens that arise from somatic SNM, INDEL, aberrant RNA splicing, and gene fusions, generating much more potent neoantigen candidates. Furthermore, we demonstrated robust T-cell response and prolonged survival by combining ICI and cancer vaccines in preclinical models. This phase I/II clinical trial is in progress to assess the safety, feasibility, and immunogenicity of personalized neoantigen vaccines in combination with pembrolizumab in patients with advanced solid cancers with a phase II extension cohort in patients with early-stage TNBC with residual disease after neoadjuvant chemotherapy.
Methods: Key eligibility criteria include females ≥ 18 years old with histologically confirmed stage I-III TNBC (ER<10%, PR <10%, and HER2 negative per ASCO/CAP guideline. Patients with evidence of residual disease ≥ 1 cm after neoadjuvant pembrolizumab-based

chemotherapy on imaging will be enrolled in the pre-screening phase prior to surgery to collect fresh frozen samples for sequencing. After surgery, patients with histologically confirmed RCB-II and RCB-III will be pre-registered, and patient samples will be sequenced to identify patient-specific neoantigens using the REAL-neo algorithm. Patients will continue with standard-of-care therapy based on the treating physician's choice. Once PNeoVCA vaccine production is completed, eligible patients will receive the vaccine consisting of up to 20 peptides delivered with GM-CSF as an adjuvant. PNeoVCA will be administered with 4-5 peptides at 300 mcg/peptide and GM-CSF 125 mcg per injection site in each limb in combination with Pembrolizumab 200 mg i.v. PNeoVCA will be given via subcutaneous injection on days 1, 4, 8, 15, and 21, with boosters in weeks 5 and 8.
Clinical trial information: NCT05269381

P3-10-17:Phase I/II study of the novel radioligand therapy [177Lu]Lu-NeoB plus capecitabine in patients w/ ER+/HER2- advanced breast cancer (ABC) w/GRPR expression after progression on prior endocrine therapy (ET) plus a CDK4/6 inhibitor (CDK4/6i) for ABC

Mario Campone, Louise Emmett, Dejan Juric, Debasish Tripathy, Sonia Pernas, Monica Zuradelli, Kevin Perraud, Aleix Prat

Background: A CDK4/6i in combination with ET has become standard of care in first-line ER+/HER2− ABC, but despite significant improvements in survival, these patients will eventually experience disease progression. The optimal treatment sequence following progression on a CDK4/6i + ET has not yet been established and is dependent on various factors. Thus, new targeted treatments are needed for patients with ET-resistant ABC. [177Lu]Lu-NeoB (177Lu-NeoB) is a first-in-class radioligand therapy that binds selectively to GRPR, which is expressed in ER+ breast cancer, including in primary lesions, lymph nodes, and distant metastases. NeoRay is a first-in-human trial testing 177Lu-NeoB in advanced solid tumors. We describe the CAAA603D12101 study (NeoB D1), a phase I/II, open-label, multicenter study evaluating 177Lu-NeoB in combination with capecitabine, a known radiosensitizer, in patients with ER+/HER2− ABC with GRPR expression after progression on prior ET plus a CDK4/6i.

Methods: The NeoB D1 study includes dose-escalation and dose-optimization parts for 177Lu-NeoB. Eligible patients include adult men and pre/peri/postmenopausal women with histologically or cytologically confirmed ER+/HER2− ABC and measurable disease per RECIST v1.1. Pre/perimenopausal women are only included in the phase II part of the trial and will require, along with men, the addition of a gonadotropin-releasing hormone analogue. Patients can have received one to three prior lines of ET in the ABC setting, one of which is required to have included a CDK4/6i. If the participant has progressive disease while on or within 12 months of adjuvant treatment, this is considered a line of therapy. For patients with a confirmed deleterious or suspected deleterious germline BRCA1/2

mutation, prior treatment with a PARP inhibitor is allowed. For patients with HER2-low disease, prior treatment with trastuzumab deruxtecan is allowed. All patients are required to have diagnostic screening with a target lesion showing uptake of [68Ga]Ga-NeoB.
In the phase I part of the study, patients will receive 177Lu-NeoB at 150 millicurie (mCi) Q6W plus capecitabine (1000 mg/m2 BID for 14 days, followed by 7 days off). If dose escalation is supported, then patients will be randomized to the two higher dose levels (200 mCi Q6W or 100 mCi Q3W); if dose escalation is not supported, then lower dose levels will be explored. The phase II part will randomize patients to two dose levels of 177Lu-NeoB plus capecitabine based on multiple different scenarios. Treatment duration of 177Lu-NeoB is planned to be six administrations for the Q6W regimen and 12 administrations for the Q3W regimen.
The primary objectives of the dose-escalation part are the incidence and severity of dose-limiting toxicities and adverse events and dose modifications of 177Lu-NeoB plus capecitabine. The dose-optimization primary objectives are objective response rate, clinical benefit rate, time to response, duration of response, progression-free survival, and overall survival. Secondary objectives include pharmacokinetic and biodistribution (dosimetry) data. Planned enrollment is approximately 58 patients across both phases. Patient enrollment is currently open. ClinicalTrials.gov ID: NCT06247995.

P3-10-18: Phase II Single Arm Trial of Low Dose Capecitabine in Patients with Advanced Breast Cancer (NCT06105684)

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Background: Capecitabine (Xeloda), an oral prodrug of fluorouracil, is approved for adjuvant and advanced stage treatment of breast cancer. The approved dose of capecitabine is determined based on studies in heavily pretreated patients, which demonstrated responses at the maximum tolerated dose of 1000-1250 mg/m2 on days 1-14 of a 21-day cycle. However, higher doses of other chemotherapy agents have resulted in increased toxicity without necessarily improving response rates.
Multiple studies used capecitabine at lower, fixed doses, demonstrating maintained efficacy and notable reductions in toxicities (diarrhea, palmar-plantar erythrodysesthesia, mucositis, and neutropenia). Older adults and frail individuals experience more severe treatment-related side effects and are often underrepresented in clinical trials due to presumed frailty or implicit bias of investigators. This study aims to evaluate both efficacy and tolerability of a daily lower dose of capecitabine in the treatment of advanced HER2-negative breast cancer in aging and frail populations.
Methods: This single-arm, open-label phase 2 study will evaluate the anti-tumor effect of continuous daily oral low-dose capecitabine at 1500mg in 40 patients of age 60 years or older, and/ or considered frail at any age (ECOG 0-2). Frailty is defined by the investigator as an individual at greater risk of complications and poorer outcomes with systemic

therapy, secondary to a lower physiologic reserve and higher comorbidities and functional deficits. Eligible patients include those with HER2-negative unresectable or metastatic breast cancer (hormone positive or triple negative) with measurable disease, who have progressed on at least 1 prior line of therapy. Major exclusion criteria include HER2-positive breast cancer, severe hepatic or renal failure, inability to swallow pills, and uncontrolled CNS/ leptomeningeal disease. Patients will be evaluated for toxicity every 4 weeks and for response every three cycles using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 criteria. Therapy will be continued until evidence of disease progression or unacceptable toxicity. The primary objective is the clinical activity of daily low dose capecitabine by overall response rates (ORR) per RECIST. The secondary objectives include progression-free survival (PFS), overall survival (OS), and determining the safety and tolerability of daily low dose capecitabine using the Common Terminology Criteria for Adverse Events (CTCAE) v.6.0. Exploratory analyses include evaluating quality of life using the European Organization for Research and Treatment of Cancer Quality of Life Core Questionnaire 30 (EORTC QLQ-C30), and the PRO-CTCAE (Patient reported outcomes-Common Terminology Criteria for Adverse Events) questionnaire. Other exploratory analyses include measurement of sarcopenia: measuring body composition including skeletal muscle index (SMI) calculated from skeletal muscle area on CT scan before, during, and after treatment, as well as evaluating geriatric assessment using the CARE (Cancer and Aging Resilience Evaluation) tool prior to registration and at the end of treatment, and evaluating adherence to capecitabine. Simon's two-stage minimax design will be used with a type I error rate of 0.05 and power of 80%. The null hypothesis response rate of 10% will be tested against a one-sided alternative response rate of 25%. In the first stage, 22 patients will be accrued. If there are 2 or fewer responses in the first 22 patients enrolled, the study will be discontinued. The trial was approved by the Institutional Review Board at the University of Alabama at Birmingham (UAB) and opened to accrual July 2024.

P3-10-19: Phase II Trial of Pembrolizumab in Combination with Olaparib in Advanced Breast Cancer with BRCA-mutation or Homologous Recombination Defect (HRD)

Jin Sun Bitar, Monica Mita, Philomena McAndrew, Dorothy Park, Reva Basho, Joey Di Padova, Marie Lauzon, Yuan Yuan

Background: Tumors with HRD showed similar propensity to be sensitive to poly ADP-ribose polymerase inhibitors (PARPi) inhibitors as BRCA-mutated tumors. The rationale for combining PARP inhibitors with immune checkpoint inhibitors (ICIs) comes from the observation that PARPi induces PD-L1 and triggers robust local and systemic antitumor immunity via STING-dependent pathways, which in turn synergizes with ICIs. This study was designed to test the effectiveness of this combination in breast cancer patients with HRD defects or BRCA mutations.
Methods: This is an open-label, single-center, phase 2 study for breast cancer with BRCA-mutated or HRD cancer progressing on prior therapies for metastatic or locally advanced

disease with no restriction of receptor status. All commercially available HRD assays are acceptable. Eligible pts received pembrolizumab 200 mg IV every 21 days combined with olaparib 300 mg PO daily. Treatment was continued until documented progression by RECIST 1.1 or unacceptable toxicity. The primary objective was the overall response rate (ORR) of the combination therapy, with secondary objectives being progression-free survival (PFS), overall survival (OS), and duration of response (DOR). Adverse events (AEs) were monitored using CTCAE v.5.0.
Results: Twelve pts were included in this preliminary analysis. The median age at study entry was 50 (Range 33-73). Of these, 8 pts(66.7%) were White, including 1 Hispanic; 1 pt was African American, and 1 pt was Asian. Ten pts had hormone receptor-positive HER2-negative disease, and 2 patients had triple-negative disease. The pts had received median 4 lines of treatment (Range 1-10) prior to study entry. Six pts and 2 pts had received prior PARPi and ICI respectively, including 1pt who had received both agents at separate times. Treatment response was not evaluable for 2 patients: 1 withdrew consent after cycle 1, and 1 had disease progression during cycle 1 of treatment. Germline BRCA1 and BRCA2 mutations were found in 3 patients (25.0%) and 4 patients (33.3%), respectively. ORR was 33.3% (3 partial responses and 0 complete responses). Median PFS was 3.7 months, and median OS was 5.7 months. Median DOR was 2.2 months. Currently, one pt has been on treatment for over 8 months, with stable disease after 4 months of partial response. The most common adverse events were nausea (7 patients; 4 pt with grade 1-2, 3 pt with grade 3) and anemia (5 patients, all grade 1-2). Other grade 3 toxicities were muscular-skeletal pain in 1pt, fatigue in 1pt, leukopenia in 1pt and pneumonia in 1pt. There was no grade 4-5 toxicity.
Conclusions: The combination of olaparib and pembrolizumab was well tolerated, with ORR of 33.3% in heavily pretreated breast cancer patients, including patients with prior PARPi exposure. This trial is ongoing to finalize the clinical benefits assessment. Clinical trial information: NCT03025035
Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA provided pembrolizumab, olaparib, and financial support for the study.

P3-10-20: Phase II study of talazoparib, a PARP inhibitor, in HER2- metastatic breast cancer (MBC) with a somatic BRCA1/2 mutation identified in cell-free DNA or tumor tissue genotyping

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Background: PARP inhibitors are associated with improved progression free survival (PFS) and patient quality of life in germline BRCA1/2 mutant MBC, leading to their approval in this setting, but their applicability is limited as germline BRCA1/2 mutations are present in 5% of breast cancer. We previously demonstrated that a subset of patients with MBC who are not germline BRCA1/2 carriers may harbor pathogenic somatic BRCA1/2 mutations

that are identified by cell-free DNA and/or tumor tissue genotyping. We developed a circulating tumor cell culture from a patient with MBC harboring a pathogenic somatic BRCA1 mutation, and demonstrated that a PARP inhibitor induced cell growth inhibition similar to a germline BRCA1/2 mutant culture. Thus, we hypothesize that a PARP inhibitor may be effective in treating somatic BRCA1/2 mutant MBC. In this clinical trial, we are studying the efficacy of a PARP inhibitor in somatic BRCA1/2 mutant MBC. Our work may expand the clinical application of PARP inhibitors in MBC.
Trial Design: This phase II investigator-initiated clinical trial is enrolling 30 patients with MBC with a pathogenic somatic BRCA1/2 mutation identified by a CLIA certified cell-free DNA and/or tumor tissue genotyping assay. Patients are treated with the PARP inhibitor talazoparib 1 mg/day until progression of disease. Imaging (CT chest, abdomen and pelvis, and bone scan) occurs for disease assessment at baseline and every 3 months. Cell-free DNA is collected at baseline and then monthly to evaluate changes in the genomic environment. Patients also undergo Cancer Risk B (CR-B) assay, a novel flow variant test to identify double-strand break repair mutations in circulating blood cells, at baseline.
Eligibility Criteria: Patients must have MBC with a pathogenic somatic BRCA1/2 mutation identified in a cell-free DNA and/or tumor tissue genotyping assay, with pathogenicity confirmed by a genetics counselor using validated genomic databases such as ClinVar. Patients may have triple-negative (receipt of at least 1 prior chemotherapy) or hormone receptor positive/HER2- (receipt of at least 1 prior hormone therapy) MBC. Patients must not be known germline BRCA1/2 carriers. Any number of prior therapies including a prior platinum (in absence of progressive disease on a platinum) are allowed, but patients should not have received a prior PARP inhibitor. Patients must have adequate organ function and performance status.
Specific Aims: The primary aim is to determine PFS (RECIST 1.1). Secondary aims include determining the objective response rate and toxicity (NCI CTCAE v 5.0). Exploratory aims include evaluating serial changes in BRCA1/2 mutant allelic frequency in cell-free DNA, understanding the impact of BRCA1/2 reversion mutations in cell-free DNA, comparing pre- and post-treatment cell-free DNA results to understand changes in the genomic environment, studying the CR-B assay positivity rate, and correlating these biomarker analyses with treatment response.
Statistical Methods: This study has 81% power to demonstrate that the 12-week PFS is 53% or higher. In contrast, there is a 4% (alpha) probability of concluding that the 12-week PFS is ≥ 53% if the true 12-week PFS is 30% or lower. If 14 or more of 30 total patients achieve PFS > 12 weeks, the null hypothesis (12-week PFS ≤ 30%) will be rejected.
Present accrual and target accrual: This study (NCT03990896) is open at Massachusetts General Hospital, MD Anderson, University of California San Francisco, Emory, Northwestern, Vanderbilt, and Cornell. As of 7/2024, 21/30 patients are enrolled.
Funding: A Pfizer ASPIRE award and Conquer Cancer Foundation of ASCO–Breast Cancer Research Foundation- Career Development Award fund this study.

P3-10-21: Phase Ib dose-finding study of [177Lu]Lu-NeoB + ribociclib + fulvestrant in patients w/ ER+/HER2- advanced breast cancer (ABC) w/ GRPR expression w/ early relapse from (neo)adjuvant endocrine therapy (ET) or progression on ET + CDK4/6i for ABC

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Background:
Ribociclib (RIB) + ET has shown significant progression-free survival and overall survival benefit in patients (pts) with HR+/HER2− ABC. However, there is still an unmet need for novel therapies for pts with disease progression on or after ET +/− a CDK4/6 inhibitor (CDK4/6i). Radioligand therapies (RLTs) have shown improvements in survival vs standard of care in castration-resistant metastatic prostate cancer and gastroenteropancreatic neuroendocrine tumors. Given their known benefit in other disease settings, RLTs are now being evaluated in ABC. We describe the CAAA603B12101 study, evaluating the addition of 177Lu-NeoB, a first-in-class RLT that selectively binds to GRPR, which is overexpressed in ER+ breast cancer cells, to the currently approved and recommended combination of ET + CDK4/6i for pts with ER+/HER2− ABC with GRPR expression who experienced early recurrence from (neo)adjuvant ET +/− a CDK4/6i or progression on ET + a CDK4/6i for ABC.
Methods:
The purpose of this Phase Ib, single-arm, multicenter, open-label, dose-finding study is to estimate the recommended dose (RD) of 177Lu-NeoB in combination with RIB and fulvestrant (FUL). Eligible pts include adult women (regardless of menopausal status) and men with ER+/HER2− ABC with GRPR expression who meet one of the following criteria: (i) experienced recurrence ≤12 mo from completion of (neo)adjuvant ET (+/− CDK4/6i) with no treatment for ABC, (ii) experienced recurrence >12 mo from completion of (neo)adjuvant ET and then subsequent progression after one line of ET (except FUL) + a CDK4/6i for ABC, or (iii) had ABC at diagnosis that progressed after one line of ET (except FUL) in combination with a CDK4/6i. Pts are required to have one target lesion (measurable per RECIST 1.1) in the baseline stand-alone CT or MRI. All pts are required to have diagnostic screening with a target lesion showing uptake of [68Ga]Ga-NeoB.
This study comprises a dose-escalation part and a concurrent backfill part. In the dose-escalation part, four provisional dose levels are planned to be tested: 100 mCi (initial dose), 150 mCi, 200 mCi and 250 mCi in cohorts of three to six pts. The initial dose of 177Lu-NeoB will be 100 mCi (every 28d for six cycles) in combination with RIB (600 mg/d; 3 wk on/1wk off) and FUL (500 mg on d1 of a 28-d cycle with an additional single dose on d15 of cycle 1). Pre/perimenopausal pts will also receive goserelin (3.6 mg on d1 of a 28-d cycle).
After inclusion of pts in each cohort, the incidence rate of dose-limiting toxicities (DLTs) will be compared with the predefined toxicity rate boundaries to decide whether the next cohort will receive a lower, higher, or same dose or whether the trial will be terminated. The backfill part will allow enrollment to a previously cleared dose level (during escalation

part) to obtain additional safety, tolerability, and preliminary efficacy data. During the backfill part, the cumulative incidence rate of DLTs will also be compared with the predefined toxicity rate boundaries to determine if escalation should be restarted from a lower dose level.
Primary outcomes include incidence and nature of DLTs, incidence and severity of adverse events (AEs) and serious AEs, and incidence of dose interruptions, discontinuations, and dose reductions. Secondary outcomes include preliminary antitumor activity and pharmacokinetic and biodistribution (dosimetry) data. The RD will be determined considering all available data from the escalation and backfill parts. Planned enrollment is approximately 48 pts. Pt enrollment is currently open. Clinical trial ID: NCT05870579.

P3-10-22: Preoperative radiotherapy versus postoperative radiotherapy after neoadjuvant chemotherapy in high-risk breast cancer: a prospective, randomized, international multicentre Phase III trial—NeoRad

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Background: Preoperative radiotherapy is a well-established treatment for various tumor types (rectal cancer, sarcoma, bronchial carcinoma). Promising studies on preoperative radiotherapy also exist for breast cancer, but most of them were not randomized or very old. Recent studies suggest that preoperative radiotherapy followed by immediate reconstruction or flap reconstruction is a low-complication method. There is evidence that the immunogenic effects of radiotherapy may lead to improved immune recognition of tumor cells. These potential immunogenic effects make preoperative radiotherapy a promising modality in interdisciplinary cancer therapy. In addition, preoperative radiotherapy may obviate the need to irradiate implants, expanders, or autologous transplants.
NeoRad (NCT04261244, GBG116) is a multicenter, prospective, international randomized Phase III trial. It aims to investigate whether pre- versus postoperative radiotherapy after neoadjuvant chemotherapy (NACT) improves disease-free survival in patients with high-risk breast cancer and show superiority of preoperative radiotherapy (PRT) of the experimental treatment schedule in terms of disease-free survival (DFS). In addition, several secondary endpoints (cosmetic outcomes, quality of life, overall survival, etc.) will be assessed to better understand the benefits and risks of preoperative radiotherapy compared to the current standard of care.

Study Design: Only patients with high-risk breast cancer who are eligible for NACT will be enrolled and randomly assigned in a 1:1 ratio to two study arms. In the standard arm, patients will undergo surgery, sentinel lymph node biopsy, possibly axillary dissection or targeted axillary dissection according to current S3/AGO guidelines. After surgery, patients will receive adjuvant radiotherapy +/- lymphatic pathways and, if indicated, systemic treatment according to S3/AGO guidelines.
In the experimental arm, patients will receive whole-breast irradiation (WBRT) +/- lymphatic pathway irradiation after NACT. Approximately 3-6 weeks after radiotherapy, patients will undergo surgery including sentinel lymph node biopsy or axillary dissection, followed, if indicated, by post-neoadjuvant systemic therapy according to S3/AGO guidelines. Enrolment in post-neoadjuvant studies is allowed. To assess the response to NACT before radiotherapy, a biopsy of the primary tumor and suspicious lymph nodes is recommended in the experimental arm.
An interim analysis on wound healing will be conducted after 100 patients have received breast-conserving surgery or autologous flap reconstruction (in both arms combined) and after 40 and 100 patients have been reconstructed with an implant (also combined from both arms).
The recruitment period will be four years. The total study duration, including follow-ups, is ten years.

Recruitment: The first patients were enrolled in 02/24 at the University Hospital of Düsseldorf. A total of 1826 patients will be recruited across 40 centers within 4 years.
Funding: This study was supported by a grant from Deutsche Krebshilfe.

P3-10-23: RecurIndex Predicts Risk of Recurrence in Early-stage Luminal Breast Cancer: APRIL Trial

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Background: Evidence has shown that Oncotype DX® (ODx) may overestimate the risk of recurrence among Asian populations. To fill this gap, RecurIndex® (RI) based on the gene-expression profiling of Asian breast cancer patients was developed. This multicenter prospective observational study named the APRIL trial aims to validate the predictive performance of the RI testing for recurrence risk prediction in Chinese women with early-stage luminal breast cancer. This trial will provide prospective evidence of the predictive value of RI testing in the adjuvant setting.

Method: The study was planning to enroll 500 individuals with stage I-II (pT1-2N0-1M0 or pT3N0M0) hormone receptor-positive, HER2-negative breast cancer treated with breast-conserving surgery or mastectomy. The patients were treated according to the low or high risk of relapse measured by RI and in conjunction with clinicopathological risk factors. The

choice of adjuvant therapy was determined by the physician's choice. This study design evaluated the utility of RI for refining recurrence risk prediction to guide personalized adjuvant management. Follow-up visits included every three months for the first two years after the definitive surgery and every six months for three to five years afterward. The primary endpoints included 5-year invasive disease-free survival (iDFS) and 5-year recurrence-free survival (RFS). Secondary endpoints included 5-year distance metastasis-free interval (DMFI), 5-year local-regional recurrence-free interval (LRFI), and 5-year overall survival (OS).
Statistical analysis: This study will test two hypotheses: 1) After 690 person-years of follow-up, the annual distant metastasis-free rate of RI low-risk patients is ≤1%. Statistically, this hypothesis is considered to fail with $p<0.05$, and the test power is 80%; 2) After 690 person-years of follow-up, the annual distant metastasis-free rate of RI high-risk patients is >2%. Statistically, this hypothesis is considered to fail with $p<0.05$, and the test power is 80%. Based on our previous results of the parallel comparison study between RI and ODx, 80% of N0 and 43% of N1 patients will be classified as RI low-risk group, indicating that 2/3 of early luminal breast cancer patients will be at low risk of recurrence. Five hundred patients will be enrolled (333 low-risk and 167 high-risk). When 690 person-years of follow-up are completed, the pre-set rate of no distant metastasis in low-risk patients will be ≤1% and these patients will be advised to receive only adjuvant endocrine therapy. It is expected that data will be collected every four months and reported to the Data and Safety Monitoring Board (DSMB). If it involves changes in patient treatment, the department will make an independent decision based on relevant policies.
Role of the funding source: The study sponsor, National Key R&D Program, oversaw data management and statistics. This study was registered with ClinicalTrials.gov, NCT04972448.

Results: The APRIL Trial opened for recruitment in May 2022 and is in progress. A total of 323 patients enrolled: 120 (37.2%) of 323 patients had clinical high-risk and genomic low-risk, 159 (49.2%) of 323 patients had clinical and genomic low-risk, 43 (13.3%) of 323 patients had clinical and genomic high-risk, and 1 (0.3%) of 323 patients had clinical low-risk and genomic high-risk.
Conclusions: In conclusion, this study will be the first prospective multicenter study to evaluate the risk-prediction value of RI testing in the Chinese population. Those findings will provide valuable evidence for the recommendation of RI testing for guiding adjuvant chemotherapy in early luminal breast cancer patients in China.

P3-10-24: Refusal of sentinel lymph node biopsy in patients with luminal A subtype of early breast cancer

Alexander Emelyanov, Zhanna Bryantseva, Irina Akulova, Pavel Krzhivitskiy, Sergey Novikov, Petr Krivorotko, Amirov Nikolay

This study is a randomized, single-center, prospective observational study that aims to de-escalate surgical treatment for patients with early breast cancer. First of all, the safety of avoiding SLNB in patients with early luminal A breast cancer (T1-2N0).

Study aim: Conduct a comparative analysis of the axillary reccurence incidence in patients without sentinel lymph node biopsy followed by radiation therapy to the remaining breast tissue and axillary lymph nodes and in patients who received standard treatment with SLNB.
Study objectives: Compare the axillary reccurence incidence in patients with early breast cancer who did not undergo SLNB and in patients who underwent standard treatment with SLNB (Time frame: 2, 3 and 5 years)
- Compare locoregional disease-free survival in the studied groups of patients with early breast cancer (Time frame: 5 and 10 years)
- Compare overall and disease-free survival in the studied groups of patients with early breast cancer (Time frame: 5 and 10 years)
- Compare the incidence and severity of adverse events using the current CTCAE version 5.0 (Time frame: 3, 5 and 10 years)
Methods of surgical and radiation treatment
1. Before surgical treatment, all patients included in the study undergo SPECT-CT of the breast with 99mTc-Technetril and SPECT-CT of sentinel lymph nodes with Tc99m-colloid. The patient undergoes a BCS, followed by a histological examination of the removed material. If the patient is allocated to group 1, then she additionally undergoes an SLNB procedure. If the patient is randomized to group 2, then SLNB is not performed.
2. At the next stage, 4 to 12 weeks after surgical treatment, 3D conformal radiation therapy of the remaining breast tissue is carried out with the inclusion of signal lymph nodes in the volume in the group of patients without SLNB (group 2). In patients after SLNB (group 1), only irradiation of the remaining breast tissue is performed without irradiation of the axillary region. In the presence of metastatic lesions of sentinel lymph nodes, additional irradiation of the axillary region is performed.
Radiation therapy to the area of non-removed sentinel lymph nodes will be carried out using modified (high) tangential fields and 3D modeling according to the atlas of sentinel lymph nodes, depending on their location (cent., pect [S], pect [Th II], pect [Th III], pect [ThIV], IP, Lat.)
To date, 21 patients have already been included in the study, and enrollment is ongoing.

P3-10-25:Repeat Breast Conserving Surgery Followed by Daily Partial Irradiation for Patients w/ Ipsilateral Breast Tumor Recurrence or new Ipsilateral Primary Breast Cancer, Previously Treated w/ Breast Conserving Surgery & Whole Breast Radiation Therapy

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Introduction: Breast cancer survival rates have improved with advances in screening and treatment. Standard of care for early-stage and selected locally advanced breast cancers involves breast-conserving therapy, which includes partial mastectomy followed by radiation. For patients with ipsilateral breast tumor recurrence/new ipsilateral primary breast cancers, traditional treatment has been salvage mastectomy. However, many patients prefer to avoid mastectomy, leading to interest in repeat breast-conserving surgery (reBCS) with partial breast re-irradiation. Based on the RTOG 1014 trial, the current standard of care for external beam re-irradiation involves partial breast re-irradiation to a dose of 45Gy delivered twice daily over 3 weeks (30 fractions), which can be burdensome for patients and lead to increased time off work, travel time and childcare challenges. We hypothesize that daily hypofractionated partial breast external beam radiation therapy after repeat BCS will be well-tolerated, achieve good local control, demonstrate comparable toxicity profiles, and offer a more convenient option than the current standard.

Methods: This multicenter, single-arm prospective phase II study is enrolling patients >18 years of age with histologically confirmed ductal carcinoma in situ (DCIS) or invasive unifocal breast cancer recurrence or new ipsilateral breast primary breast cancer measuring <3 cm after repeat BCS, with a minimum of 3 years since previous whole breast irradiation. Patients receive radiation within 21 days after treatment planning CT and no more than 15 weeks post-surgery. Hormonal therapy is allowed and chemotherapy follows radiation if planned, though neoadjuvant chemotherapy is allowed. The radiation dose is 40.05 Gy in 15 fractions of 2.67 Gy, with an option for 48 Gy in 15 fractions of 3.2 Gy for high-risk patients requiring a boost. Target volumes are defined based on the excision cavity, surgical changes, and clips.

Findings: The trial opened in October 2023, aiming to accrue 55 patients over six years (powered similar to RTOG 1014). The primary endpoint is the rate of grade 3+ treatment-related skin, fibrosis and breast pain adverse events within one year post-reirradiation, graded by CTCAE criteria. Secondary endpoints include in-breast tumor recurrence, freedom from mastectomy, overall survival, and mastectomy-free survival. Cosmesis will be evaluated at 1 and 3 years using the BCTOS quality of life questionnaire (ePRO), the Global Cosmesis Score (ePRO) and Worksheet for Breast condition (ePRO). By July 2024, seven patients were screened, with three enrolled. Of those enrolled, 2 completed treatment and one is currently on treatment. For the two patients that have completed treatment, there has been no significant acute toxicity. Two patients with tumors >3 cm were deemed ineligible and treated off-trial with this regimen. Two others are pending further procedures.

Conclusion: This trial has shown high patient interest and acceptance. If outcomes match the current standard, this approach will provide a more convenient option and increase acceptance of repeat attempts at breast conservation reBCS.

P3-10-26: SERIES: SEquencing Sacituzumab Govitecan (SG) AfteR Trastuzumab Deruxtecan (T-DXd) In ER+/HER2 LOW MetaStatic Breast Cancer

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Background: SG is approved for patients (pts) with unresectable locally advanced or metastatic hormone receptor-positive, HER 2-negative breast cancer who have received endocrine-based therapy and at least 2 additional systemic therapies in the metastatic setting. T-DXd is approved in pts with unresectable or metastatic HER2-low breast cancer who have received a prior chemotherapy in the metastatic setting or developed disease recurrence during or within 6 months of completing adjuvant chemotherapy. Currently, there are no prospective data evaluating the efficacy of sequential use of these antibody drug conjugates. Tissue and blood biomarkers are needed to clarify mechanisms of response and resistance. Trial Design: This multi-center phase 2 trial includes pts (N=75) with ER+/HER2 low MBC, who progressed or were intolerant to T-DXd in the metastatic setting and will receive SG on day 1 and 8 every 21 days until disease progression or intolerable toxicity. To be eligible, pts must have ECOG PS ≤2 , be considered endocrine refractory, pre-treated with a CDK4/6 inhibitor (in adjuvant/metastatic setting) and must have received ≥ 1 but ≤ 4 lines of chemotherapy in the metastatic setting. T-DXd is not required as the immediate prior therapy to SG. Clinically inactive brain metastases are permitted. Pts must have ≥1 measurable lesion per RECIST 1.1. Correlative NGS analyses will be conducted on tissue and blood at various timepoints: archival tissue (prior to T-DXd, if available), tissue biopsy prior to SG and at study discontinuation, blood at baseline, cycle 3, day 1 of SG, and at study discontinuation. The primary endpoint is investigator assessed overall response rate. Secondary endpoints include clinical benefit rate, progression free survival, overall survival, duration of response, global quality of life, and adverse events. The trial is currently enrolling patients (NCT06263543).

P3-10-27: SMALL: Open Surgery versus Minimally invasive vacuum-Assisted excision for small screen-detected breast cancer – a UK phase III randomized multi-centre trial

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Background: Mammographic screening programmes reduce breast cancer mortality but detect many small good-prognosis tumours which may not progress. Screen-detected cancers are currently treated with standard surgery and adjuvant therapies, with associated morbidities. There is a need to reduce overtreatment of good prognosis tumours and

numerous studies are evaluating omission of radiotherapy in low-risk disease. However, there is little evidence to support surgical de-escalation, although percutaneous minimally invasive treatment approaches have been described. Vacuum-assisted excision (VAE) is in widespread use for management of lesions of uncertain malignant potential and benign lesions. SMALL (ISRCTN 12240119) is designed to determine the feasibility of using this approach to treat small invasive tumours detected within the UK NHS Breast Screening Programme.

Methods: SMALL is a phase III multicentre randomised trial comparing standard surgery with VAE for screen-detected cancers. Main eligibility criteria are age ≥47 years, unifocal grade 1 tumours with maximum diameter 15mm, strongly ER/PR+ve and HER2-ve, with negative axillary staging. Patients are randomised 2:1 to VAE or surgery, with no axillary surgery in the VAE arm. Completeness of excision is assessed radiologically, and if excision is incomplete, patients undergo surgery. Adjuvant radiotherapy and endocrine therapy are mandated in the VAE arm but may be omitted following surgery.
Co-primary end-points are:
1. Non-inferiority comparison of the requirement for a second procedure following excision
2. Single arm analysis of local recurrence (LR) at 5 years following VAE

Recruitment of 800 patients will permit demonstration of 10% non-inferiority of VAE for requirement of a second procedure. This ensures sufficient patients for single arm analysis of LR rates, where expected LR free survival is 99% at 5 years, with an undesirable survival probability after VAE of 97%. To ensure that the trial as a whole only has 5% alpha, the significance level for each co-primary outcome is set at 2.5% with 90% power. The Data Monitoring Committee will monitor LR events to ensure these do not exceed 3% per year. Secondary outcome measures include time to ipsilateral recurrence, overall survival, complications, quality of life and health economic analysis.
A novel feature of SMALL is the integration of a QuinteT Recruitment Intervention (QRI), which aims to optimise recruitment to the study. Recruitment challenges are identified by analysing recruiter/patient interviews and audio-recordings of trial discussions, and by review of trial screening logs, eligibility and recruitment data and study documentation. Solutions to address these are developed collaboratively, including individual/group recruiter feedback and recruitment tips documents.

Results: SMALL opened in December 2019, but recruitment halted in 2020 for 5 months due to COVID-19. At 8th July 2024, 46 centres are open, with 449 patients randomised, with a randomisation rate of approximately 45%, and a per site recruitment rate of 0.4-0.5 patients/month.
Drawing from preliminary QRI findings and insights from patient representatives, a recruitment tips document has been circulated (on providing balanced information about treatments, encouraging recruiters to engage with patient preferences, and explaining randomisation). Individual recruiter feedback has commenced, and wider feedback is being delivered across sites via recruitment training workshops. Patient interviews are ongoing to

explore patient views and experiences of the trial.

Conclusion: SMALL continues to have excellent recruitment, is expected to complete recruitment in 2025 and to have a global impact on treatment of breast cancer within mammographic screening programmes.

SMALL is funded by the UK NIHR HTA programme award 17/42/32

P3-10-28: Sentinel Lymph Node Biopsy vs Observation After Neoadjuvant Treatment (SLOAN)

Alexander Emelyanov, Petr Krivorotko, Nikolay Amirov, Zhanna Bryantseva, Irina Akulova, Pavel Krzhivitskiy, Sergey Novikov

Study design: SLOAN is a prospective study to evaluate the incidence of axillary recurrence and survival of breast cancer patients who received neoadjuvant therapy and achieved pCR of the primary lesion in the breast without surgical staging of the axillary region.
The hypothesis of the SLOAN study is to prove the oncological safety of excluding SLNB when achieving pCR of the tumor in breast after NST in patients with HER2-positive and TNBC with initially clinically negative lymph nodes (cN0).
SLOAN is a prospective, non-randomized, single-arm surgical trial. A randomized design is not useful because of the expected extremely low rates of axillary recurrence at 3 and 5 years for the experimental group. In the case of a two-arm randomization, the risk of insufficient testing power due to a small number of events would be significantly higher.
Primary endpoints:
- 3-year assessment of axillary recurrence (AR) rate, defined as the presence of tumor in the lymph nodes of the ipsilateral axilla, sub/supraclavicular fossa, or interpectoral region. Recurrences must be confirmed by biopsy or local surgical excision (open biopsy).
Secondary endpoints:
5-year disease-free survival (RFS)
5-year overall survival (OS)
5-year locoregional disease-free survival (LRFS)
5-year distant metastasis-free survival (DFFS)
3-year survival without axillary recurrence
5-year survival without axillary recurrence
Inclusion criteria
- Signed informed consent form
- Invasive HER2-positive or triple-negative breast cancer (confirmed by core biopsy)
- The tumor has not spread to the lymph nodes in the armpit.
- Tumor stage cT1 to cT2
- Planned chemotherapy before surgery OR chemotherapy already completed before surgery
- Female patients ≥ 18 years of age

- Achieving complete clinical regression of the tumor in the breast
- Patients suitable for breast-conserving treatment followed by radiation therapy
- Breast pCR after breast - conserving surgery

Exclusion criteria

- Distant metastases
- Recurrence of breast cancer
- Inflammatory forms of breast cancer
- Extramammary breast cancer
- Bilateral breast cancer
- History of breast cancer or any other type of cancer
- Surgical intervention performed on the axillary region before NCT
- Confirmed involvement of axillary lymph nodes
- Confirmed involvement of supraclavicular lymph nodes
- Confirmed involvement of parasternal lymph nodes
- Pregnancy
- Conducting less than 4 cycles of NCT
- Patients not suitable for surgical treatment

To date, 6 patients have already been included in the study, and enrollment is ongoing.

P3-10-29: Start of SerMa – EUBREAST 5 (Seroma of the Mammary Gland) study (NCT05899387) - On the way to identify women at risk of developing seroma after mastectomy

Nina Ditsch, Melitta Koepke, Nicole Pochert, Udo Jeschke, Mariella Schneider, Matthias Reiger, Claudia Traidl-Hoffmann, Angelika Mattmer, Shirin Hunstiger, Jacqueline Sagasser, Christian Dannecker, Mathis Wild, Christian Hinske, Sarah Friedrich, Steffi Hartmann, Henning Kahl, David Krug, Natalia Krawczyk, Kerstin Stemmer, Regina Fluhrer, Andreas Raue, Maria Luisa Gasparri, Roland Reitsamer, Justyna Jelinska, Güldeniz Cakmak, Eduard Bonci, Rachel Wuerstlein, Visnja Fink, Vesna Bjelic-Radisic, Marc Thill, Christoph Heitmann, Walter Weber, Annette Lebeau, Hans-Christian Kolberg, Toralf Reimer, Rosa di Micco, Jana de Boniface, Oreste Gentillini, Michael Untch, Maggie Banys-Paluchowski, Thorsten Kuehn

Background; Postoperative seroma formation is one of the most common and serious complications after breast surgery for primary breast cancer, especially in patients undergoing mastectomy with or without implant-based breast reconstruction. Seromas may lead to infections and wound dehiscence, which can result in implant loss. To date, the cause of seroma development has not yet been clarified. Recent data of the unicenter SerMa pilot study identified an association with immunological-inflammatory processes as a potential reason for seroma development. Trial Design:The main objective of the multicenter SerMa study is to identify a subgroup of patients with an increased risk of developing seromas based on immunological or inflammatory markers in a planned cohort of 2200 subjects. The study is designed as international, prospective cohort study in cooperation with the

EUBREAST Network (European Breast Cancer Research Association of Surgical Trialists). Currently participating study groups are AGO-B, AWOgyn and OPBC. Patients with primary breast cancer or ductal carcinoma in situ (DCIS) planned for mastectomy with or without implant insertion can participate in this study (study group 1 and 2). Study group 3 includes patients at high risk for breast cancer planned for risk-reducing subcutaneous mastectomy and implant reconstruction. Study group 4 includes healthy women with implant insertion for exclusively cosmetic reasons. Furthermore, it is planned to initiate a registry for postoperative breast seromas (SerMaReg). Primary endpoints: To achieve the primary endpoint – the identification of a patient group with an elevated risk of developing a seroma - it is planned to examine the groups with and without postoperative seroma formation regarding immunological markers in seroma fluid as well as in blood samples. In addition, local microbiome analyses, as well as tumor analyses with focus on microenvironment will be performed to differentiate possible carcinoma-specific immunological processes. Secondary endpoints: To answer the question whether differences are caused by immunological or cancer-related reasons, groups with and without postoperative seroma formation as well as groups with or without breast cancer are compared. Current status of the study: The study started recruiting in April 2024, the first patients have been enrolled and the protocol has been confirmed for practicability. The study is currently expanding worldwide. Target accrual: 550 participants per group, therefore 2200 participants in total.

P3-10-30: Stereotactic Body Radiation Therapy and FES PET/CT Imaging for the Treatment of Oligoprogressive Estrogen Receptor Positive Metastatic Breast Cancer

Jose Bazan, Joanne Mortimer, Yun Rose Li, Rebecca Nelson, Sharon Yim

Background: The term oligoprogression (OP) refers to a clinical scenario in which patients with diffuse metastatic disease on systemic therapy have a limited number of metastases that have progressed or are new whereas the majority of metastases are stable or improved. OP disease is increasingly being encountered in clinical practice due to improvements in systemic therapy. Treating OP disease with local ablative therapies may therefore prolong the time to more diffuse progression necessitating a change in systemic therapy and may therefore lead to improved overall survival in these patients. Only one study has evaluated the role of SBRT in patients with OP MBC. This trial accrued 47 patients with OP MBC, with 2/3 having triple negative disease. There was no difference in PFS between patients that received SBRT versus those that did not (4.2 months vs. 4.4 months, p=0.2). Whether ablative therapies are beneficial in subtypes of breast cancer that have many effective systemic therapies available, such as ER+ breast cancer, remains an open question and an unmet clinical need.

Trial Design: Phase II study for patients with OP ER+ MBC (1-4 new and/or progressing metastatic lesions). Eligible patients will receive stereotactic body radiation therapy (SBRT) to the OP lesions. SBRT will be delivered to each lesion in 3-5 fractions. Each patient's

systemic therapy regimen will be held during study therapy and will resume upon completion of study therapy. Patients will then be restaged at 12 weeks post-SBSRT. Patients that have at least stable disease at that time point will continue on their systemic therapy and then be re-staged 12 weeks later (24 weeks after SBRT). This study will also study the role of ER-targeted positron emission tomography (PET) imaging with 16α-18F-Fluoro-17β-Fluroestradiol (FES) in the OP ER+ patient population with FES PET scans obtained at baseline, and at each of the 2 follow-up imaging timepoints. A key secondary hypothesis is that the use of FES PET in addition to standard imaging at baseline and in follow-up will help confirm patients have OP disease and will help assess for new lesions on subsequent restaging.

Eligibility: Key inclusion criteria: age≥18 yo; histologically confirmed ER+/HER2- metastatic breast cancer; the presence of metastatic breast cancer at the time of study entry with progression in 1-4 lesions (including new lesions); SBRT must be feasible for all progressing lesions.

Key Exclusion Criteria: >2 lines of systemic therapy for metastatic disease; intracranial disease progression

Primary Objective: To determine whether using SBRT to treat OP lesions allows ER+ breast cancer patients to continue on their current systemic therapy for at least 24 weeks post SBRT.

Select Secondary Objectives: To assess whether FES-PET increases the number of lesions found prior to SBRT; To determine the impact of SBRT on patient quality of life; time to next line systemic therapy; PFS

Statistical Methods Simon 2 stage optimal design with $\alpha=0.05$ and $1-\beta=0.80$. The null hypothesis is that the proportion of patients that remain on their original systemic therapy ≥24 weeks (2 restaging scans) post-treatment is 20%. The alternative hypothesis is that the proportion of patients that remain on their original systemic therapy after 2 restaging scans is 50%. In stage 1, 8 patients that proceed to SBRT will be enrolled. The study will be terminated if only 0 or 1 subjects remain on original systemic therapy after 2 restaging scans. However, if ≥2 patients remain on original systemic therapy after 2 restaging, then an additional 10 patients would be enrolled for a total of 18 patients. The null hypothesis will be rejected if ≥6 patients remain on their original systemic therapy after 2 restaging scans.
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P3-11-03: De-escalation of chemotherapy in patients with HER2-positive, hormone receptor negative, node-negative early breast cancer: primary results of the phase II DECRESCENDO trial

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Background: Excellent survival outcomes have been achieved with anti-HER2 therapies in patients with HER2-positive early breast cancer. For these patients, the long-term and irreversible side effects of chemotherapy remain a major concern.
Methods: DECRESCENDO (NCT04675827) is an investigator-initiated multicenter, single-arm phase II trial in patients with HER2-positive, hormone receptor negative, node-negative early breast cancer with tumor size 15-50 mm. Patients received neoadjuvant treatment with taxanes and subcutaneous fixed dose combination pertuzumab and trastuzumab (P+T) for a total duration of 12 weeks followed by surgery. Adjuvant treatment was determined according to response at surgery: patients with a pathologic complete response (pCR, defined as pT0/Tis pN0) received adjuvant P+T for additional 14 cycles, while patients with residual disease received adjuvant trastuzumab emtansine (T-DM1) for 14 cycles. Patients with a residual cancer burden (RCB) score ≥2 received 3-4 cycles of anthracycline-based chemotherapy prior to T-DM1.
Primary endpoint is 3-year relapse-free survival in patients with pCR and PAM50 HER2-enriched subtype.
PAM50 subtypes were centrally determined on baseline tumor samples.
Results: DECRESCENDO was terminated early due to slow recruitment that led to financial support withdrawal. Among 139 patients enrolled, median age was 58 years (range 29-86) and median tumor size was 24.5 mm (range 15-50 mm). Most patients had no-special type histology (N=112, 81%), and grade 3 (N=71, 51%) or grade 2 (N=51, 37%) tumors.
131 patients underwent surgery, with 113 (86%) achieving pCR/RCB 0, 10 (8%) RCB 1, and 8 (6%) RCB ≥2.
No new safety signals were identified.
PAM50 subtype was successfully determined in 124 patients (insufficient material or non-contributive test in 7 patients).
Of these, among patients who achieved pCR/RCB 0 (n=107) the most represented PAM50 subtype was the HER2-enriched one (n=87, 81%) followed by the basal-like (n=13, 12%) and luminal-A (n=7, 7%) subtypes.
Among patients with RCB 1 (n=10), 7 (70%) had HER2-enriched and 3 (30%) basal PAM50 subtype.
Among patients with RCB2 ≥ 2 (n=7), 4 (57%) had HER2-enriched, and 3 (43%) basal-like PAM50 subtype.
Conclusions: Neoadjuvant treatment with an anthracycline-free regimen in patients with HER2-positive, hormone receptor negative, node-negative early breast cancer resulted in a

high rate of pCR.
The majority of patients achieving pCR had the HER2-enriched PAM50 subtype.

P3-11-04: Treatment Patterns and Outcomes associated with the use of Neoadjuvant Chemotherapy (NACT) among Patients with Early-stage HER2-Positive Breast Cancer (BC)

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Background: HER2-positive BC is a clinicopathologically aggressive subtype. The emergence of targeted therapies changed the treatment landscape of this disease by decreasing the risk of recurrence and improving survival. We examined the treatment patterns and trends of NACT use, subsequent pathological complete response (pCR), and the association between pCR and overall survival (OS) in a large cohort of patients with early-stage HER2-positive BC.

Methods: Patients 18 years with clinical stage I-III HER2-positive BC diagnosed between 2010-2021 who had surgery and received chemotherapy were identified in the National Cancer Database. Trends in NACT use and pCR were assessed using the Cochran-Armitage test for time trends. Multivariable logistic regression was used to evaluate the factors associated with NACT use and pCR. Furthermore, a multivariable cox proportional hazards model with propensity score (PS) adjustment and PS matching (1:1 matching performed by exact year of diagnosis, clinical stage, and hormone receptor [HR] status) was used to examine the impact of pCR on OS.

Results: Of the 179,202 patients with HER2-positive BC who received chemotherapy, 35.39% received NACT while the rest had chemotherapy in the adjuvant setting. The rate of NACT receipt increased from 18.4% in 2010 to 60.3% in 2021 ($p<0.001$). Among patients treated with NACT, pCR rates increased from 17.5% in 2010 to 38.6% in 2021 for HER2-positive/HR-positive disease ($p<0.001$) and from 27.1% in 2010 to 65.8% in 2021 for HER2-positive/HR-negative disease ($p<0.001$). In the multivariable model, compared to non-Hispanic Whites, Black patients (aOR=0.96; 95% CI 0.92–0.99) were less likely to receive NACT while Hispanic patients were more likely (aOR=1.10; 95%CI 1.05–1.15). Among patients who received NACT, Black patients were less likely (aOR=0.88; 95%CI 0.83–0.92) to achieve a pCR, but Hispanic (aOR=1.07; 95%CI 1.01–1.14) and other (aOR=1.07; 95%CI 1.01–1.14) patients were more likely to achieve a pCR relative to White patients after adjusting for confounders. More comorbidities, older age, negative HR status, and public insurance were associated with lower odds of NACT receipt and pCR. Conversely, higher income, treatment in academic centers or integrated network cancer programs were associated with higher odds of NACT receipt and pCR. Higher tumor size and nodal stage were associated with higher odds of NACT receipt but lower pCR.

Among patients treated with NACT, having a pCR was associated with a reduction in the risk of death (aHR= 0.44; 95%CI 0.41–0.47). Based on matched pairs, the 5-year OS in patients who achieved pCR was 96% versus 89% among patients who did not (p<0.001). The 3-year OS increased from 89% in 2010 to 96% in 2018 for patients without a pCR (p<0.001), and from 97% to 99% for patients with a pCR (p=0.005).

Conclusions: NACT receipt and rates of pCR among patients with HER2-positive BC have remarkably increased in the past decade. These coincide with the improvements in treatment regimens, especially the introduction of dual anti-HER2 targeted therapy in the management of this aggressive disease. Notably, OS trends have improved for patients with and without pCR. In contrast to the Hispanic patients, Blacks were less likely to receive NACT and achieve pCR, possibly contributing to the worse outcomes seen in Black patients when compared to Whites. Further investigation is warranted to understand and address these disparities to facilitate improvement in survival outcomes.

P3-11-05: Three-year efficacy and safety of neoadjuvant chemotherapy with pertuzumab, atezolizumab, docetaxel, and trastuzumab in patients with stage II/III ERBB2-positive breast cancer (NeoPATH): a nonrandomized, multi-institutional, phase 2 trial

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Background: In the primary analysis of the NeoPATH trial, neoadjuvant pertuzumab, atezolizumab, docetaxel, and trastuzumab (PATH) led to 61% of patients with stage II/III ERBB2-positive breast cancer having a pathological complete response (pCR; defined as no invasive cancer cells in the breast and regional lymph nodes [ypT0/isN0] and the absence of lymphovascular cancer invasion). Here, we report the analysis of secondary endpoints, including a 3-year event-free survival rate, overall survival, and long-term safety.
Methods: In this nonrandomized, multi-institutional, single-arm phase 2 trial, patients with stage II/III ERBB2-positive breast cancer received up to six cycles of neoadjuvant PATH, followed by definitive surgery. Patients achieving pCR received 12 cycles of adjuvant atezolizumab, trastuzumab, and pertuzumab (AHP) every 3 weeks, while those without pCR received 14 cycles of atezolizumab plus trastuzumab emtansine (T-DM1) every 3 weeks.
Results: Of the 67 enrolled patients, 65 (97%) completed six cycles of neoadjuvant therapy, and 41 (61%) achieved pCR, all completing 12 cycles of adjuvant AHP. Among the 26 patients without pCR (including one with disease progression that precluded surgery), 21 (81%) completed the 14 cycles of adjuvant atezolizumab plus T-DM1. The median follow-up was 39.4 months. The estimated 3-year event-free survival rate was 89% (95% confidence interval [CI], 81–98) in the intention-to-treat population, with rates of 97% and 75% for patients with and without pCR, respectively (hazard ratio [HR], 0.1; 95% CI, 0.01–0.83).

Five events occurred: one local disease progression that precluded surgery, two distant breast cancer recurrences, and two second primary malignancies (thymic carcinoma and glioblastoma). Only two patients died; the estimated 3-year overall survival rate was 98% (95% CI, 96–100). Grade 3 or higher adverse events occurred in 29 patients (43%), primarily during the neoadjuvant phase, most commonly neutropenia (n=11) and febrile neutropenia (n=6). Grade 3 or higher immune-related adverse events (n=4) occurred exclusively during the neoadjuvant phase, and one patient died of sepsis during adjuvant treatment.
Conclusion: In patients with stage II/III ERBB2-positive breast cancer, replacing carboplatin with atezolizumab in neoadjuvant therapy and adding atezolizumab to adjuvant therapy shows promising long-term efficacy and acceptable safety profile.

P3-11-06: Predicting the pathological response to neoadjuvant therapy using untargeted metabolomics in HER2-positive breast cancer

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Background: The standard treatment for HER2-positive breast cancer is the use of neoadjuvant chemotherapy with dual HER2 blockade (trastuzumab+pertuzumab). In addition to providing equal efficacy in preventing cancer recurrence and a less invasive surgical approach, response to neoadjuvant therapy (NAT) provides prognostic information for determining further adjuvant treatment. Pathologic complete response (pCR) has become a surrogate endpoint in neoadjuvant randomized clinical trials because it has been found to correlate with better long-term treatment outcomes. Many studies are currently focused on discovering predictors of response to NAT. Metabolomics is a relatively new research field focused on the analysis of small molecules (metabolites) with promising applications in personalized medicine and oncology. Neoadjuvant chemotherapy with dual HER2 blockade has led to high pCR rates, but there is still a significant number of patients with early HER2-positive breast cancer who have a partial or no response to NAT and represent an unmet medical need. The aim of this study was to find differences in the pre-NAT serum metabolomic profile of patients who achieved pCR and those with residual disease in order to discover predictive biomarkers of response to NAT.
Methods: This prospective pilot study cohort included 36 patients with HER2-positive breast cancer treated with NAT at the University Hospital Center Zagreb from August 2017 to December 2021 with prior approval of the Ethics Committee. Patients' plasma samples were collected before the start of NAT and before the last cycle of NAT. Untargeted metabolomic analysis was performed using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS). Multivariate statistical analyses, including principal component analysis (PCA) and partial least squares analysis (PLS-DA) models, were performed. Metabolites for which the observed Variable Influence in Projection (VIP) was higher than one were considered statistically significant. The tentative

identification of metabolites was confirmed by LC–MS/MS analysis. For univariate statistical comparisons between groups Student's t-test or Mann-Whitney U test were performed, depending on data distribution, followed by Bonferroni post hoc correction for multiple comparisons ($p \leq 0.050$).
Results: Of the 36 patients included in the study, 55.5% (N=20) had pCR, while 44.5% (N=16) were classified as non-PCR. PCA and PCA DA analyzes showed different plasma metabolomic profiles before NAT in the pCR group and the group with residual disease. By tentative identification of metabolites, three metabolites were found with different concentrations in the two observed groups. Ganglioside GD3 and norajmalin were significantly higher in the group with residual disease, while undecaketide was significantly higher in the pCR group. A comparison of samples before and after NAT revealed different metabolomic signatures in which discriminatory metabolites belong to different metabolic pathways. In the group with residual disease, the concentration of ganglioside GD3 was lower in control samples taken after NAT than before NAT.
Conclusion: This study demonstrated that there are distinct plasma metabolomic profiles in patients with HER2-positive breast cancer who achieve a pCR on NAT and those with residual disease after NAT. Ganglioside GD3, previously linked to tumor progression and aggressiveness, could be a potential predictive marker of poor response to NAT in patients with HER2-positive breast cancer treated with dual HER2 blockade combined with chemotherapy.

P3-11-07: HER2 determination by ERBB2-mRNA expression analysis

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Background and Aim: The human epidermal growth factor receptor 2 (HER2) is overexpressed in 15-20% of breast carcinomas (BC). Patients with inoperable or metastatic BC and low HER2 expression (HER2-low) may benefit from trastuzumab-deruxtecan. The aim of this study was to compare HER2 status with ERBB2-mRNA expression analysis, and to analyze the association of ERBB2-mRNA expression with disease progression of the patients.

Material and Methods: The HER2 determination (i.e. clinical testing) in a sample of 694 BC patients from a prospective, multicentre cohort (n=1,270, NCT 01592825, 2009-2011) was performed according to ASCO/CAP of the respective time by the local pathology (n=3), and provided the following distribution: HER2-zero (IHC 0) 34% (n=237), HER2-low (IHC 1+, IHC 2+, ISH negative) 52% (n=360), and HER2-positive (IHC 2+, ISH positive, IHC 3+) 14% (n=97). ERBB2-mRNA expression was determined using high-throughput qPCR. The cut-off values for three ERBB2-mRNA categories were calculated by ROC analyses, adjusted to the HER2 results. The associations with prognostic markers and survival (recurrence-free interval, RFI) were estimated by linear regression (odds ratios, OR) and Kaplan-Meier

analyses.

Results: Relative mRNA expression < 9.7 classified samples as ERBB2-weak, ≥ 9.7 and < 23.2 as ERBB2-intermediate (AUC 0.72), and ≥ 23.2 as ERBB2-high (AUC 0.86). In our cohort 48% (n=333) of the samples were ERBB2-weak, 36% (n=252) ERBB2-intermediate and 16% (n=109) ERBB2-high tumours. Thus, compared to the tumors that were classified as HER2-low by clinical testing, ERBB2-mRNA expression analyses reduced the proportion by 30%. With regard to the HER2-zero samples, 32 % (n=76) were categorized as ERBB2-intermediate (n=61) or ERBB2-high (n=15), and 68% (n=161) were identified as ERBB2-weak. From 597 HER2-negative tumors, 43 (7.2%) tumors were classified as ERBB2-high, and from 97 HER2-positive tumors, 32% were reclassified as ERBB2-intermediate (n=21) or ERBB2-weak (n=10). Compared to ERBB2-weak, ERBB2-intermediate tumours were significantly more frequently associated with a positive hormone receptor status (HR) (OR 4.0; 95% CI 2.25-7.05), favourable grade (G1/2 vs 3; OR 2.1; 95% CI 1.45-3.04), and smaller tumor size (T1 vs >T1; OR 1.4; 95% CI 1.04-1.93).These patients had fewer disease related events after five years (no RFI events: 94%, 95% CI 90.9 – 97.1) than patients with an ERBB2-weak tumours (no RFI events 89%, 95% CI 86.0 – 92.6, p=0.008), presumably since they received endocrine therapy more frequently.

Conclusion: In this retrospective analysis, ERBB2-mRNA analyses provided results that were different from clinical HER2 testing, and may be more accurate to define HER2-zero, low and positive status. In our study, using routine clinical HER2-testing, the proportion of low HER2 expressors (i.e. ERBB2-intermediate) was reduced by 30%. On the other hand, ERBB2-mRNA analysis increased the proportion of tumours that potentially would respond to anti-HER2 therapy. Considering the tumor characteristics (e.g. HR, grade, size), our data suggest that ERBB2-intermediate/HER2-low may be an own entity.

P3-11-08: Treatment patterns and impact of adjuvant chemotherapy and targeted therapy on outcomes in HER2+ microinvasive breast cancer

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Microinvasive breast carcinoma (MiBC) is defined as invasive breast carcinoma ≤1 mm in size. MiBC is most commonly encountered in the setting of high-grade human epidermal growth factor receptor (HER2) positive ductal carcinoma in situ (DCIS) and may consist of one or more foci. Most MiBC is HER2+, and some studies suggest it may be associated with a worse prognosis than pure DCIS. Evidence-based guidelines on the role for adjuvant systemic therapy in HER2+ lymph node negative (LN-) MiBC are lacking, particularly for cases with multifocal microinvasion. Our objectives were to analyze treatment patterns and assess clinical benefit of adjuvant chemotherapy with or without HER2-targeted therapy (CT/H2TT) in this subgroup. We conducted a retrospective analysis of patients with

HER2+ LN- MiBC treated at Memorial Sloan Kettering Cancer Center from 1/1/09-9/30/23. Patients with macroinvasive breast cancer in the preceding 20 years or concurrent to the index MiBC were excluded. Pathologic and clinical data were obtained by a review of pathology reports, slides, and medical records. Pathology slides were reviewed whenever available to confirm MiBC focality. The primary outcome was invasive disease-free survival (iDFS) defined as time from date of surgery to invasion, recurrence, or death. Alive patients without recurrence were censored at last known no evidence of disease (NED) date. The Kaplan-Meier method was used to estimate iDFS. The log-rank test was used to compare iDFS between different groups. We identified 213 patients with HER2+ LN- MiBC who met inclusion criteria. The number of MiBC foci ranged from 1 to 41. Most patients had <5 foci (141, 68%). Compared to patients with <5 foci of MiBC (n=141), patients with ≥5 foci (n=65) were more likely to undergo total mastectomy than breast conserving surgery (72% vs 55%, p=0.02), and receive adjuvant CT/H2TT (39% vs 6.4%, p<0.001), predominantly (94%) trastuzumab/paclitaxel. Age (56 vs 51 years, p=0.1), rates of ER/PR+ (27% vs 18%, p=0.12), adjuvant antiestrogen therapy (28% vs 22%, p=0.3), and adjuvant radiotherapy (44% vs 34%, p=0.2) did not differ significantly between the <5 foci and ≥5 foci cohorts. In the overall cohort, there were 12 invasive disease-free survival (iDFS) events: 2 distant recurrences, 7 local recurrences, 1 contralateral HER2-negative new breast primary, 1 non-BC related death, and 1 death from unknown cause. There was no significant difference in iDFS between patients with <5 vs ≥5 foci (p=0.7). The 5-year iDFS rates in patients with <5 and ≥5 foci were 94% and 95%, respectively. Among patients with ≥5 foci, there was no significant difference in iDFS between those who received adjuvant CT/H2TT (25, 39%) and those who did not (39, 61%) (p=0.4). iDFS did not differ between those with a single focus (n=58) and multiple foci (n=155) of MI (p=0.7). Our study found that there is no difference in iDFS between the patients with <5 and ≥5 foci or those with a single focus and multiple foci of MI. In the cohort with ≥5 foci, we did not detect a statistically significant iDFS advantage for adjuvant CT/H2TT compared to observation following local therapy. Although the small number of events limits statistical power, these findings suggest that patients with HER2+ LN- MiBC and ≥5 foci of MI are generally treated more aggressively than those with <5 foci without significant clinical benefit. Further investigation in a larger cohort is underway to determine optimal management of HER2+ LN- MiBC.

P3-11-09: Incidence and Treatment Patterns of High-Risk HER2+ Early-Stage Breast Cancer Patients in the United States Community Oncology Setting

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Background: Clinical staging of HER2+ early-stage breast cancer (eBC) is key to patients receiving appropriate care. Patients categorized as high-risk are at increased risk of recurrence, hence neoadjuvant treatment is recommended for these patients. With the advent of novel antibody drug conjugates for treatment of high-risk HER2+ eBC, it is

important to understand risk-status assessment and current treatment landscape. This real-world study aimed to describe the incidence of high-risk HER2+ eBC, treatment patterns and rates of recurrence with distant metastases during the follow-up period within community oncology settings in the United States (US).

Methods: A retrospective study utilizing electronic medical record data from The US Oncology Network was conducted. Adult (≥21 years) patients with diagnosis of HER2+ eBC and not enrolled in a clinical trial between 01/01/2017 and 03/31/2023 (earliest diagnosis date as index date) were included in the study and were followed until 09/30/2023. Risk assessment was defined as high risk (Tumor size: T0-T4, nodal involvement: N1-3, distant metastases: M0), moderate to high risk (T2, N0, M0), or low risk (T1a-T1c, N0, M0). Incidence of high-risk HER2+ eBC, patient characteristics, neoadjuvant and adjuvant treatment patterns, and rates of distant metastases during the follow-up period were analyzed descriptively. Among high-risk HER2+ eBC patients, factors associated with receipt of neoadjuvant treatment were assessed using multivariable logistic regression.

Results: 5,487 HER2+ eBC patients met study criteria, of which 4,125 had documented TNM staging. Among the 4,125 patients with available risk assessment, 38% were high-risk (n=1,567), 24.6% were moderate to high risk (n=1,016) and 37.4% were low risk (n=1,542). High-risk HER2+ eBC patients had a mean (SD) age of 56.6 (14.1) years, were majority White (n=967, 61.7%), with nodal involvement (n=1,413, 90.17%) and postmenopausal (n=909, 58%). Neoadjuvant and adjuvant treatments were initiated among 62.6% (n=981) and 91.4% (n=1,432) of high-risk patients, respectively. Of high-risk patients with neoadjuvant treatment (n=981), majority received TCHP (docetaxal + carboplatin + trastuzumab + pertuzumab) regimen (n=807, 82.3%). Of those with adjuvant treatment (n=1,432), about 35.9% (n=514) received trastuzumab+pertuzumab based regimen (±hormonal therapy), 22% (n=318) received T-DM1 and 14.4% (n=206) received TCHP. During the follow-up period (median 31.9 months), 4.6% (n=45) of high-risk patients with neoadjuvant treatment progressed to distant metastases compared to 10.9% (n=64) of high-risk patients without neoadjuvant treatment. Multivariable logistic regression model adjusting for age, race, practice location, menopausal status, and hormone receptor status revealed that older high-risk patients, those in perimenopausal phase (vs. premenopausal) were less likely to receive neoadjuvant treatment; whereas patients with a negative hormone receptor status (vs. positive) were more likely to receive neoadjuvant treatment (p-value <0.05).

Conclusion: In this large sample of real-world HER2+ eBC patients treated in the US community practice setting, over one-third of patients met criteria of being at a high-risk of recurrence. Neoadjuvant treatment for these patients primarily comprised of dual HER2 targeted treatment and two different chemotherapies and adjuvant treatment varied. Despite established guidelines for preoperative systemic therapy for high-risk HER2+ eBC

patients, many patients did not initiate neoadjuvant treatment and had higher rates of metastases, demonstrating unmet need and opportunity to improve health outcomes.

P3-11-10: Benefit/risk relationship of TCH versus AC-TH in BCIRG 006: analysis using generalized pairwise comparisons

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Purpose: Given the nominally superior disease-free and overall survival for doxorubicin, cyclophosphamide, docetaxel, and trastuzumab (AC-TH) versus docetaxel, carboplatin, and trastuzumab (TCH), but significantly improved cardiac safety and lower incidence of leukemia with TCH than with AC-TH, we formally assessed the benefit/risk relationship of these two regimens using Generalized Pairwise Comparisons, a novel method that yields a single efficacy measure that represents multiple outcomes under a single statistical test.

Patients and Methods: We used data from BCIRG 006 with a median follow-up of 10.5 years in order to compare AC-TH and TCH regarding the following outcomes analyzed in this order of priority: time to death; time to distant metastases; time to second primary cancer, including leukemia; time to locoregional relapse; occurrence of congestive heart failure; time to left-ventricular ejection fraction (LVEF) loss >30%; time to LVEF loss >25%; and time to LVEF loss >20%. We computed the Net Treatment Benefit (NTB), a measure of benefit/risk that can be interpreted as the net probability that a random patient from the TCH arm would have a more favorable outcome than a random patient from the AC-TH arm, given the prioritized outcomes.

Results: In keeping with the original analysis at a median follow-up of 65 months, there was no significant difference between TCH and AC-TH in individual outcomes related to survival or to cancer-related events but, TCH was significantly superior to AC-TH for all individual outcomes related to cardiac safety. In multivariate analysis with all outcomes combined in the prespecified order, 36.0% of the pairwise comparisons favored TCH and 29.1% favored AC-TH, resulting in an NTB of 6.9% in favor of TCH (P=0.025). This translates into a number needed-to-treat of approximately 15 patients in favor of TCH.
Conclusion: Using a methodologically rigorous, single measure of treatment effect, the NTB significantly favors TCH when outcomes are prioritized as specified here.

P3-11-11: Ductal Carcinoma in Situ: Molecular and Cellular Basis of Malignant Transition

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Background: The goal of this study is to test whether cellular stemness drives the metastatic progression of human DCIS. DCIS is the most common type of non-invasive breast cancer and is considered a precursor to invasive ductal carcinoma (IDC). The 20-year breast cancer-specific mortality rate following a DCIS diagnosis is approximately 3.3%. The current radiation and anti-hormonal therapy have not reduced mortality associated with DCIS, which is quite intriguing. Moreover, significant racial disparities exist in mortality outcomes since Black women with DCIS compared to White women demonstrate a 7.5-fold increase in breast cancer-related mortality as well as a 12-fold risk when diagnosed before the age of 50. While the reasons for these racial disparities are not known, previous studies have suggested increased stem cell-like characteristics in breast cancer cells from women of African ancestry as compared to those with Caucasian ancestry. Additionally, studies have shown that HER2 could be a potential biomarker for DCIS, indicating future risk of regional and distant metastasis. Approach: We have developed a model referred to as the Mouse INtraDuctal (MIND) model, which mimics the non-invasive to invasive transition of human breast cancers. To generate the models, DCIS tissues were obtained from the clinics and digested into single cells followed by their intraductal injection to generate the MIND models, and a portion of the cells were subjected to single-cell transcriptomic and epigenomic analysis. Patient DCIS and DCIS with associated IDC were also subjected to spatial transcriptomics. Results: We conducted scATAC/RNA sequencing on epithelial cells from ten patients with DCIS and DCIS with associated IDC. The analysis of stemness using CytoTRAE as defined by Gulati et al., revealed that two clusters (9 & 0) had the highest stemness scores. These two clusters also exhibited high expression of FOXA1, and a strong correlation was observed between stemness score and FOXA1 expression, particularly in HER2-3+ DCIS/IDC samples compared to HER2-3+ pure DCIS, luminal or TNBC. We also found that the open chromatin regions in the stemness clusters were highly enriched for FOXA1 binding motifs, particularly in HER2-3+ DCIS/IDC samples. Additionally, single-cell RNA sequencing was performed on 60,000 cells from 17 samples of DCIS, including 9 that progressed in the MIND models and 8 that remained non-invasive. The stemness scores, determined using CytoTRACE were significantly higher in progressed DCIS cells compared to non-progressed cells. There was also a notable correlation between the expression of FOXA1 and the stemness scores. Additionally, we performed spatial transcriptomics (10x Genomics-Xenium) on sixteen patient DCIS samples, including eleven HER2-3+ (five pure DCIS and six DCIS/IDC) and five HER2-negative (three pure DCIS and two DCIS/IDC). As expected, the CytoTRACE analysis indicated that luminal HR+ epithelial cells in DCIS/IDC HER2-3+ samples exhibited the highest overall level of stemness. FOXA1 was identified as one of the top 10 genes associated with stemness. Ligand receptor (LR) interaction analysis identified the highest dissimilar LR pairs was CEACAM6-EGFR. CEACAM6 was upregulated on luminal HR+ cells with the highest stemness scores and interacted with EGFR expressed

by luminal secretory, B cells, T cells, myeloid, mast cells vascular and lymphatic cells. High CEACAM6 expression is associated with poor survival and metastasis in several cancer types, including pancreatic cancers and breast cancers. Conclusion: The presence of a spatial niche containing stem-like cells with increased epigenetic activity of FOXA1 may drive the invasive and metastatic progression in DCIS. We suggest that targeting the activity of FOXA1 can inhibit the function of these stem-like cells and prevent metastatic or invasive progression of human DCIS. Additionally, CEACAM6-EGFR may facilitate communication between epithelial stem cells and their stromal niches, promoting progression.

P3-11-12: (Neo)adjuvant Therapy Patterns and Outcomes in Patients with HR-Positive/HER2-Positive Early or Locally Advanced Breast Cancer: a Real-World Study Using National Cancer Information Database, China

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Background: Our previous national-wide analysis in representative large samples identified molecular subtype of hormone receptor-positive/human epidermal growth factor receptor 2-positive (HR+/HER2+) accounting for 18.0% of all breast cancers (BC), and 25.4% of HR+/HER2+ early or locally advanced (EBC or LABC) patients received neoadjuvant therapy in the real-world setting in China. This study reported further results regarding short-term response and mid-term survival outcomes.

Methods: A representative sample of 51 hospitals (31 cancer centers and 20 general hospitals) covering 27 provinces or municipalities from the National Cancer Information Database (NCID) in China was used. Anonymous individual patient data from electronic medical records (EMR) were retrieved to extract information on demographics, disease characteristics, clinical features, therapy modalities, and outcomes. HR+/HER2+ EBC or LBC patients initially diagnosed between 1 January 2019 and 31 May 2022 were included if they had (1) received surgery and adjuvant treatment and (2) had post-surgery pathologic reports. Descriptive statistics was used to illustrate the distribution of patients in different subgroups with or without neoadjuvant therapy. Survival probability was calculated by the Kaplan-Meier method.

Results: 13,323 Chinese patients initially diagnosed with HR+/HER2+ EBC or LABC were identified during the study period. Most patients' data were derived from tertiary hospitals (n = 13321 [99.98%]), Cancer Centers (n = 11212 [84.16%]), and second-tier cities (n = 10089 [75.73%]). For the clinical stage, patients with cT2 (58.87%), cN0 (45.83%), or cN1 (35.01%) tumors were predominated. 3,791/13323 patients received neoadjuvant therapy. Combinations of two anti-HER2 agents + Chemo (2089/3791) topped the list of neoadjuvant regimens, of which trastuzumab-pertuzumab (TP) + chemotherapy (Chemo) (1970/3791[52.0%]) was the most frequently used therapy, while TP+ a tyrosine kinase

inhibitor (n = 41) was used less frequently. T+ Chemo was reported in 649 patients and Chemo only was documented in 980 patients. Compared to neoadjuvant T + Chemo, combinations of two anti-HER2 agents + Chemo yielded significantly higher total pathological complete response (tpCR, 29.86% vs. 49.14%) and breast pCR (bpCR, 34.39% vs. 53.30%), both $p<0.0001$. Generally, patients who achieved tpCR had a lower recurrence rate than those who did not. Neoadjuvant TP followed by adjuvant TP appeared to be the most selected sequential regimen, the recurrence rate is 2.6% (3-yr-DFS: 94.83%) for patients with tpCR and 5.4% (3-yr-DFS: 80.86%) for who without tpCR. For patients that received neoadjuvant TP, following adjuvant TP led to a lower recurrence rate than T (2.6% vs. 4.9% in tpCR patients and 5.4% vs. 15.9% in patients without tpCR).

Conclusions: To our knowledge, this is the first and largest longitudinal real-world study focusing on perioperative therapy utilization patterns, and short-term and mid-term outcomes in HR+/HER2+ EBC or LABC patients in China. The study, whether in terms of geographical coverage, hospital level, number of patients population, or the study period (that followed the launch of pertuzumab, which is one of the current standard-of-care treatments for patients with HER2+ BC), can represent the up-to-date diagnosis and treatment status quo in China. Our results provide evidence from clinical practice to verify the remarkable pCR and DFS outcomes derived from perioperative dual HER2 blockade with TP.

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P3-11-13: Efficacy and Safety of Chemo-Free Regimens in HER2-Positive Early Breast Cancer: A Comprehensive Systematic Review and Meta-Analysis

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Background: Human epidermal growth factor receptor-2 (HER2)-positive breast cancer is a rapidly growing cancer that has poor outcomes if left untreated. Generally, this cancer requires treatment with aggressive chemotherapy and HER2-targeting therapies. However, there is increasing evidence in randomized clinical trials (RCTs) that chemotherapy-free regimens can improve outcomes of HER2-positive early breast cancer while maintaining high oncogenic safety.

Objective: To examine the efficacy and safety of chemotherapy-free regimens in patients with HER2-positive early breast cancer.

Methods: A systematic literature search for randomized trials published until July 2024 was conducted on PubMed, CENTRAL, Google Scholar, and Embase. Trials were eligible if they compared chemotherapy-free HER2-targeted therapies to HER2-targeted therapies combined with chemotherapy in women with early-stage HER2-positive breast cancer. Conversely, trials that examined chemotherapy-free regimens in advanced HER2-positive breast cancer and observational studies were excluded. The primary endpoint of our study was pathological complete response (pCR), and the secondary outcomes were event-free survival (EFS), invasive Disease-free survival (iDFS), and discontinuation from treatment due to drug-related adverse events. EFS encompassed both relapse-free survival and progression-free survival. All outcomes were pooled together using the random-effects model. Moreover, subgroup analysis was performed to outline the effect of each chemo-free therapy on pCR and assess survival outcomes over different assessment periods. Furthermore, we examined the impact of hormone receptor (HR) status on pCR in patients receiving chemo-free therapies.

Results:Six randomized trials, including 1976 patients with early-stage HER2-positive breast cancer, met the inclusion criteria for this meta-analysis. The pooled results showed that about 35.5% (269/757) of patients receiving chemo-free therapies achieve pCR. However, the subgroup analysis revealed that patients treated with trastuzumab plus pertuzumab and those treated with trastuzumab emtansine plus pertuzumab had significantly inferior pCR than those receiving chemotherapy combined with anti-HER2 drugs (OR: 0.22, p=0.0002 and OR: 0.69, p=0.04). Additionally, the pooled results showed that among patients receiving chemo-free therapies, those with HR-positive disease had inferior pCR than those with HR-negative disease (OR: 2.92, p=0.05). The subgroup analyses also demonstrated that patients receiving chemo-free therapies have high 3-year and 5-year iDFS (96.84% and 87%) and EFS (94.75% and 81.13%). Regarding safety, the pooled results revealed that the discontinuation rates due to adverse events were statistically similar between the two groups (OR: 0.69, p=0.55).

Discussion/Conclusion: Chemotherapy-free regimens demonstrate promising responses and survival outcomes in patients with HER2-positive early breast cancer. However, combining anti-HER2 drugs with chemotherapy still demonstrates better clinical outcomes. Therefore, omitting chemotherapy outside clinical trials might expose patients to unnecessary risks. Moreover, pCR was more significant in patients with HR-negative disease, suggesting that chemo-free therapies might be more beneficial in these patients.

P3-11-14: Differential response to neoadjuvant antiHER2 therapy between HER2 2+ISH+ and HER2 3+ in HER2-positive breast cancer: Is HER2 2+ISH+ a distinct subtype?

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Background: Neoadjuvant chemotherapy with dual anti-HER2 therapy has become the standard neoadjuvant systematic treatment (NST) for HER2-positive breast cancer patients. However, the efficacy of neoadjuvant anti-HER2 therapy varies greatly, different HER2 protein expression level of tumor cell is one of the reasons. Several studies have shown that the HER2 protein expression level is an independent impact factor of pathologic complete response (pCR) rate, HER2 2+ISH+ group patients have a poorer response to NST. However, few studies have explored the relationship between the use of single or dual anti-HER2 agents with HER2 protein expression and its impact of efficacy in pCR and survival.
This study analyzes the pCR rate of patients with different HER2 protein expression level receiving single or dual anti-HER2 therapies. We aimed to identify poor respondors to anti-HER2 therapy, whom in need of optimization and individualization of NST regimen.

Methods: A total of 575 HER2-positive breast cancer patients from multiple centers in Nanjing, China during 2013 to 2022 were retrospectively analyzed, containing of 117 HER2 2+ISH+ patients (20.3%) and 458 HER2 3+ patients (79.7%). All patients received neoadjuvant chemotherapy and anti-HER2 therapy, which is Trastuzumab with or without Pertuzumab. We performed subgroup analysis according to different HER2 IHC classes (HER2 2+ISH+ or HER2 3+), evaluating their difference in clinicopathological features, response to NST under different HR (hormone receptor)status and anti-HER2 therapy. Category changes of HER2 IHC from core needle biopsy(CNB) previous to NST to residue disease after NST and the impact of different HER2+classes on survival were analyzed as well.

Results: [Difference in Clinicopathologic characters]
The HER2 2+ISH+ subgroup had a higher proportion of HR+ status than HER2 3+ patients (76.1% vs 48.7%, $p < 0.001$).
[Different response of HER2+ classes to NST stratified by anti-HER2 therapy]
HER2 2+ISH+ subgroup had significantly lower pCR rate (16.24% vs 46.07%, $p < 0.001$). Multivariate regression analysis based on HER2 IHC subgroups showed that premenopausal status (OR=4.435, 95%CI 1.205-16.324, p=0.025) and HR-status (OR=4.863, 95%CI 1.373-17.228, p=0.014) were favorable factors for pCR in HER2 2+ISH+ patients. However, the use of single or dual anti-HER2 therapy was not an independent impact factor (OR=0.537, 95% CI 0.171-1.685, p=0.287), especially in HER2 2+ISH+/HR+ subgroup, the pCR rates were 11.11% and 13.21%, respectively, with no significant difference (OR=0.821, 95%CI 0.222-3.04, p=0.768). While in HER2 +ISH+/ER- subgroup, the pCR rates were 15.38% and 40.0%, respectively, showing an increase in pCR rate with the use of dual anti-HER2 therapy, but has no statistic significance (OR=0.273, 95%CI 0.044-1.695, p=0.163).

In HER2 3+ patients, dual anti-HER2 therapy were beneficial for pCR (OR= 2.760, 95%CI 1.734-4.392, p < 0.001), while HR status shows no significant impact (OR=1.382, 95%CI 0.934-2.044, p=0.105).
[Category change of HER2 IHC from CNB to residue disease]
We also found that HER2 2+ISH+ subgroup tend to has more HER2 protein expression loss than that in the HER2 3+ patients. The proportion of patients who converted to HER2 0 or 1+ after NST was 22.2% vs. 3.7%, respectively in HER2 2+ISH+ and HER2 3+ subgroup (Kappa=0.329, p < 0.001).
[Impact of different HER2+ classes to survivial]
After a median follow-up of 49.6 months, survival analysis showed that patients who received pCR had significantly better disease-free survival (DFS) rate than those who did not (Log-rank p=0.0003). There was a trend toward a longer DFS in patients with HER2 IHC 3+ than in HER2 2+ISH+ patients (the estimated 5-year DFS: 89.6% vs. 83.8%; estimated 10-year DFS: 89.6% vs. 67.1%), wheras with no statistic significance (p=0.354).

Conclusion: In HER2+ patients, HER2 2+ISH+ subgroup showed a poor response to NST. So we consider it to be a distinct subtype and define it as the HER2-weak subgroup. In HER2-weak subgroup, receiving dual anti-HER2 therapy did not exert significant improvement in pCR rate compared to single anti-HER2 therapy, especially in HR+ patients. Therefore, further optimize and individualize NST regimen is needed for HER2-weak patients, among which ADC drugs and neoadjuvant endocrine therapy are potential optimization agents. Additionally, considering the increasing HER2 protein expression loss in HER2-weak group, a secondary detection to HER2 IHC and after NST seems necessary. Furthermore, researches with large sample sizes and long time follow-up are still in need to confirm the impact of different HER2+ classes to survivial.

P3-11-15: De-escalating neoadjuvant chemotherapy in early HER2+ breast cancer: a retrospective case series

Patricia Avancena, Sabina Hajiyeva, Elena Katz, Sandy Ching, Sharon Rosenbaum-Smith, Janet Yeh, Paul Baron, Francisco J Esteva

Purpose: In HER2-positive breast cancer (HER2+ BC), neoadjuvant chemotherapy (NACT) with dual HER2-targeted therapy achieves high pathologic complete response (pCR) rates. One of the most used regimens is docetaxel, carboplatin, trastuzumab, and pertuzumab (TCHP). Several studies have shown that a shorter weekly paclitaxel and carboplatin (wPC) regimen can be as effective as the standard TCHP, with less toxicity. Here, we report our de-escalation experience using neoadjuvant wPCHP in early HER2+ BC. Methods: This retrospective review included patients diagnosed with non-metastatic HER2+ breast cancer who completed HER2-targeted neoadjuvant systemic therapy and underwent surgery at Lenox Hill Hospital between January 1, 2023, and May 2024. The clinical stage was calculated based on tumor size and nodal status. The received operative treatment was recorded to determine the pathologic stage and chemotherapy response. The chemotherapy

backbone consisted of weekly paclitaxel 80 mg/m^2 x 12, with or without weekly carboplatin AUC2. All patients received pertuzumab and trastuzumab hyaluronidase (Phesgo) subcutaneously every three weeks before surgery. The study endpoint, pCR, was assessed after definitive surgery. Residual cancer burden (RCB) was calculated using the method validated by I-SPY investigators. pCR was defined as no evidence of invasive disease in the breast or lymph nodes (RCB 0). Dose modifications and post-op therapy were at the physician's discretion.

Results: In patients treated with neoadjuvant wTHP (n=9), the pCR rate was 44%; 56% achieved pCR or RCB1, indicating a good prognosis. Within this RCB 0-1 subset, the pCR rate was 75% in patients with hormone receptor-negative BC. In the cohort treated with neoadjuvant wPCHP (n=21), the pCR rate was 62%; 67% scored in the RCB 0-1 range; of this subset, the pCR rate was 87% in patients with hormone receptor-negative disease. No patients transitioned to an anthracycline-based regimen for non-response. There were no episodes of febrile neutropenia or grade ≥3 peripheral neuropathy. The most common toxicities were neutropenia and grade 1-2 peripheral neuropathy. No patients developed heart failure. Postoperative treatment was based on pathological response, following national guidelines.

Conclusion: pCR rates with wPCHP are as high as those reported with the docetaxel-based TCHP regimen, with fewer grade ≥3 toxicities. A subset of patients, particularly those with stage I and ER/PR-negative disease, may not need carboplatin. The wPCHP regimen should be considered an alternative to standard TCHP as neoadjuvant therapy for HER2+ BC.

P3-11-16: Personalized Neoadjuvant Therapy Guided by Drug Screening of Patient-Derived Tumor-Like Cell Clusters in HER2-Positive Breast Cancer: A Prospective Phase II Study and Exploratory Analysis

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Background: HER2-positive breast cancer exhibits unique characteristics that can significantly influence patients' response to neoadjuvant therapy. There is a critical need for a platform that facilitates in vitro drug screening and tailors personalized treatment regimens for such patients. Previously, we introduced an in vitro model named 'patient-derived tumor-like cell clusters' (PTCs), a cell cluster including tumor cells, mesenchymal cells, and lymphocytes, which accurately replicates the structural, functional, and micro-environmental features of the original tumors. Our retrospective research has shown that PTCs could serve as a reliable preclinical model for drug screening, and thereby, to guide neoadjuvant therapy strategies for patients with breast cancer. This prospective, phase II, open-label study (ClinicalTrials.gov No. NCT04750122) aims to prospectively investigate efficacy of personalized neoadjuvant therapy guided by drug sensitivity profiles of PTCs in HER2-positive breast cancer and to reveal biomarkers associated with resistance to anti-HER2 targeted therapies.

Methods: Tissues were obtained by core needle biopsy of candidate patients and cultured to generate PTCs, which were subsequently subjected to drug sensitivity testing and bulk RNA sequencing. Patients with HER2-positive breast cancer were assigned to neoadjuvant therapy according to their individual drug sensitivity profiles. The treatment regimens included THP (taxanes, trastuzumab, and pertuzumab), TCbHP (taxanes, carboplatin, trastuzumab, and pertuzumab), or treatment of physician's choice (TPC). After completing neoadjuvant therapy, surgery was performed to evaluate the pCR rates of PTC-guided treatment (PGT) and TPC. For the PTCs subjected to RNA-seq, drug sensitivity testing was conducted using H, P, trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-Dxd), respectively. The gene expression data was then analyzed to identify potential biomarkers by differentially-expressed-gene analysis and to establish a predictive model for therapeutic efficacy by logistic regression analysis.

Results: From April 2021 to July 2023, a total of 729 samples were collected by core needle biopsy from candidate patients, among whom 592 patients were diagnosed as invasive breast cancer. The success rate of generating PTCs through invasive breast cancer was 83.4%. Overall, 24 patients with HER2+ breast cancer that successfully constructed PTCs and drug sensitivity tests were enrolled in this study. Nineteen patients (79.2%) had T2-3 tumors and nineteen (79.2%) had node-positive disease. The in vitro drug sensitivity profiles suggested that 6 patients (25%) were sensitive to THP, 14 (58.3%) were sensitive to TCbHP, and 4 (16.7%) were resistant to both. Following neoadjuvant treatment, the patients that received THP treatment achieved a pCR rate of 83.3%, while the patients that were sensitive to TCbHP rather than THP achieved a pCR rate of 78.6%. Compared to only 1 out of the 4 patients with resistant PTCs achieved pCR, 16 of the 20 patients receiving PGT achieved pCR (80.0% vs 25.0%, p=0.027). In subgroup analyses of the PGT counterparts, the pCR rates were 62.5% for HR+/HER2+ patients and 91.7% for HR-/HER2+ patients. Besides, 14 PTC samples carried on bulk RNA sequencing. As a result, 5 co-upregulated genes (PLBD1, BMP3, HLA-DRB5, IGLV2-14, TIAF1) and 5 co-downregulated genes (EPN3, KCNK15, ACSF2, AC025048.2, PIP4K2B) were identified in the resistant group compared with the sensitive group. The multidrug resistance signature was utilized to establish a predictive model, of which the area under curves were 100.0% for predicting response to H, P, T-DM1, and T-Dxd.

Conclusion: The in vitro drug screening PTC platform could serve as a promising approach for tailoring personalized neoadjuvant treatment strategies for HER2-positive breast cancer. The identification of multidrug resistance signature emerges as a practical and effective biomarker for forecasting the response to anti-HER2 targeted therapies.

P3-11-17: A Randomized Phase II Trial of Neo-adjuvant Chemotherapy with Metformin or Placebo for HER-2 Positive Operable Breast Cancer (The HERMET Trial)

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Background: Human epidermal growth factor receptor 2 (HER-2) is overexpressed in 15-20% of breast cancer (BC). Pathologic complete response (pCR) after HER-2 directed chemotherapy is an important surrogate marker for survival in HER-2+ operable BC. Patients with diabetes who receive metformin have a lower risk of metastasis in HER-2+ breast cancer. Metformin's inhibition of PI3K/AKT/mTOR pathway through IGF receptor inhibition has been proposed as a possible mechanism. However, its clinical benefit as an anticancer agent is not clear. We conducted a randomized, placebo-controlled study to assess the impact of adding metformin or placebo to neoadjuvant docetaxel, carboplatin, trastuzumab and pertuzumab (TCH-P) in HER2 positive breast cancer.

Methods: Patients with HER-2+ operable breast cancer with at least 2 cm breast primary and/or axillary lymph node positive disease were eligible. HER-2 positivity was defined by the current ASCO-CAP guidelines. Patients were randomized 1:1 to receive neoadjuvant TCH-P every 3 weeks for 6 cycles, plus placebo (N=30) or metformin (N=31). Metformin dose was ramped up over the first 3 cycles to a target of 1500 mg twice daily on days 8-21 of each cycle. Breast surgery was performed 3-6 weeks after final chemotherapy cycle. The primary endpoint was pCR in breast and axilla. Subjects with unevaluable pCR were treated as non-responders for intent-to-treat analyses.

Results: 61 patients were enrolled between 8/17/2017 and 03/21/2023. Median age was 51 years. 51% of the patients had node positive disease and 67% had estrogen receptor (ER) positive breast cancer. Among all patients pCR in both breast and axilla was 43.3% in TCH-P plus placebo group and 54.8% in the metformin group (p= 0.37). In patients with ER+ breast cancer pCR was 42.9% in placebo group and 40.0% in metformin group (p=0.85).
Grade ≥3 adverse events occurred in 40% of placebo subjects and 48% of metformin subjects. Grade 3 diarrhea occurred in 29% of the patients in the metformin group compared to 17% in the placebo group.

Conclusions: Metformin added to TCH-P neoadjuvant chemotherapy does not significantly improve pCR rate in HER-2+ breast cancer. Grade ≥3 diarrhea was greater in the metformin group.

P3-11-18: Prognostic significance of lobular versus ductal histology in HER2-positive breast cancer

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Background: Lobular HER2-positive (HER2+) breast cancer is a rare entity. As such, oncologic outcomes as compared to HER2+ breast cancer with ductal histology are not well-defined.

Methods: We identified all non-metastatic HER2+ breast cancers treated with surgery in the province of Alberta, Canada from 2010-2017. We excluded those who had no exposure to trastuzumab. We compared clinicopathologic characteristics by histologic type, categorized as lobular, mixed ductal/lobular or ductal. We used Kaplan Meier methods and the log-rank test to compare recurrence-free survival (RFS) by histologic type. Multivariable Cox proportional hazards analyses were performed to evaluate the association between histology and RFS, adjusting for age, grade, ER status, pT stage and pN stage.

Results: Among 1515 HER2+ breast cancer patients, 74 (4.9%) had lobular or mixed ductal/lobular histology (34 pure lobular and 40 mixed) and 1441 (95.1%) had ductal histology. Patients with lobular/mixed histology were older (median age 58.2 vs. 52.9, $p<0.001$) and had higher proportions of grade 1-2 disease (52.8% vs. 22.1%, $p<0.001$), ER-positive disease (95.9% vs. 74.3%, $p<0.001$) and pT3 disease (21.6% vs. 7.1%, $p<0.001$). Among 398 (26.3%) patients treated with neoadjuvant chemotherapy, the rate of pathologic complete response was 10.0% for lobular/mixed histology vs. 43.9% for ductal ($p=0.04$). At median follow-up of 4 years, there were 187 recurrences, 92 deaths with recurrence and 20 deaths without recurrence. 5-year RFS was 94.4% (95%CI: 66.6-99.2%) for lobular histology, 86.1% (95%CI: 66.7-94.6%) for mixed ductal/lobular histology and 87.0% (95%CI: 84.4-89.1%) for ductal histology ($p=0.75$) (Figure). In both unadjusted and adjusted analyses, neither lobular (adjusted HR 1.05, $p=0.93$) nor mixed histology (adjusted HR 0.71, $p=0.46$) were associated with worse RFS as compared to ductal histology. pT2-4 (vs. pT1) disease and pN2-3 (vs. pN0) disease were associated with worse RFS (all $p<0.01$). Results were unchanged in subgroup analysis restricted to ER-positive disease.

Conclusion: While lobular cancers are known to be less sensitive to cytotoxic chemotherapy, our data supports that for HER2+ breast cancer treated with HER2-directed antibody therapy, lobular or mixed ductal/lobular histology is not a poor prognostic factor.

P3-11-19: Efficacy of different neoadjuvant systemic treatment regimens in Chinese patients with HER2-positive advanced breast cancer: a real-world retrospective multi-center cohort study

Xiaoming Zha, Weiwei Zhang, Jue Wang

Background: Neoadjuvant systemic treatment (NST) is often used to treat inoperable locally advanced breast cancer. For human epidermal growth factor receptor 2 (HER2) positive patients, we usually choose chemotherapy combined with targeted therapy, such as epirubicin/cyclophosphamide followed by taxanes/trastuzumab (EC-TH), epirubicin/cyclophosphamide followed by taxanes/trastuzumab/pertuzumab (EC-THP), taxanes/trastuzumab/pertuzumab followed by epirubicin/cyclophosphamide (THP-EC), and taxanes/carboplatin/trastuzumab/pertuzumab (TCbHP).

Methods: The study aims to design a real-world study to retrospectively evaluate the effects of different regimens on the efficacy of NST with HER2-positive disease. The efficacy is further subdivided into total pathological complete response (tpCR), breast pathological complete response (bpCR), and axillary pathological complete response (apCR) for more detailed analysis.

Result: A total of 505 patients from 5 centers were included in this study from May 2014 to April 2022. In terms of tpCR and bpCR, the THP-EC regimen is superior to the EC-TH regimen ($p=0.046$ and 0.037), but there is no difference in efficacy compared to EC-THP and TCbHP (all $p>0.05$). The selection of four regimens has no effect on apCR. Patients with HER2 3+ in immunohistochemistry (IHC) are more likely to achieve pCR (tpCR, bpCR, and apCR) than those with HER2 2+ in IHC and HER2 amplification. Regarding the selection of taxanes for target therapy, in this study, liposomal paclitaxel, docetaxel, nanoparticle albumin-bound paclitaxel, and solvent-based paclitaxel have no significant difference in the efficacy of NST.

Conclusion: EC-THP, THP-EC, and TCbHP regimens are superior to the EC-TH regimen in NST with HER2-positive disease. The selection of chemotherapy regimens, medication sequence, and choice of taxanes have no effect on the efficacy of NST. The higher the expression of HER2, the more benefits it can benefit from targeted therapy.

P3-11-20: 2018 and 2024 surveys of clinical investigator (CI) use of postoperative systemic therapy after prior neoadjuvant treatment of HER2-positive breast cancer (HER2+BC)

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Background: The presentation in December 2018 of the KATHERINE trial data demonstrated the marked benefit of TDM-1 in patients with HER2+BC and residual disease at surgery after neoadjuvant treatment. Prior to that in 2017 the FDA approved "postadjuvant" neratinib (N) based on the ExteNET trial demonstrating significant benefit, particularly in patients with HER2+/ER+ disease. We were interested in how these data affected CI clinical practice and the potential benefits of treatment CIs were communicating to patients.

Methods: To evaluate not only how treatment practice patterns have changed during this time, but also the estimated impact on risk of recurrence, we surveyed 28 clinical investigators from multiple U.S. institutions shortly after the KATHERINE presentation in 2018 and in April 2024 surveyed 24 investigators, 14 of whom were 2018 participants. We asked respondents to provide not only their preferred management approaches but also what "numbers" they would provide to interested patients about the absolute impact of both adjuvant and "postadjuvant" treatment. Key variables in these HER2+ scenarios were ER/PR- and residual disease status.

Results: For scenarios with postneoadjuvant pathologic complete response (pCR), in 2018 there was heterogeneity in terms of whether to add pertuzumab (P) to trastuzumab (T). However, by 2024 TP had become standard. The use of N in the pCR setting was minimal at both time points. For patients with residual disease, in 2018 TP or T was used but by 2024, now years past the initial KATHERINE data, TDM-1 had become standard for patients with both minimal and more extensive residual disease. In terms of postadjuvant N, CIs were divided on whether to use N, with more use with greater residual disease and much greater use in patients with ER+ disease that was somewhat increased in 2024 compared to 2018. Interestingly, of the 285 treatment decisions related to postadjuvant N in the survey, in total there were 114 instances in which CIs believed a DFS advantage existed for N, but the agent was not recommended. Of these, 41 were situations where the perceived benefit was 1%, 37 where it was 2% and 17 situations in which a predicted benefit of 5% or greater was believed to exist but treatment was not given.
Of related interest, the predicted disease-free survival of the subsets in this survey shifted significantly upward in patients with residual disease, but was similar in 2018 and 2024 in scenarios of pCR.

Conclusions: Neoadjuvant treatment of localized HER2+BC has been utilized extensively for many years, but the postoperative management of the disease has shifted significantly since 2018 particularly for patients with residual disease, because TDM-1 became an almost instant standard when KATHERINE was presented. The use of postadjuvant neratinib — while increasingly utilized, particularly for patients with "triple-positive" disease — is more heterogenous and a number of CIs don't use this form of therapy despite their recognition that it likely improves outcomes. Additional efforts should be undertaken to understand

why some individuals decline to recommend a therapy with perceived benefits and one that is employed by many of their colleagues, and how factors such as treatment-related toxicities and shared decision-making factor into this decision.

P3-11-21: Sociodemographic Factors Associated with Use of Adjuvant Therapy in Stage I Human Epidermal Growth Factor Receptor 2 (HER2)-Positive Breast Cancer

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Background: Approximately 40% of new HER2-positive (HER2+) breast cancer diagnoses are classified as Stage I. Extensive retrospective studies have failed to demonstrate a substantial improvement in breast cancer outcomes and survival rates for those with Stage I HER2+ breast cancer, irrespective of whether adjuvant therapy is administered. Large scale retrospective studies have demonstrated that even without adjuvant therapy, this patient population has a very favorable rate of 5-year breast cancer-specific survival ranging from 91% to 99%, particularly in those with T1a/b lesions. Despite this, many still receive adjuvant therapy. We investigated both clinical and sociodemographic factors associated with use of adjuvant HER2-targeted therapy with/without systemic chemotherapy in patients with Stage I HER2+ breast cancer.

Methods: This observational study used the Oncoshare database, which integrates electronic medical record and California Cancer Registry data for patients treated in Northern California in the Stanford University Health Care Alliance. We included patients treated for Stage I HER2+ breast cancer from 2000-2024. Descriptive statistics are reported, and a multivariate analysis is forthcoming.

Results: The study population included 512 patients with T1aN0/T1bN0 disease and 515 with T1cN0 disease. Approximately 68% of patients with pT1cN0 HER2+ breast cancer and 56% of patients with T1a or T1b disease received adjuvant therapy. We have collected extensive data on sociodemographic factors, including age, race and ethnicity, neighborhood socioeconomic status (reported as statewide quintiles), distance traveled for care, rurality, Charlson comorbidity scores, and insurance status.

Initial analysis was completed to determine the standardized mean differences between those who received adjuvant systemic therapy and those who did not. For those with T1aN0/T1bN0 HER2+ breast cancers, we found moderate differences between those who received adjuvant therapy and those who did not based on insurance type (SMD 0.416) and grade of the tumor (SMD 0.472). Of those who received adjuvant therapy, 54.5% had private insurance, whereas only 25.5% had government-based insurance. However, in those patients who did not receive adjuvant systemic therapy, 44.2% of patients had government-based insurance and 38.5% had private insurance. Patients with grade II and III tumors made up 75.9% of patients who received adjuvant therapy, whereas only 6.6% of

patients with grade I tumors received adjuvant therapy.
For T1cN0 HER2+ breast cancers, we found moderate differences in receipt of adjuvant therapy between insurance type (SMD 0.442), age (SMD 0.626), and grade of the tumor (SMD 0.424). Those with government-based insurance represented the highest number of patients who did not receive adjuvant therapy (51.8%), whereas those with private insurance represented the highest number of patients who did received adjuvant therapy (49.9%). In patients under 50, 84% received adjuvant therapy, whereas only 45% of those over 70 received adjuvant therapy. Of those patients who received adjuvant therapy, only 3.7% had a grade I tumor, whereas 40.4% and 55.6% had grade II and III tumors, respectively.

Conclusions: Despite excellent outcomes in patients with T1N0 HER2+ breast cancer regardless of receipt of adjuvant therapy, 62% of patients received adjuvant systemic therapy. Initial data shows that there are differences in the clinical/sociodemographic factors between the patients receiving adjuvant systemic therapy and those who are not. Although it is expected that younger patients and those with high grade tumors have higher adjuvant systemic therapy use, it was interesting to see that patients with government-based insurance received less adjuvant therapy than those with private insurance. Forthcoming multivariate analysis will help us better understand the association between sociodemographic factors and receipt of adjuvant therapy.

P3-11-22: Extended adjuvant neratinib in HER2+/HR+ early breast cancer (eBC) in clinical routine – interim analysis of the multinational, prospective, non-interventional study ELEANOR (n=300)

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Background: Despite modern human epidermal growth factor receptor (HER2)-directed treatment options, relevant recurrence risk persists in patients (pts) with extensive local disease and/or lack of pathologic complete response (pCR) to neoadjuvant therapy. Neratinib is approved in Europe for extended adjuvant therapy in adult pts with HER2+/hormone receptor positive (HR+) eBC who completed adjuvant trastuzumab-based therapy less than one year ago. In this population, the ExteNET study demonstrated an absolute 5.1% improvement in 5-year invasive disease-free survival (iDFS) with neratinib compared to placebo (90.8% vs. 85.7%; hazard ratio 0.58 [95% confidence interval (CI), 0.41-0.82]). Exploratory post-hoc analyses demonstrated a more pronounced benefit in non-pCR pts and/or in those completing neratinib therapy. Diarrhea was the most common adverse event (AE) in the neratinib arm (39% grade 3 without primary diarrhea

prophylaxis). ELEANOR is the first non-interventional study of the real-world use and management of neratinib given after the current adjuvant standard of care in pts with HER2+/HR+ eBC in Germany, Austria, and Switzerland.

Methods: A total of 300 pts diagnosed with HER2+/HR+ eBC were planned to be enrolled in accordance with the EMA/Swissmedic product specifications. The primary objective is to assess patient adherence to neratinib (i.e., ≥75% of prescribed treatment days). Secondary objectives focus on patient and tumor characteristics, pretreatment and neratinib treatment details, effectiveness, safety, and health-related quality of life. Results of the preplanned interim analysis based on 300 enrolled pts observed for a minimum of three months are reported.

Results: At the data cut-off on 5th August 2023, 286 pts qualified for the main analysis set and 288 pts for the safety analysis set, respectively. Median age at inclusion was 52.0 years, 73.4% (210/286) of pts were at increased risk of disease recurrence having AJCC stage > I or N+ or non-pCR after neoadjuvant treatment. Overall, 37.4% (107/286) and 31.8% (91/286) of pts received adjuvant/post-neoadjuvant trastuzumab monotherapy or trastuzumab/pertuzumab, respectively, and 22.4% (64/286) of pts received post-neoadjuvant trastuzumab-emtansine (T-DM1). In pts who had non-pCR following neoadjuvant treatment, 55.4% (62/112) received post-neoadjuvant T-DM1. Dose escalation strategy (neratinib starting dose <240 mg) was used in 44.4% (128/288) of pts, and 86.8% (250/288) of pts received diarrhea prophylaxis. AEs were consistent with the known safety profile of neratinib with incidence of grade ≥3 diarrhea by worst grade less common in pts starting on a lower dose of neratinib (15.6% vs. 23.8%).
At the time of this preplanned interim analysis with an estimated median observation time for DFS of 14.0 months, six pts (6/286, 2.1%) had experienced a relapse and one patient died since starting neratinib treatment, resulting in a 12-month DFS rate of 97.4% (95% CI, 94.6-98.7). At the end of neratinib treatment, physicians evaluated treatment satisfaction for 85 pts with effectiveness and for 88 pts with safety. Satisfaction rates were 88.2% for effectiveness and 86.4% for safety.

Conclusion: This preplanned interim analysis provides data on the use of neratinib in a contemporary cohort of predominantly higher-risk pts and after completion of pertuzumab- and T-DM1-containing adjuvant regimens in routine clinical practice. Preliminary effectiveness data appears to be consistent with previous data and the integration of treatment management strategies such as diarrhea prophylaxis and neratinib dose escalation were shown to enhance treatment tolerability.

P3-11-23: A multicentre UK Study of the impact of progesterone receptor status on pathological complete response rate and outcomes in 1037 patients with HER-2 positive, early breast cancer treated with neoadjuvant chemotherapy, trastuzumab and pertuzumab

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Background: Pathological complete response (pCR) rates are lower in ER positive, HER-2 positive early breast cancers (EBC) as compared to ER negative, HER2-positive disease when treated with neoadjuvant chemotherapy, trastuzumab and pertuzumab (T+P). To date, pivotal phase III neoadjuvant studies have not explored the impact of progesterone (PgR) status on pCR rates and outcomes. Given this, we explored the impact of PgR expression on pCR rates as well as clinical outcomes following neoadjuvant chemotherapy, and T+P in HER-2 positive EBC in patients treated at 4 cancer centres in the UK.

Methods: Patients with HER2-positive EBC, treated with neoadjuvant chemotherapy, and T+P, between 2013-2024 at 4 cancer centres in the UK were identified. Clinicopathological information including ER and PgR status taken from core biopsy, with positivity defined as Quick Score >2, histopathological response, disease relapse and survival outcomes were collected. Data cut off was 1st March 2024.

Results: 1037 patients were identified; 17 had bilateral disease making 1054 assessable tumours. Hormone receptor status was as follows: ER+, PgR+: 495 of 1054 (47%); ER+, PgR-: 158 of 1054 (15%); ER-, PgR+: 30 of 1054 (3%); ER-, PgR-: 297 of 1054 (28%); ER or PgR missing data: 74 of 1054 (7%). The overall pCR rate (ypT0/is, ypT0) was 52% (552/1054). pCR rates by ER status (regardless of PgR) were: ER+: 45% (293/653); ER-: 70% (229/327) (P < 0.0001). pCR rates by PgR status were: PgR+: 42% (221/524); PgR-: 66% (302/456) (P<0.0001). pCR rates by ER & PgR were: ER+, PgR+: 41% (204/495); ER+, PgR-: 56% (89/158); ER-/PgR+: 57% (17/30); ER-,PgR-: 71% (212/297). At a median follow-up of 34 months (95% CI 33-36) disease-free survival (DFS) and overall survival (OS), for the overall population was not reached. pCR was associated with improved DFS (HR 0.30, 95% CI 0.17-0.50) and OS (HR 0.29, 95% CI 0.13-0.62) in the overall patient group. In ER+ disease, regardless of PgR status, pCR was associated with improved DFS (HR 0.38, 95% CI 0.18-0.78) but not OS (HR 0.46, 95% CI 0.16-1.33). pCR in the ER- disease group, was associated with significantly improved DFS (HR 0.17 95% CI 0.07-0.38) and OS (HR 0.11, 95% CI 0.03-0.35). In PgR+ disease, achieving pCR lead to an improved DFS (DFS: HR 0.26, 95% CI 0.11-0.65) but not OS (HR 0.30, 0.08-1.09). In PgR- disease achieving pCR was associated with both improved DFS and OS (DFS: HR 0.29, 95% CI 0.15-0.59; OS: 0.22, 95% CI 0.08-0.58).
In ER+/ PgR+ disease, an association was found for DFS (HR 0.26, 95% CI 0.09-0.69) but not

for OS (HR 0.24, 95% CI 0.05-1.14). In ER+/ PgR- disease, pCR was not statistically significant for an improved DFS (HR 0.81, 95% CI 0.23-2.80) or OS (HR 1.22, 95% CI 0.20-7.34). pCR was significant for both DFS and OS in patients with ER-, PgR- disease (DFS: HR 0.18, 95% CI 0.07-0.42; OS: HR 0.10, 95% CI 0.02-0.36).
An updated analysis with data from an additional 1 centre will be presented as well as updated outcomes.

Discussion: In this multicentred, real-world study achieving pCR was associated with ER and PgR status, with higher rates in ER-/PgR- & ER+/PgR- cases versus ER+/PgR+ cases. DFS and OS in the overall population was associated with pCR, however this did not hold for all hormone receptor subgroups. Whilst an association was found for achieving pCR and DFS in the ER+/PgR+ patient group, only in ER-/PgR- disease was pCR significant for both DFS and OS. These data indicate that in ER+ HER2+ EBC PgR status can influence pCR, and survival outcomes.

P3-11-24: Neoadjuvant Nab-paclitaxel combined with Trastuzumab and Pyrotinib for HER2-enriched subtype of HER2-positive early or locally advanced breast cancer: A multicenter, single-arm, phase 2 trial

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Background: Target therapies significantly improved the pathological complete response rate (pCR) of human epidermal growth factor receptor 2 (HER2) positive breast cancer. A tyrosine kinase inhibitor (TKI) plus trastuzumab-based regimen showed inconsistent efficacy in neoadjuvant therapy. As we know, HER2-positive breast cancer consists of four intrinsic molecular subtypes of luminal A, luminal B, HER2-enriched, and basal-like. This trial was aimed to evaluate the efficacy of chemotherapy de-escalated neoadjuvant regimen of pyrotinib, trastuzumab and nab-paclitaxel in HER2-enriched subtype of HER2-positive early breast cancer.

Methods: This multicenter, single-arm, phase 2 trial was conducted in nine hospitals in China (ClinicalTrials.gov, NCT05659056). Simon's two-stage design was adopted, and 65 eligible patients with HER2-positive, stage IIA-IIIC invasive breast cancer regardless of hormone receptor status were scheduled to be enrolled. Patients received pyrotinib (400mg, continuous orally), trastuzumab (loading dose of 8 mg/kg, followed by 6 mg/kg every 3 weeks intravenously), and nab-paclitaxel (260mg/m2 every 3 weeks intravenously) for six cycles. The primary endpoint was the total pCR (tpCR, ypT0/Tis and ypN0) of HER2-enriched subtype patients. Intrinsic molecular subtypes of tumor biopsy samples taken at baseline were determined via the BluePrint predictor.
Findings: In the first stage, 21 patients were enrolled and tpCR exceeding 35%, so between

Nov 22, 2022, and Jul 1, 2024, we recruited 65 patients, of whom 52 (80%) completed neoadjuvant therapy and received surgery as planned, and BluePrint results were available in 50 of them. Of the 50 patients, 32 (64%) were HER2-enriched subtype, 2 (4%) were luminal A, 9 (18%) were luminal B, and 7 (14%) were basal-like. Twenty-two (42.3%, 95% CI 29.0%-56.7%) of 52 patients had tpCR at the time of surgery. Eighteen (56.3%, 95% CI 37.9%-73.2%) of 32 patients with the HER2-enriched subtype and 4 (22.2%, 95%CI 7.3%-48.1%) of 18 patients with non-HER2-enriched subtypes achieved tpCR. Forty-five (86.5%, 95% CI 0.74-0.94) of 52 patients achieved objective response. The treatment related any grade adverse events was 91.2%, and 6 patients changed treatment regimen due to adverse events. And the most common grade 3 or 4 adverse events were diarrhea (12 [21.1%]), and neutrophil count decreased (3 [5.3%]).

Conclusion: The trial reached the pre-specified tpCR, had met the primary end point. Neoadjuvant pyrotinib, trastuzumab and nab-paclitaxel reached a high tpCR rate, with manageable toxicity, for patients with HER2-enriched subtype of HER2-positive breast cancer. Chemotherapy can be de-escalated in patients with HER2-enriched subtype breast cancer with BluePrint intrinsic subtyping.

P3-11-25: Use of the MammaTyper® Platform to Reclassify Breast Tumors by the Expression of HER2

Serafin Morales, Ana Velasco, Izaskun Rdanibia, Ariadna Gasol, Noemi Tuset

The molecular classification of breast cancer is essential for selecting the appropriate treatment. Most of the time, this classification is carried out by the immunohistochemical (IHC) status of hormonal receptors (estrogen and progesterone), HER2 protein and the Ki67 proliferation index.
MammaTyper® is a gene expression assay based on RT-qPCR, developed to provide enhanced sensitivity in testing these markers by determining the levels of their messenger RNA.
We analyzed the concordance between immunohistochemistry and MammaTyper® in the expression of hormonal receptors (estrogen and progesterone), Ki67, and HER2, with a particular focus on HER2 expression.
We examined a cohort of 30 cases, of which 27 were classified as HER2 +2. Among these, 19 were HER2 positive by FISH, 6 were luminal, and 5 were triple-negative. We found concordance in the expression of estrogen receptors (p: 0.001), with 83% agreement for negative receptors (10 out of 12) and 90% agreement for positive receptors.
Of the 19 cases considered HER2 positive by FISH, only 7 were confirmed by MammaTyper®, 15 were HER2 low and 5 ultralow. Additionally, none of the 11 HER2 negative cases were positive by MammaTyper®.
MammaTyper®reclassifies cases considered HER2 +2 by IHC and positive by FISH, identifying 36% (7 out of 19) as HER2 positive and 42% (8 out of 19) as HER2 low. Given

the critical role of accurately quantifying HER2 expression for selecting appropriate anti-HER2 treatments, this technique should be considered.

P3-11-26: A Phase II study of dalpiciclib combined with letrozole, pertuzumab and trastuzumab as neoadjuvant therapy in HR+/HER2+ breast cancer

Xinrui Liang, Ningning Zhang, Jing Wu, Xiaohua Zeng

Background: HR+/HER2+ breast cancer is a unique subtype of breast cancer. Between ER and HER2 crosstalk pathways may affect the sensitivity to both HER2-targeted and endocrine therapy in patients with HR+/HER2+ breast cancer. Current therapies for HER2-positive breast cancer have demonstrated limited efficacy in patients with HR+/HER2+ breast cancer. This Phase II trial aimed to assess the efficacy and safety of a neoadjuvant regimen combining trastuzumab, pertuzumab, dalpiciclib (a cyclin dependent kinase 4/6 inhibitor) and letrozole in patients with HR+/HER2+ breast cancer, alongside the exploration of efficacy biomarkers.

Methods: Patients were administered six 28-day cycles of dalpiciclib (150 mg once daily on days 1-21) and letrozole (2.5 mg once daily), plus six 21-day cycles of trastuzumab (8 mg/kg loading dose followed by 6 mg/kg maintenance dose on day 1), pertuzumab (840mg loading dose followed by 420mg maintenance dose on day 1), prior to surgery. The primary endpoint was total pathological complete response (tpCR, ypT0/is, ypN0) rate. Secondary endpoints were objective response rate (ORR), breast pathological complete response (bpCR; ypT0/is) rate, change in Ki-67 level from baseline to one cycle of treatment and to surgery, and safety. For biomarkers analysis, next-generation sequencing was performed on pre-treatment tissue and circulating tumor DNA (ctDNA) at various time points before surgery.

Results: A total of 29 patients have been enrolled from July 12, 2022 to July 4, 2024. Among these patients, 19 patients had completed the neoadjuvant treatment and surgery. The median age of these 19 patients was 52 years, with 57.9% being postmenopausal. Stage II and III accounted for 52.6% and 47.4%, respectively. Additionally, 89.5% of the patients had lymph node metastasis. The tpCR and bpCR rate were 42.1% (8 out of 19) and 52.6% (10 out of 19), respectively. The ORR was 84.2 %. Significant reductions in Ki-67 levels were noted after both one ($P < 0.05$) and six cycles of neoadjuvant therapy ($P < 0.05$). Grade 4 neutropenia was reported in one case, with no fatalities. The most common grade 3 adverse events were neutropenia (10 [71.4%]) and leukopenia (8 [57.1%]). NGS identified frequent mutations in TP53 (73.7%), PIK3CA (47.4%), and ARID1A (21.1%). Cell cycle pathway gene alterations correlated with a higher pCR rate (80% vs 25%, P=0.101). Ten (55.6%) patients had positive ctDNA at baseline, and ctDNA positivity decreased significantly post-neoadjuvant treatment ($P < 0.05$). Patients with positive ctDNA at baseline without

clearance post-treatment exhibited a trend towards a lower pCR rate.

Conclusions: This combination neoadjuvant regimen of dalpiciclib, letrozole, pertuzumab and trastuzumab demonstrated promising pathological responses and manageable safety in patients with HR+/HER2+ breast cancer. The predictive value of genetic alterations and ctDNA status for treatment efficacy warrants further investigation.

P3-11-27: Pathologic Complete Response and Residual Cancer Burden as Powerful Prognostic Markers in HER2-Positive Early Breast Cancer: A Comprehensive Real-World Study

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Background: Pathologic complete response (pCR) following neoadjuvant therapy in HER2-positive early breast cancer (eBC) is recognized as a significant prognostic marker. This study aims to evaluate the impact of pCR and residual cancer burden (RCB) on survival outcomes in a real-world cohort of women with HER2-positive eBC.
Methods: A retrospective review was conducted on 385 newly diagnosed women with HER2-positive eBC who underwent neoadjuvant therapy between June 2016 and December 2022. Clinicopathological data were collected, including age, menopausal status, tumor grade, stage, ER/PR status, and neoadjuvant treatment regimens. Overall survival (OS) and event-free survival (EFS) were analyzed using Kaplan-Meier estimates, stratified by pCR and RCB status. Prognostic factors were identified using Cox proportional hazards modeling.

Results: Among the 385 patients, 55.1% achieved pCR. Those treated with trastuzumab plus pertuzumab exhibited a higher pCR rate (69%) compared to trastuzumab alone (53.8%). Five-year OS was 82.4%. Patients who achieved pCR had significantly improved OS (HR: 0.373, p = 0.005), while those who did not achieve pCR had a lower OS (HR: 2.676). Three-year EFS was 88.9%. Although not statistically significant, patients achieving pCR had better EFS (HR: 0.56, p = 0.087). Residual cancer burden analysis showed that patients with minimal residual disease (RCB-0) had significantly improved OS (HR: 0.178, p = 0.003) and EFS (HR: 0.300, p = 0.023) compared to those with higher RCB scores. When stratified by hormonal receptor status, hormone receptor-negative (HR-) patients who achieved pCR had improved OS (HR: 0.366, p = 0.006) and EFS (HR: 0.302, p = 0.017) compared to those who did not. Similar trends were noted in hormone receptor-positive (HR+) patients.

Conclusion: This real-world study demonstrates that achieving pathologic complete response (pCR) and having minimal residual cancer burden (RCB-0) are strongly associated

with improved survival outcomes in HER2-positive early breast cancer. These findings underscore the critical importance of pCR and RCB as prognostic markers in guiding neoadjuvant therapy and optimizing treatment strategies.

P3-11-28: HER2+ Breast Cancer: Loss of HER2 and Disease Prognosis After Neoadjuvant Treatment - A Pertuzumab effect?

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Introduction: HER2 overexpression/amplification occurs in 15-20% of breast cancers (BC) and is associated with worse prognosis. The addition of anti-HER2 treatment to neoadjuvant chemotherapy significantly improves pathological complete response (pCR) rate. Changes in HER2 status after neoadjuvant treatment (NAT) may affect prognosis (Mittendorf EA, Cancer Res 2009; Guarneri V Ann Oncol 2013). Our group previously studied this topic in the era of trastuzumab (T) single treatment, finding that patients (pts) who did not achieve a pCR and had loss of HER2 expression/amplification during NAT had worse disease-free (DFS) and overall survival (OS) (Branco FP, Am J Transl Res 2019). The present study aimed to confirm these findings in a more recent and larger cohort.

Methods: A retrospective chart review and pathologic evaluation was conducted on all consecutive pts with HER2+ BC (defined as IHC 3+ or IHC 2+ with amplification by ISH) treated with NAT between Jan 2015 and Dec 2017 at 8 Portuguese hospitals.

Results: 304 female pts were included, with a median age of 42 years (range 24-82); 9 pts (3%) with stage I, 187 (62%) with stage II, 104 (34%) with stage III and 4 (1%) unknown. Hormone receptors were positive (>1% tumor cells) in 201 tumors (66%). The majority of pts (293, 96%) received NAT with anthracyclines and taxanes, while 11 (4%) received non-anthracycline NAT; all received anti-HER2 treatment with T and 250 (82%) also received pertuzumab (P) (dual blockade). pCR (ypT0/is ypN0) was achieved in 172 pts (57%), with increase in 5-year DFS (5yDFS) compared to pts with residual disease, 95% (SE 1.7%) versus 85% (SE 3.3%), respectively (p=0.02). With a median follow-up of 62 months, among the 132 pts (43%) with residual disease at surgery, in 79 (60%) the residual tumor remained HER2+, 32 (24%) lost HER2 overexpression/amplification, and 21 (16%) had insufficient residual tumor to allow HER2 determination. The 5yDFS was 79% (SE 4.9%) for pts with HER2+ residual tumors, 90% (SE 5.4%) for those who became HER2-negative, and 100% (SE 4.9%) for those with insufficient material. In the overall population, the 5yDFS was 79% (SE 5.5%) in pts who received neoadjuvant T alone and 93% (SE 1.6%) for T+P (p=0.004). Among pts with residual disease, the 5yDFS difference was even greater: 66% (SE 9.3%) with T versus 91% (SE 3.0%) with T+P (p=0.001). Among pts treated with T-only NAT whose tumors lost HER2, 2 out of 5 relapsed (5yDFS 60%, SE 21%) compared to 7 out

of 20 who remained HER2+ (5yDFS 64%, SE 11%). In contrast, pts treated with T+P NAT whose tumors lost HER2 only 1 out of 27 pts relapse (5yDFS 96%, SE 3.9%) compared to 8 of 59 with HER2+ residual tumors (5yDFS 85%, SE 4.9%). In multivariate survival analysis, among the subgroup of pts with residual disease, the inclusion of P in NAT was the only factor with a statistically significant impact on DFS (HR=0.21, p=0.005) after adjusting for age (above or below 50), estrogen receptor expression, grade, stage, dose-dense versus standard AC chemotherapy, and HER2 in residual tumor (positive or negative). A similar result was observed when stage was substituted by nodal status (positive or negative), with only P impacting DFS (HR=0.22, p=0.007).

Discussion: This real-world cohort study confirms the benefit of dual HER2 blockade in increasing pCR rate after NAT but also in improving 5yDFS. Contrary to our previous findings, loss of HER2 in residual tumor at surgery was not associated with worse DFS. The main difference between studies was the use of preop P. In this cohort, pts treated only with T maintained a trend towards worse prognosis with loss of HER2 overexpression/amplification, while the addition of P to NAT impacted positively the prognosis of HER2 BC despite HER2 loss. Pts with insufficient material in the surgical specimen for HER2 determination likely had a near pCR, which may explain why none relapsed. Future clinical trials in HER2+ BC should take into consideration HER2 status in the surgical specimen post NAT.

P3-11-29: Early change in lymphocyte count on neoadjuvant therapy (NAT) and treatment response in patients with HER2+ breast cancer (BC)

Colin Bergstrom, Ingrid Luo, Eran Kotler, Mina Satoyoshi, Candice Thompson, Christina Curtis, Summer Han, Jennifer L. Caswell-Jin

Introduction: While immune infiltration of the tumor after initiation of NAT has been shown to predict response in HER2+ BC, it is unknown if peripheral blood changes correlate with effective immune activation. This study aimed to evaluate the association between the change in lymphocyte count after the initial cycle of NAT and pathological complete response (pCR) among patients with HER2+ BC.

Methods: Patients with HER2+ BC who received NAT, had baseline absolute lymphocyte count (ALC) pre-therapy (within 90 days of diagnosis), and on-treatment (7-35 days after first dose) were identified from the Oncoshare database. Oncoshare merges data from the California Cancer Registry and electronic medical records of women diagnosed with BC between 2000-2022 from the Stanford Health Care Alliance, encompassing an academic hospital, a community hospital, and a community practice network. Data collected included demographic (age, race/ethnicity), laboratory (ALC values across therapy), clinical (stage, grade), and pathological (estrogen receptor (ER) and pCR status) variables. For the primary analysis, patients were excluded if the baseline ALC was < 0.5. ALC change was defined as

ALC after the first cycle of NAT divided by baseline ALC prior to NAT. Univariate (Mann Whitney U test) and multivariate (logistic regression) analyses between pCR and ALC change were performed. An exploratory analysis examined changes in the ALC trajectory across the entire treatment course between patients with and without pCR.

Results: The study included N=207 patients with HER2+ BC. The median diagnosis age was 51 (interquartile range[IQR] 43-61), 51% were non-Hispanic White, the majority had ER+ disease (69%) and received pertuzumab-based NAT (58%). pCR occurred in 47% of patients. One patient was excluded for sub-threshold baseline ALC (<0.5). Among all patients, the median ALC decreased from 1.8 (IQR 1.4-2.3) at baseline to 1.3 (IQR 1.0-1.9) on-treatment. Greater ALC reduction occurred in patients who ultimately achieved pCR: on-treatment ALC was 72% (SD 30%) of baseline in patients with pCR, as compared to 86% (SD 40%) in patients without (P=0.013). In multivariate analysis, ER status (P<0.001) and pertuzumab-based NAT (0.016) were associated with pCR, and a significant association persisted between pCR and ALC change (p=0.017) after adjusting for these factors. In the exploratory analysis, patients who underwent pCR not only experienced a greater early decline in their ALC, but a greater recovery toward the end of NAT.

Conclusion: In patients with HER2+ BC undergoing NAT, a significant association was found between an early ALC decline after first treatment and subsequent pCR. These results suggest that an effective immune response to NAT in HER2+ breast cancer may be detectable within a few weeks of initiation of therapy, opening new avenues to development of biomarkers of treatment response.

P3-11-30: Low Ki67 is associated with a lower pathological complete response rate after neoadjuvant therapy in HER2-positive breast cancer patients.

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Introduction: The expression of the Ki67 protein assessed by immunohistochemistry (IHC) can estimate the cell proliferation rate and, therefore, be used as a prognostic biomarker to stratify breast cancer patients who harbor a high risk of disease relapse and can benefit more from chemotherapy. However, the prognostic and predictive value of Ki67 for HER2-positive breast cancer is not clearly understood. This study aimed to test the relationship between Ki67 expression and the pathological response in HER2-positive breast cancer. Materials and Methods: We included 260 patients with HER2-positive breast cancer treated with neoadjuvant chemotherapy plus anti-HER2 therapy at A. C. Camargo Cancer Center, Brazil, from January 2010 to December 2022. Information regarding demographics,

comorbidities, immunohistochemical features, treatment, clinical staging, and toxicity was collected from patients' electronic medical records. Ki67 expression information was collected from pathological reports and categorized as <30% or >30%. Descriptive statistics were used to summarize data. Multivariate analysis using Cox's regression models was performed to integrate factors associated with pathological complete response(pCR).

Results: The number of stage II and III patients was 154 (58.3%) and 93 (35.2%), respectively. Histological grade II and III were observed in 136 (51.5%) and 121 (45.8%) tumors. Ki67 >30% was seen in 185 (70%) tumors, and 172 (65.2%) had hormonal receptor expression. The median follow-up was 41 months (37.13-44.87). One-hundred and forty-four (55.4%) patients achieved pCR. The Ki67 <30% was significantly associated with a lower rate of pCR, 33 patients (42.3%) compared to 111 patients (61%) in the group with Ki67>30% (p=0.002). On the multivariate analysis, after adjustment for the use of neoadjuvant trastuzumab plus pertuzumab, use of neoadjuvant anthracyclines, and HER2 score + 3 on IHC (vs. HER2 amplified by FISH/ISH), Ki67 remained as an independent factor associated with pCR.

Conclusion: Tumors with Ki67 <30 were associated with a lower rate of pCR after completing neoadjuvant treatment. Ki67 was kept as an independent predictor for pCR regardless of the use of neoadjuvant antracycline or trastuzumab plus pertuzumab, and HER2 score as evaluated by IHC. In the future, PAM 50 or another multigenic test may better fit these patients into different prognostic subgroups and help to de-escalate treatment.

P3-12-01: Eribulin in HER2-negative breast cancer. Results of real world data (RWD) in the era of precision oncology.

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Background: Eribulin is a standard of care for HER2-negative metastatic breast cancer (MBC) regardless of hormone receptor expression. It is approved for use after progression to anthracyclines and taxanes based on the phase 3 EMBRACE trial. Despite its widespread use, there is a paucity of real-world data (RWD).

Methods: Patients with HER2-negative MBC treated with eribulin at Virgen del Rocio and Insular de Gran Canaria Hospital (Spain) between January 2016 and December 2022 were reviewed. IBM SPSS Statistic 27.0.1.0 was used for statistics.

Results: 211 patients were identified and retrospectively followed up until March 2024. Median age at diagnosis was 62 years. 66.4% were luminal phenotype and 53.1% showed high Ki67 expression (>30%). 18.3% were diagnosed with metastatic disease at debut.

Prior to eribulin treatment, 85.1% of patients had visceral disease, 55% liver involvement and 11% brain metastases. 6.2% of patients received eribulin as 1st line, 17.1% as 2nd line, 27.5% as 3rd line and 49.3% as 4th line or later. Progression-free survival (PFS) with eribulin was 4.76 months (95% CI, 4.06-5.47) and overall survival (OS) 10.75 months (9.37-12.12), median follow-up of 12 months. PFS was 5.53 months (4.60-6.46) in the luminal phenotype and 3 months (2.67-3.74) in the triple negative population. OS was 11.43 (9.79-13.06) and 9.31 (6.79-11.82), respectively. Overall response rate was assessed in 160 patients and was 43.12%. Median survival in this subgroup was 7.6 months. The presence of visceral disease and more than three lines of treatment before eribulin seems to decrease survival but did not reach statistical significance. 33.49% required dose reductions of eribulin. Most common toxicities were asthenia (73.7%), neutropenia (45.5%) and nausea (27.3%).

Conclusions: In our population, the efficacy and toxicity of eribulin are consistent with published data. The RWD complements the results of clinical trials by providing information on the efficacy and safety of treatments under conditions of routine clinical practice. This is the first phase of a multi-center RWD on the use of eribulin in the context of personalized medicine after the incorporation of targeted therapies.

P3-12-02: Ribociclib in Combination with Aromatase Inhibitors or Fulvestrant in Hormone Receptor (HR)-Positive, HER2-Negative Premenopausal Women with Metastatic Breast Cancer. Real World Data from a Resource-Restricted Country

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Background: Cyclin-dependent kinase (CDK) 4/6 inhibitors when combined with aromatase inhibitors (AI) or fulvestrant have revolutionized the treatment landscape of hormone receptor (HR)-positive, human epidermal growth factor receptor-2 (HER2)-negative metastatic breast cancer (MBC). Here we report real-world clinical outcomes and toxicity data in premenopausal patients treated at a single tertiary cancer center in a resource-restricted country; patients of which were not well represented in clinical trials that led to the approval of these drugs.

Methods: This is a retrospective analysis of individual patients' data. All consecutive premenopausal women with HR-positive and HER2-negative MBC, treated at our institution with ribociclib plus AI or fulvestrant, between July 2017 and December 2023 were reviewed. Progression-free survival (PFS) was defined as the time from treatment initiation with CDK4/6 inhibitor (all with ribociclib) until the first documented progression, death

from any cause, or last follow-up, whichever occurred first. The Overall survival (OS) was defined as the time from treatment initiation with ribociclib until the date of death from any cause or last follow-up.

Results: A total of 164 patients, median age 43 (27-54) years were enrolled; almost one-third (n=52, 31.7%) were below the age of 40. All patients had metastatic disease; de novo (n=83, 51.6%), or recurrent following a presentation as early-stage (n=81, 49.4%). Although bone metastasis was reported in 100 (61.0%) patients, bone-only metastasis was identified in 87 (53.0%), while visceral metastasis was reported in 60 (36.6%), including the lung (n=23, 14.0%) and liver (n=40, 24.4%). Majority of the tumors were high grade; G3 (n=50, 31.2%) or G2 (n=102,63.8%). Ribociclib combined with AI were used in 147 (91.3%) patients and with fulvestrant in only 14 (8.7%). Dose reduction was required in 43 (26.2%) patients, mostly because of neutropenia (n=34, 20.7%) and abnormal liver enzymes (n=17, 10.4%), while 18 (11.0%) discontinued treatment or switched to another CDK4/6 inhibitors, including 3 (1.8%) due to cardiac toxicities. Patients were followed up for a median of 41.0 (range 27-54) months. Overall response rate (ORR) was 64.6% and median PFS, irrespective of the line of therapy, was 35.0 months (95% CI, 30.6-42.2). PFS was longer in those with bone-only metastasis; 48.1 (95% CI, 35.0-59.1) versus 33.3 (95% CI, 29.0-40.2) months for patients with visceral metastasis (HR 0.45; 95% CI 0.25-0.83; P=0.011.), and for those with de novo MBC; 42.1 months (95% CI, 37.1-55.2) vs. 29.0 months (95% CI, 25.4-35.9) in patients with recurrent disease. In multivariate analysis, only timing of metastasis (de novo versus recurrent) had significant impact on PFS (HR, 2.82; 95% CI, 1.46-5.43; p=0.002). The median OS for the whole group was 56.8 (95% CI ,44.9%-NR).

Conclusions: Outside the stringent clinical trials settings, and in a resource-restricted country, treatment outcomes of ribociclib and AI or fulvestrant in premenopausal patients with MBC, in real-world settings, are comparable to those reported in major clinical trials, including the MONALEESA-7.

P3-12-03: Optimizing Treatment Sequence for Inoperable LABC: Long-Term Outcomes of Surgery First vs. Neoadjuvant Chemotherapy in a Real-World Setting

Yu Song, Bowen Liu, Yan Lin, Ying Xu, Xin Huang, Yidong Zhou, Qiang Sun

Background: Breast cancer is the most common cancer in Chinese women. Locally advanced breast cancer (LABC) is a subtype with a high incidence and poses a significant treatment challenge. Traditionally, inoperable LABC patients receive neoadjuvant chemotherapy (NAC) followed by surgery. However, not all patients benefit from NAC, raising questions about the need for a more precise definition of inoperability and the potential benefits of upfront surgery.

Objectives: This retrospective cohort study aimed to assess the feasibility and efficacy of upfront surgery compared to NAC as the initial treatment approach for inoperable LABC patients. The study also evaluated the impact of various factors on disease-free survival (DFS) and overall survival (OS).

Methods: The study included 243 patients diagnosed with inoperable LABC (excluding T3N1M0) at PUMCH between January 2017 and December 2018. Patients were divided into two groups: initial surgery (n=187) and NAC (n=56). Propensity score matching (PSM) was used to minimize selection bias. The primary endpoint was investigator-assessed DFS and OS. Secondary endpoints included confirmed objective response rate and subgroup analyses based on age, tumor stage, lymph node stage, molecular subtypes, and treatment response. Survival was estimated using the Kaplan-Meier method with log-rank tests for comparisons. Cox proportional hazards models were used for univariate analyses in different subgroups (Figure 1).

Results: The median follow-up was 60.9 months. The 5-year OS was 89.6% for the upfront surgery group and 81.9% for the NAC group (p=0.12). Similarly, the 5-year DFS was 73.0% and 67.1%, respectively (p=0.24). No significant difference in survival outcomes was observed between the two groups overall (Figure 2).
Subgroup analyses revealed that upfront surgery resulted in significantly better OS for patients younger than 60 years old (HR=0.32; 95% CI, 0.10 to 0.96; P=0.0429) and those with stage IIIA disease (HR=0.22; CI, 0.06 –0.86; P = 0.03). There was a trend towards better OS for patients with tumors smaller than 5cm receiving upfront surgery (HR=0.37; 95% CI 0.13 –1.03; p=0.056). NAC response also played a role, with patients experiencing progressive disease (PD) or stable disease (SD) after NAC having a significantly worse DFS (HR, 0.27; 95% CI, 0.09 to 0.79; P = 0.017) and OS (HR, 0.09; 95% CI, 0.02 to 0.48; P =0.004) compared to those who responded well to NAC (Figure 3).

Conclusion: This study suggests that upfront surgery may be a viable and potentially beneficial treatment option for a subset of inoperable LABC patients, particularly those younger than 60 years old, with stage IIIA disease, or smaller tumors. Additionally, the response to NAC can be a valuable factor in guiding treatment decisions. These findings highlight the need for a more nuanced approach to LABC treatment, considering patient characteristics and NAC response. Further research is warranted to refine the definition of inoperability and develop personalized treatment strategies for LABC patients.
Keywords
Locally advanced breast cancer (LABC); inoperable breast cancer; neoadjuvant chemotherapy (NAC); prognosis; real-world study
Funding:To be added.
This study was conducted according to ICH-GCP guidelines and was approved by the Ethics Committee of PUMCH. This study is registered with ClinicalTrials.gov.

Figure 1. The flowchart of this study.
Figure 2. The survival analyses of the upfront surgery group and neo-adjuvant chemotherapy group.
Figure 3. The forest plot of the subgroup analysis of this study.

P3-12-04: Aromatase Inhibitors versus tamoxifen for Elderly Breast Cancer Patients: A Retrospective Study on the Survival Outcomes

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Background: The use of aromatase inhibitor (AI) significantly reduced breast cancer recurrences in postmenopausal women with hormone receptor (HR)-positive breast cancer when compared to tamoxifen. However, there is limited data on the efficacy of AI in elderly patients. This study aimed to compare the outcomes of adjuvant AI versus tamoxifen in elderly breast cancer patients.

Methods: This retrospective cohort study used the clinical records of postmenopausal patients who underwent breast cancer surgery and adjuvant treatment at Seoul National University Hospital between January 2000 and December 2020. Postmenopausal women with HR-positive and human epidermal growth factor receptor 2 (HER2)-negative stage I to III breast cancer were included for analysis. Elderly patients were defined as patients aged 70 or older.

Results: Among a total 3,952 postmenopausal patients, 3,315 (83.9%) were age <70 and 637 (16.1%) were >=70. Patients aged <70 included 2,434 patients in the AI group and 881 patients in the tamoxifen group. The median age of the participants was 57 years [IQR, 53 to 62]. After a median follow-up of 95.2 months [IQR, 71.5 to 126.0], there was no significant differences in overall survival (OS), breast cancer-specific survival (BCSS), and distant metastasis-free survival (DMFS) between the AI and tamoxifen groups. However, a significant difference was observed in disease-free survival (DFS) between the two groups (Hazard ratio, 1.36; 95%CI, 1.09-1.68, p=0.005), with the tamoxifen group showing a higher risk of disease recurrence compared to the AI group. In the multivariate analysis, the tamoxifen group continued to demonstrate a higher risk of recurrence compared to the AI group in patients under the age of 70 (OR, 1.46; 95%CI, 1.13-1.87).
We identified a total of 637 breast cancer patients aged ≥70 during the study period who met the inclusion criteria. Among them, 505 patients were treated with AI and 132 with tamoxifen. The median age of patients was 74 years [IQR, 72 to 77]. After a median follow-up of 62.3 months [IQR, 47.2 to 80.7], there was no significant difference in OS, BCSS, DFS, and DMFS between the AI and tamoxifen groups. The 10-year BCSS and 10-year OS for patients treated with AI was 96.1% and 62.3%, respectively, suggesting most of the mortality was unrelated to their breast cancer.

Conclusion: These findings suggest that in elderly breast cancer patients aged ≥70, the use of AI did not provide survival benefit over tamoxifen. Most of the mortalities in these elderly patients were unrelated to breast cancer. Our data indicates that the choice of endocrine therapy in elderly patients can be tailored to each patient's comorbidity and quality of life with no meaningful difference in oncologic outcomes.

P3-12-05: Real-world use of sacituzumab govitecan (SG) in the management of metastatic triple-negative breast cancer (mTNBC) through a Canadian Patient Support Program (PSP)

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Background: In the ASCENT trial, SG demonstrated significant survival improvements compared to single agent chemotherapy in previously treated mTNBC. Based on these findings, SG was approved by the FDA and Health Canada in 2021 for the treatment of adult patients with unresectable locally advanced or metastatic TNBC who have received ≥2 prior systemic therapies, at least one of them in the metastatic setting. Here, we report the first study to characterize the real-world use of SG in patients with previously treated mTNBC in Canada.

Methods: This was a cohort study of Canadian patients with unresectable locally advanced or metastatic TNBC who received at least one dose of SG between November 2021 and December 2023 via the Gilead Sciences Canada's Patient Support Program post-approval. Demographic and clinical data were collected as part of PSP enrollment. Patients were followed up until the end of treatment, defined as death, SG discontinuation or lost to follow up, whichever occurred the first. The Kaplan-Meier method was used to estimate treatment duration.

Results: A total of 453 patients were identified with a median age of 58 years and mean weight of 71 kg. 245 patients (54%) were primarily treated at a cancer centre and 208 patients (46%) were treated at private infusion Innomar Clinics location. Patients were enrolled in the PSP to receive SG in second-line (2L, 29%); 3L (43%) or 4L+ (28%). The median treatment duration was 4.2 months (95% CI 3.7-4.9). The median dose at treatment initiation was 10.0 mg/kg (IQR, 10.0-10.0) and 355 patients (78%) experienced at least one dose delay and 250 patients (55%) experienced at least one dose reduction. Treatment delay and dose reduction were due to any reason. Among the 323 patients who discontinued treatment, 197 (61%) discontinued due to physician decision in the context of change in patient condition or disease progression. Patients aged < 65 years at enrollment had a median treatment duration of 4.4 months (95% CI 3.7-5.1), whereas those aged ≥ 65 years had a median treatment duration of 3.8 months (95% CI 3.0-5.3). The median treatment duration of SG was 3.7 months (95% CI 3.0-5.3), 4.7 months (95% CI 3.9-5.6), and 3.5 months (95% CI 2.8-4.9) when used in 2L, 3L, and 4L+, respectively with a logrank

p=0.2.

Conclusions: This study reports real-world SG treatment patterns that are generally consistent with those of the ASCENT trial. These findings support the clinical benefit of SG demonstrated by existing clinical trial data. Potential limitations include the limited capacity of the PSP, the presence of unmeasured confounding, purposive sampling during PSP enrollment, and lack of data linkage.

P3-12-06: Locoregional and contralateral breast tumor events within 5 years after diagnosis of primary breast cancer in the Netherlands: a population-based study including 121,426 breast cancer patients

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Background: Due to an increasing incidence, improving survival rates and changes in treatment patterns, a comprehensive overview of trends in breast tumor events (BTE) in breast cancer survivors is highly needed. In this study, contemporary trends in the occurrence of locoregional and contralateral BTE in patients with previous non-metastatic invasive breast cancer in the Netherlands were investigated.

Methods. In this nationwide population-based study, women ≥18 years diagnosed with primary non-metastatic invasive breast cancer between 2003-2008 and 2012-2016, treated with local surgery in the Netherlands, were selected. Data on patient-, tumor-, and treatment-related characteristics were retrieved from the Netherlands Cancer Registry. Data on BTE of breast cancer patients diagnosed between 2003 and 2008 were collected 5 years after primary diagnosis from all hospitals in the Netherlands in retrospect by checking all patient files. For patients diagnosed between 2012 and 2016, data on locoregional BTE were collected by linking the NCR to the Nationwide Pathology Archive (PALGA). Patients with suspected locoregional recurrence were selected through an algorithm based on diagnostic codes within the database and dates of occurrence. Descriptive statistics and Chi-squared tests were performed to compare both cohorts with regard to patient-, tumor-, and treatment-related characteristics and to identify potential differences over time. Five-year event rates were calculated per type of BTE (ipsilateral, regional, and contralateral), and stratified for age at primary diagnosis (<50, 50-69, or ≥70), primary tumor stage (stage I, II, or III), primary subtype based on hormone receptor status and human epidermal growth factor receptor 2 (HR+/HER2-, HR+/HER2+, HR-/HER2+, or HR-/HER2-), and primary tumor grade (grade I, II or III). An ipsilateral breast event was defined as an event in the same breast as the primary breast cancer, i.e. an in-breast recurrence or a second primary breast cancer. A regional event was defined as a regional lymph node metastasis on the same side as the primary breast cancer, where regional lymph nodes were defined as either axillary, periclavicular, or internal mammary lymph

node regions. A contralateral breast event was defined as an event in the contralateral breast as the primary breast cancer, which could be either in situ or invasive breast cancer. Findings. Of the 121,426 included breast cancer patients, at least one type of BTE was observed in 6,256 patients (5.2%), of which 422 patients (6.7%) had multiple events at the same time (occurring within 30 days). Five-year ipsilateral, regional and contralateral breast event rates decreased from 3.3%, 1.8% and 2.7% in 2003 to 2.0%, 1.3% and 2.0% in 2016, respectively. The largest decreases were shown in ipsilateral breast event rates for stage III (4.7% in 2003 to 1.7% in 2016) and grade III (5.5% in 2003 to 2.1% in 2016). Interpretation. Taking the change of way of notification into account, our study indicates a slight decrease in or at least stable event rates of ipsilateral, regional, and contralateral BTE in breast cancer patients with a primary diagnosis between 2003-2008 and 2012-2016 in the Netherlands. As breast cancer treatment has been personalised increasingly over the last decades, with de-escalation of local treatment and more options in systemic treatment, it is reassuring that BTE have not increased.

P3-12-07: SARELIFE study: Safety and efficacy of Sacituzumab Govitecan (SG) in pretreated metastatic triple negative breast cancer (mTNBC): a multicentric, real-life study

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Background: mTNBC is an aggressive subtype of breast cancer with poor prognosis, so treatment primarily aims to prolong patients (pts) survival and improve their quality of life. SG is a new antibody-drug conjugate that incorporates the anti-TROP2 antibody hRS7 conjugated to a topoisomerase-1 (TOP1) inhibitor payload, that increased overall survival (OS) in pretreated mTNBC. Based on the results of ASCENT pivotal study, SG was approved in Italy since August 2022 for mTNBC pts, pretreated with at least 2 previous lines of chemotherapy. To date, very fragmentary real-world data are available. In order to study safety and efficacy of SG outside of a registrative trial, the SARELIFE study was designed.

Patients and Methods: This is an observational, retrospective and prospective study, whose primary objective was to evaluate the safety of SG in a real-world population. The secondary objectives included: treatment adherence, safety of concomitant radiation therapy (RT) and drug-drug interactions (DDI), response rate according to RECIST v1.1 criteria, progression free survival (PFS) and OS. Treatment-related adverse events (AEs) were categorized and graded according to NCI-CTCAE v5.0. Data were analysed for safety, activity and survival

outcomes, and the results were reported using descriptive statistics.

Results: Since August 2023 to July 2024, 121 women were enrolled from 27 Italian centres. Median age was 59 years (range: 31-86). PS (ECOG) was 0-1 in 89.7% of pts; 12% had ≥ 2 comorbidities; 10.4% of pts were gBRCAmut carriers; 23.1% were metastatic at the diagnosis. Visceral metastases (mts) were present in 62.6% of pts, the commonest metastatic site was lung (40.9%), 12.2% had brain mts. Median number of prior treatment lines was 2 (range: 0-8): 29.3% of pts had received >2 prior lines of therapy, 26.7% had been treated with immunotherapy and 9.5% with PARP inhibitors.
For the safety analysis, 116 pts were considered: 68.1% experienced at least one AE of any grade. The most frequent were: neutropenia (51.9%, G3/G4: 24.1%), fatigue (45.6%, G3/G4: 3.8%), nausea (44.3%), diarrhoea (32.9%, G3: 5.1%), anaemia (27.8%, G3: 6.3%) and febrile neutropenia (6.3%, G4: 3.8%). 1 case of G3 pneumonitis was recorded. No G5 AEs observed. Median time to the onset of the first AE is 21 days. Dose reductions were needed for 43 pts. Median time to first dose reduction: 39 days (range 7 -161). 4 pts discontinued SG due to toxicities. UGT1A1 status was evaluated in 18 pts: 4 were (*28/*28), 6 were (*1/*28). In the (*28/*28) group only 1 case of G3 anaemia was reported. Interestingly, 21 pts received RT during SG treatment, including 10 concurrent treatments, but no additional AEs occurred. Data analyses on DDI are still ongoing.
With a median follow-up of 11.5 months, the median PFS was 5.9 months (95%CI: 4.5-6.8), while the median OS was 14.1 months (95%CI: 11-16.3) these data were consistent with those reported in the ASCENT study (mPFS: 5.6 months; mOS: 12.1 months). Best response was evaluable in 95 pts: 25.2% obtained partial response (PR), 1% complete response (CR), 40% stable disease (SD) and 31.5% progression disease (PD).

Conclusions: SARELIFE offers an interesting overview on the performance of SG in an unselected real-world mTNBC population. Of note, in our study was included also a small percentage of pts with PS (ECOG): 2, excluded in the ASCENT trial. No new safety signals were reported. Survival outcomes are surprisingly good. Concomitant RT appears safe, although few pts underwent to both treatments.

P3-12-08: Results of the cohort w/ central nervous system (CNS) metastasis in the real-world evidence observational study SACISUR, patients treated w/ Sacituzumab-Govitecan (SG) in triple neg metastatic breast cancer (mTNBC) clinical practice in the south

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Vicente-Rubio Elena, De Toro-Salas Rubén, Cano-Jiménez Alicia, Alba-Conejo Emilio, Sánchez-Guisado Antonia, Rodríguez José Francisco, Cruz-Jurado Josefina, Gil-Torralvo Ana, De la Fuente-Domínguez Icíar, Rodríguez-García Jose Andrés, Cejuela-Solís Mónica, Torres-Zurita Alberto, Benavent-Viñuales Marta, Henao-Carrasco Fernando

Background: mTNBC patients will develop metastasis in CNS in between 25-46% along the course of the treatment. SG is a new antibody-drug conjugate against Trop-2 that has been approved for the treatment of mTNBC after progression on at least one previous line of chemotherapy (CTX) for advance disease. In the ASCENT trial, progression-free survival (PFS) and overall survival (OS) were longer with SG compared with CTX (5.6 vs 1.7 months PFS and 12.1 vs 6.7 months respectively). In this trial, 11.5% of patients (61/529) had metastasis in CNS but they were excluded of the final survival analysis.

Methods: We included 159 patients with mTNBC treated at least with 1 cycle of SG between 1 January 2022 and 31 December 2023. This study has been supported by SAOM (Andalusian Society of medical oncologist). The aim of this abstract is presenting the results in the cohort of metastasis in SNC. Results of all the patients will be presented in a different poster.
Statistical analysis was made by IBM SPSS statistics, including descriptive results of the population and survival analysis by Kaplan-Meier.

Results: We report the data of 22 female patients treated in 18 different hospitals of Andalucía, Canarias and Extremadura (Spain). These patients represent a 13.9% of total of 159 patients treated in our study. All of them were treated with radiotherapy previous to the use of SG.
The median age was 47 years (50 % premenopausal, 50% Her2low). 22.7% were de novo, stage I-III patients were treated with CTX in the early setting in 54.5% in neoadjuvant and 41.2% in the adjuvant setting. Pathological complete response was 9% in the case of neoadjuvant treated patients.
Medium previous line of therapy was 2.4 (2-4) with a medium of 4.1 cycles of SG. SG was received in second line in 68.2% patients, 22.7% patients in third line and 9.1% in fourth line. 18.2% received immunotherapy in first line.
With a median follow-up of 11.8 months, PFS was 2.27 months (95% CI, 1.36-3.17) (fig 1). The percentage of patients with an objective response was 13.6% (36.3% with clinical benefit rate).
The incidence of diverse adverse events were neutropenia 45.5% (G3-4 27.2%), diarrhea 36.8% (G3-4 9.1%), nauseas 21.1% (G3-4 5.3%) and ALT/AST elevation 30% (no G3-4). The use of GCSF were 18.2 % as primary prophylaxis and 18.2 % as secondary prophylaxis. None of the patients finished SG due to adverse events and 31.8% had at least one dose reduction.

Conclusions: SG has worse results in survival for patients with CNS metastasis. Despite these results, patients could be treated with this drug safely taking in account the bad prognosis of this situation.

P3-12-09: Evaluation of the Early Risk of Recurrence in HR+/HER2- Early Breast Cancer Patients – A Retrospective Study Based on the Chinese National Cancer Database

Qiao Li, Mingxia Jiang, Jiaxuan Liu, Mengqi Zhang, Binghe Xu

Background: Recent published studies have demonstrated the value of CDK4/6i in the adjuvant setting by significantly reducing the risk of recurrence (ROR) in high-risk and intermediate-risk patients (pts). However, the ROR and adjuvant treatment pattern has not been reported in China. This study explored the ROR of 3-year and 5-year in HR+HER2- eBC pts who were treated with standard of care in China within the last decade.

Methods: This study retrospectively analyzed the electronic medical record (EMR) of a random sample of the pts with HR+/HER2- eBC (stage I-III) that were diagnosed from Jan. 2013 to Dec. 2022 from the Chinese National Cancer Database, which is the largest oncology database in China. Pts received primary breast tumor surgery and adjuvant therapy were included into the analysis. while pts with concurrent malignant tumors other than BC were excluded. RFS was defined as time from surgery to recurrence. RFS analysis was performed using SAS v9.4 software with cutoff date of Dec 31, 2023. Hazard ratio (HR) and 95% confidence interval (CI) were derived from Cox proportional hazards regression models.

Results: A total 3295 of HR+/HER2- eBC pts from the database were included in the analysis. Median age at diagnosis was 49.0 years (range,22-80 years). Majority (98.4%) were women with 54.5% in premenopausal status. There were 728 pts (22.1%) , 1558 pts (47.3%) and 1009 pts (30.6%) in stage I, stage II and stage III respectively. 62% of pts had lymph node metastasis (LN+). 3006 pts (91.2%) received AET, which contained 96 pts (3.2%) who received CDK4/6i combination treatment. The analysis showed that with a median follow up of 20.8 months (range, 0.5-121.7 months), the 3-y and 5-y RFS rates were 87.3% (95% CI, 85.7-88.9%) and 77.5% (95% CI, 74.9-80.2%), respectively. The 3-y RFS rates in stage III, II and I were 75.1%, 91.2% and 95.2% respectively. Compared to stage I, the ROR of pts in stage III (HR 4.62, 95% CI 3.25-6.56) and stage II (HR 1.75, 95% CI 1.21-2.52) were significantly higher. More importantly, the ROR of pts eligible for NATALEE trial (NATA-Group) was higher than those not eligible for NATALEE trial (nonNATA-Group) (3-y RFS rate 83.9 % vs 95.3%; HR 3.02, 95% CI 2.17-4.19). NATALEE eligible pts were defined as pts in stage II and stage III who with LN positive, or LN negative with high-risk. The ROR of pts who received AET showed consistent trend with total population.
Conclusions
This analysis from the Chinese National Cancer Database indicates that the ROR for stage II

and III breast cancer within 5 years is relatively high in China. Pts eligible for the NATALEE trial exhibits a significantly higher ROR compared to lower risk pts who are not eligible for NATALEE trial and warrant the consideration for tolerable ET adjuvant treatment escalation.

P3-12-10: Utilizing Registry Data to Explore Treatment Patterns in HR+ HER2-Negative Metastatic Breast Cancer: Insights from Denmark and Norway

Anja Reithmeier, Alina Carmen Porojnicu, Morten Johnsen, Trude Ågesen, Johan Liseth Hansen

Background: Breast cancer (BC) represents the most common cancer in women worldwide [1] and within the metastatic setting (mBC) the treatment options are continually evolving [2]. Using the unique, nationwide, population-based registries in Denmark and Norway linked on individual level, we aimed to identify patients with BC and define treatment lines in the metastatic setting exemplified by forming a patient cohort of hormone receptor-positive and human epidermal growth factor receptor 2-negative (HR+/HER2) metastatic BC (mBC).
This registry-based study aims to link data from national registries in both Denmark and Norway to explore/provide real-world data on treatment pattern exemplified by patients treated in the metastatic setting.

Methods: This is a cohort study utilizing the BC registries in Denmark and Norway linked to information from hospital administered treatment from prescription databases and patient registers to describe the treatment pattern in a selected BC cohort.
The cohort included both de novo mBC patients (Stage IV) or recurrent BC (incident Stage I-III) patients between 2017-2021 for Denmark, and 2017-2022 for Norway. De novo mBC patients were identified by using the national cancer registries (ICD-10 code: C50) with confirmed metastatic disease. Recurrent patients included those who had incident stage I-III diagnosis between 2008-2021 and 2008-2022, for Denmark and Norway respectively, and that progressed to metastatic disease from 2017 and onwards. An algorithm to identify recurrence was built including information from patient registers, hospital treatments, and pathology reports (only in Denmark).
HR and HER2-status were collected from the Danish nationwide pathology register and Norwegian quality of care register for BC.

Results: We identified 449 and 597 patients with HR+/HER2- de novo mBC and 1373 and 1218 with HR+/HER2- recurrent mBC in Denmark and Norway, respectively. Hereof, n=1651 (90.6%) patients in Denmark, and n=1562 (86.1%) of patients in Norway received a 1L treatment. In Denmark the most common first line (1L) treatment was endocrine treatment monotherapy, n=603 (36.5%), followed by CDK 4/6 inhibitor + endocrine

combination therapy with n=512 (31.0%), and chemotherapy, n=423 (25.6%). In Norway, the most common 1L treatment was CDK 4/6 inhibitor + endocrine combination therapy with n=672 (43.0%), followed by endocrine treatment monotherapy n=549 (35.2%), and chemotherapy, n=252 (16.1%). The number of patients that received a second line treatment and beyond (≥2L) in Denmark was n=812 (48.3%), and in Norway was n=930 (59.0%). The number of patients who stayed on their 1L treatment until the end of follow-up was n=478 (28.4%) and n=273 (17.3%), and the number of patients who stayed on 1L until death was n=391 (23.3%) and n=373 (23.7%) in Denmark and Norway, respectively.

Conclusion: Endocrine treatment, either as monotherapy or combination therapy with CDK4/6 inhibitors are the predominant recommended treatment for patients with HR+/HER2- mBC in 1L in Norway and Denmark. Our results show that patients are generally treated according to these guidelines. Hence, the administrative Danish and Norwegian registers are well suited to study treatment lines among patients with HR+/HER2- mBC and to identify recurrent patients. This work enables further studies on treatment outcomes, comparative effectiveness, and healthcare costs.

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P3-12-11: Effect of pretreatment lab abnormalities on the time-to-treatment discontinuation and overall survival on hormone receptor-positive metastatic breast cancer patients receiving targeted therapies

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Background: Metastatic breast cancer (MBC) randomized controlled trials (RCTs) enroll healthier patients than the general population. However, given that over one quarter of women have a comorbidity at the time of their breast cancer diagnosis, RCTs may inadequately represent the patient population. Therefore, the purpose of this study was to estimate time-to-treatment discontinuation (TTD) and overall survival (OS) for patients with and without lab abnormalities receiving a targeted therapy for MBC.

Methods: This retrospective study used the nationwide, de-identified electronic health record-derived Flatiron Health database to include women with hormone receptor-positive, Human epidermal growth factor receptor 2- negative MBC with receipt of a cyclin dependent kinase (CDK) 4/6 inhibitor everolimus, or alpelisib between 2011 and 2020. Lab tests included platelet count, neutrophil count, lymphocyte count, hemoglobin, glucose, hemoglobin A1c, creatinine, Alanine transaminase, Aspartate transaminase, and bilirubin.

Abnormalities were defined by common exclusionary cut-offs in targeted therapy clinical trials. TTD was defined as time from treatment initiation to the first occurrence of either treatment change or death. The secondary outcome was OS defined as time from treatment initiation to death from any cause. Parametric accelerated failure time (AFT) models estimating the survival time ratio, predicted mean survival time differences, and 95% confidence intervals (CIs) were used for the association between lab abnormalities and TTD or OS. All models were adjusted for age at treatment initiation, race and ethnicity, site of metastasis, concurrent treatment, and line of therapy.

Results: Among patients with receipt of a CDK 4/6 inhibitor, patients with at least one lab abnormality had 33% (MR, .67; 95% CI, .59, .68) lower TTD than those with no lab abnormalities. On average, the predicted TTD for those with no lab abnormalities was 8 months longer than those with lab abnormalities. Similar differences in the CDK 4/6 cohort were observed in OS where those with at least one abnormality had 25% (MR, .75; 95% CI, .70, .81) lower OS than those with no lab abnormalities. In our sample of patients with receipt of everolimus, more modest differences were seen in TTD. After adjusting for covariates, patients with at least one lab abnormality had 10% (MR, .90; 95% CI, .83, .98) lower TTD than those with no lab abnormalities. Similarly, patients with receipt of Everolimus and at least one abnormality had 12% (MR, .88; 95% CI.76, .98) lower OS compared to those without an abnormality. Finally, among those with receipt of alpelisib, those with any lab abnormality had 25% (MR, .75; 95% CI, .64, .87) lower TTD than those without any abnormalities. On average, patients without any lab abnormalities had 1.5 months longer survival than those with abnormalities. For OS, patients with receipt of Alpelisib with lab abnormalities had 37% (MR, .63; 95% CI, .51, .78) lower survival than those without a lab abnormality.

Conclusion: Patients with lab abnormalities saw significantly lower TTD and OS than those without abnormalities. More real-world studies of patients with lab abnormalities are needed to empower oncologists when making treatment decisions in high-risk populations, to discuss prognosis and to inform RCT eligibility criteria.

P3-12-12: Real-World Efficacy and Outcomes of CDK4/6 Inhibitors in HR+ Metastatic Breast Cancer: A Retrospective Analysis from Russia

Anastasia Danilova, Daniil Stroyakovsky, Polina Shilo, Irina Nigmatullina, Ignat Profatilo, Nikolai Ryabishin, Valery Nyashin, Vera Vashenko, Dmitry Kanner

Background: The use of CDK4/6 inhibitors has transformed the treatment landscape of HR+ metastatic breast cancer, improving progression-free survival and, in some studies, overall survival. Most of the available data come from randomized clinical trials, but the importance of data from real-life settings cannot be understated. This is the largest data set to date on the use of CDK4/6 inhibitors in routine clinical care in Russia, describing the patient

population, efficacy of treatment, comparing CDK4/6 inhibitors head-to-head, and describing post-treatment outcomes.

Materials and Methods: We retrospectively evaluated all orders of CDK 4/6 inhibitors prescribed at Moscow 62 Oncology Center in Russia and identified 803 patients who received CDK4/6 inhibitors plus endocrine therapy for advanced HR+ breast cancer. We then performed survival analysis to identify differences between CDK4/6 subgroups using Kaplan-Meier curves, the log-rank test, and Cox regression. Differences were considered statistically significant when $p<0.05$.

Results: 803 patients were included in the final analysis with a median follow-up of 37 months. Patient characteristics are described in the table below:
Patient Characteristics (N=803)
Median age: 57 years
HistologyNOS: 58 (77.3%)Lobular: 137 (18.2%)Other: 34 (4.5%)
Tumour grade1: 21 (3.4%)2: 384 (62.3%)3: 34.3%
Her2 status0: 172 (22.9%)1: 522 (69.5%)2 (FISH -): 57 (7.6%)Her2 low: 77.1%
Mutational StatusBRCA1/2 (status known in 14.4% of patients): 9.5% (12 patients positive)PIK3CA (status known in 20.2% of patients): 34.6% (56 patients positive)
StagingDe novo metastatic: 41.1%Progression: 58.9%
Metastatic SitesBone metastases present: 546 (68.3%)Liver metastases present: 134 (16.8%)Lung metastases present: 221 (27.7%)Brain metastases present: 20 (2.5%)
Line of CDK4/6 Therapy (median): 1
CDK4/6 InhibitorsPalbociclib: 408 (50.9%)Ribociclib: 346 (43.2%)Abemaciclib: 47 (5.9%)
Endocrine TherapyAnastrozole/Letrozole: 495 (62.0%)Fulvestrant: 277 (34.7%)Exemestane: 26 (3.3%)
Treatment EffectComplete response: 27 (3.4%)Partial response: 194 (24.7%)Stable disease: 518 (65.9%)Progression: 46 (6.0%)
Reason for DiscontinuationProgression: 410 (80.7%)Toxicity: 40 (7.9%)Patient refusal: 15 (3.0%)Other: 8.5%
The main efficacy endpoints in the overall population were progression-free survival (PFS) of 27 months (95% CI [23.6-30.4]) and overall survival (OS) of 47 months (95% CI [42.6-53.4]). Median PFS2 was 7.1 months.
Post progression therapy
Chemotherapy 63%Endocrine 37%
The baseline characteristics of the different CDK4/6 inhibitor subgroups were well balanced without any adjustment. There were no statistically significant differences between CDK4/6 inhibitors: the median PFS was 24 months for Palbociclib (95% CI [19.3-28.4]), 30 months for Ribociclib (95% CI [24.7-35.3]), and 32 months for Abemaciclib (95% CI [18.4-45.7]), $p = 0.743$. The follow-up periods were different between subgroups, with less mature data for Abemaciclib and the most mature for Palbociclib. We adjusted for follow-up to address this, but there were still no statistically significant differences between

CDK4/6 inhibitors (p = 0.689). Multivariable Cox regression showed that the type of CDK4/6 drug is not an independent predictor of survival. There were no statistically significant differences between metastatic site subgroups:mPFS by site of metastasis Palbo vs Ribo
Bone only disease 27 months vs 30Bone metastases present 23 vs 25 monthsBone metastases absent 41 vs 44 monthsLiver metastases present 15 vs 14 monthsLiver metastases absent 28 vs 32 monthsLung metastases present 23 vs 29 monthsLung metastases absent 26 vs 30 monthsVisceral metastases present 20 vs 25 monthsVisceral metastases absent 30 vs 32 months
InterpretationThese are the largest real-world data presented from routine clinical practice in Russia. These data confirm the results of several randomized clinical trials, providing even more information on the use of CDK4/6 inhibitors. We performed an analysis comparing the two most widely used CDK4/6 inhibitors, showing similar efficacy outcomes. These data provide essential information for physicians and patients reflecting real-world use of CDK4/6 inhibitors, and further analyses are underway.

P3-12-13: Malignant phyllodes tumor of the breast

Tatiana Titova, Elena Artamonova, Alexander Petrovsky

Purpose: Malignant phyllodes tumor of the breast (MPTB) is a rare type of breast cancer, with an incidence of less than 1%

Methods: Data on patients with a malignant MPTB were treated in N.N. Blokhin NMRCO from July 2018 to July 2023

Results: 31 MPTB patients were included in this study. Median age was 46 years. Median follow-up was 26.3 months. 13 cases (41,9%) experienced local recurrence, 7 cases (22.6%) exhibited distant metastasis, and 8 cases (25.8%) resulted in death. MPTB patients more often had breast-conserving surgery (BCS) as a surgery (67,7%). In MPTB adjuvant radiotherapy was given in 25.8%. The observed PFS at 12 months was 69% and PFS at 24 months was 27%. The observed OS at 12 months was 90%. Among patients with initial margins < 1 cm, LRFS (P = 0.004) and DFS (P = 0.003) were improved in patients reoperated to achieve margins ≥ 1 cm. Surgical margin < 1 cm (HR = 1.567, 95%CI 1.137-2.793, p = 0.043) and age < 45 years (HR = 1.059, 95%CI 1.022-3.008, p = 0.047) were identified as independent risk factors for local recurrence-free survival.

Conclusions: Surgical margin < 1 cm and age < 45 years were identified as independent risk factors for local recurrence-free survival for MPTB.

P3-12-14: Real world data with standard (SD) and reduced dose (RD) sacituzumab-govitecan (SG) in metastatic triple negative (TNBC) and ER+/HER2- metastatic breast cancer (mBC)

Naji Mallat, Nicholas Stabellini, Alexis Bennett, Naji Mallat, Bahar Moftakhar, Cynthia Owusu, James Martin, Takae Mizukami, Amanda Amin, Corey Speers, Alberto J. Montero

Background: SG is a Trop-2–directed antibody-drug conjugate currently FDA approved for: (i) unresectable, locally advanced or metastatic TNBC following ≥ 2 prior systemic therapies; and (ii) pretreated HR+/HER2- mBC following endocrine-based therapy. In the ASCENT and TROPiCS phase 3 trials, SG was shown to be superior to chemotherapy of physician's choice with superior median progression free survival (PFS), overall survival (OS), as well as higher overall response rates (ORR). This activity, however, comes with increased toxicities, primarily diarrhea and neutropenia. Cancer drugs have long been administered to patients (pts) at the maximum tolerated dose, which is selected on the basis of evaluating increasing doses in small groups of pts for short periods of time. It is therefore likely that a slightly lower SG dose may have comparable efficacy and less toxicity than the FDA approved SG dose. This study aimed to assess treatment patterns and clinical outcomes in mBC pts who received SD versus RD of SG.

Methods: This retrospective single-institution cohort study utilized de-identified patient data obtained from chart reviews. We included mBC pts who received at least 1 cycle of SG between April 30, 2021 and April 30, 2024. SG treatment parameters and patient characteristics were obtained. PFS and OS estimates from the date of SG initiation to the date of the last follow-up were assessed using the Kaplan-Meier method. Differences of OS and PFS between SD and RD groups were examined by log-rank test.

Results: We analyzed 47 mBC pts (median age 63 at SG initiation; 36% non-White), with 24 receiving SG at an initial SD (10 mg/kg) and 23 receiving a RD (median 8 mg/kg). In the overall cohort, 70% had TNBC (19/24 SD vs. 14/23 RD, p=0.29), and 30% had HR+/HER2- mBC (9/24 SD vs. 5/23 RD, p=0.45). Moreover, the median number of distinct metastatic sites of disease was 3; 83% had baseline visceral metastases and 23% had brain metastases. In the overall cohort, pts had received a median of 2 prior metastatic lines of therapy, with SD pts having received more prior metastatic lines of therapy (3 vs 1, p<0.01). All SD TNBC pts had previously received neoadjuvant chemo-immunotherapy with the Keynote-522 regimen (11 vs 0; p<0.001). For the overall cohort, 8.5% had received prior trastuzumab deruxtecan; 100% of HR+/HER2- pts had received prior CDK4/6 inhibitors; 42.6% of TNBC pts had received prior PD-1 inhibitor therapy. The median treatment duration for SD and RD were similar (96 vs 100 days; p=0.62). For the entire cohort, the ORR was 21% (17% RD and 26% SD). Among TNBC and ER+/HER2- pts, the ORR was 39.1% (36% SD & 21% RD) and 35.7% (20% SD & 11% RD), respectively. At the last follow-up, 40.4% of pts were alive. From the start of SG, the median PFS was 3 months for SD (95% CI 2-10) and 3

months for RD (95% CI 1-8; p=0.9). Similarly, the median OS was not statistically different between SD and RD: 9 months ([95% CI 7-NA] vs. 13 months [95% CI 5-NA]; p=0.7). From the start of SG, the overall median OS was 9 months (95% CI 6-15). From the date of initial mBC diagnosis, the median OS was 64 months (95% CI 29-92).
For TNBC pts, the median PFS and OS in months for the SD and RD groups, from the date of first SG, were 3 ([95% CI 2-10] vs. 4 months [95% CI 2-NA]; p=0.4); and 10 ([95% CI 7-NA] vs. 25 months [95% CI 8-NA]; p=0.5) respectively. Myeloid growth factor use was significantly higher in the SD group (58% vs. 0; p=<0.01). More SD pts required hospitalization due to SG toxicity compared to RD (20.8% vs. 4.3; p=0.20).

Conclusions: In this small data set, the median SG treatment duration, PFS, and OS did not appear to differ between SD and RD groups. A prospective trial with RD SG is warranted.

P3-12-15: Oncologists' Prescribing Habits for Adjuvant Therapy in Luminal Breast Cancer: Insights from a Low Middle Income Setting

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Background: In low- and middle-income settings where genomic profiling is often inaccessible, oncologists must rely on clinicopathological factors to make crucial decisions regarding adjuvant therapy in luminal breast cancer. This study provides an in-depth analysis of how demographic and tumor characteristics influence the selection between combined chemotherapy and hormonal therapy (CTH&HT) versus hormonal therapy alone (HT) in a resource-limited environment, focusing on patients treated at the Shefa Orman Comprehensive Cancer Center in Luxor, Egypt.

Methods: We performed a comprehensive retrospective analysis of 675 luminal breast cancer patients treated from June 2016 to December 2022. The study examined the influence of variables such as age, tumor size, nodal involvement, and comorbidities on therapy choices using Chi-square tests for categorical outcomes and logistic regression for continuous predictors.

Results: The analysis revealed significant age-dependent differences in therapy choices. Patients aged ≤45 years predominantly received CTH&HT, a trend that extended up to the age of 65, suggesting a preference for more aggressive treatment in the absence of genomic risk stratification. Tumor size and nodal involvement were also critical determinants, with larger tumors and higher nodal involvement favoring CTH&HT. Specifically, 82% of chemotherapy cases employed Anthracycline-based regimens. For hormonal therapy, Aromatase Inhibitors were used in 52.3% of cases, followed by Tamoxifen (24.3%) and combinations of LHRH agonists with either AIs or Tamoxifen (23.4%). Additionally, older

patients and those with multiple comorbidities were less likely to receive aggressive treatments, even when presenting with higher-risk clinicopathological factors.

Conclusions: This study highlights a heavy reliance on traditional clinical indicators for adjuvant therapy decisions in luminal breast cancer in the absence of genomic tools. The findings stress the importance of developing tailored treatment strategies that are sensitive to the constraints of local resources and the availability of diagnostic tools. Such strategies are essential to optimizing patient outcomes in global low-resource settings.

P3-12-16: MELODY: A prospective non-interventional multicenter cohort study to evaluate different imaging-guided methods for localization of malignant breast lesions (EUBREAST-4 / iBRA-NET, NCT 05559411)

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Background: In the last decades, the proportion of breast cancer patients receiving breast-conserving surgery has increased, reaching 70-80% in developed countries. In case of non-palpable lesions, surgical excision requires some form of breast localization. While wire-guided localization has long been considered gold standard, it carries several limitations, including logistical difficulties, the potential for displacement and patient discomfort, and re-excision rates reaching 21%. Other techniques (radioactive seed or radio-occult lesion localization, intraoperative ultrasound, magnetic, radiofrequency and radar localization) have been developed with the aim of overcoming these disadvantages. However, comparative data on the rates of successful lesion removal, negative margins and re-operations are limited. In the majority of studies, the patient's perspective with regard to discomfort and pain level has not been evaluated. The aim of MELODY (MEthods for LOcalization of Different types of breast lesions) is to evaluate different imaging-guided localization methods with regard to oncological safety, patient-reported outcomes, and surgeon and radiologist satisfaction.

Methods: The EUBREAST and the iBRA-NET have initiated the MELODY study to assess breast localization techniques and devices from several perspectives (NCT05559411, http://melody.eubreast.com). MELODY is a prospective intergroup cohort study which

enrolls female and male patients requiring breast-conserving surgery and image-guided localization for invasive breast cancer or DCIS. Multiple or bilateral lesions and neoadjuvant chemotherapy are allowed. Primary outcomes are: 1) Intended target lesion and/or marker removal, independent of margin status on final histopathology, and 2) Negative resection margin rates at first surgery. Secondary outcomes are, among others: rates of second surgery and secondary mastectomy, Resection Ratio (defined as actual resection volume divided by the calculated optimum specimen volume), duration of surgery, marker dislocation rates, rates of marker placement or localization failure, comparison of patient-reported outcomes, rates of "lost markers" and diagnostician/radiologist's and surgeon's satisfaction as well as the health economic evaluation of the different techniques. Target accrual: 7,416 patients. Enrollment started in January 2023. The study is being conducted in 30 countries and is supported by the Oncoplastic Breast Consortium (OPBC), AWOgyn, AGO-B and SENATURK. Financial support was provided by Endomag, Merit Medical, Sirius Medical and Hologic.

P3-12-17: NCT05074290 Phase I/II study of Pharmacokinetics and Safety of Epidiferphane and Taxanes in Breast Cancer Patients

Coy Heldermon, Shu Wang, Danielle Ogden, Debra Lyon, Brent Reynolds, Karen Daily

Patients with breast cancer are commonly treated with taxane class chemotherapy agents such as paclitaxel, Nab-paclitaxel or docetaxel. Some very common side effects of taxanes, such as anemia and peripheral neuropathy, result in dose reductions, dose delays and early discontinuation (collectively called relative dose intensity) of these chemotherapy agents in 15-80 % of patients on these drugs. This reduction in relative dose intensity (RDI) results in worse clinical outcomes such as survival. Pre-clinical studies in mouse models given chemotherapy regimens containing paclitaxelhave shown that Epidiferphane (EDP) delays tumor progression, and reduces both neuropathy and anemia.This is an Investigational New Drug Study that will investigate Epidiferphane in patients with breast cancer receiving a taxane-containing chemotherapy regimen in a Phase I/II trial. In phase I, we are evaluating the safety and pharmacokinetics of two EDP dose levels when given with taxane containing chemotherapy regimens to neoadjuvant and metastatic breast cancer patients. In phase II, we will assess the safety and efficacy at reducing neuropathy and anemia and improving tumor response and patient quality of life in breast cancer patients of adding EDP to taxane containing chemotherapy regimens.We will be assessing the correlation of neuropathy and anemia with the level of neurofilament light chain, VEGFA, Nrf2, NF-κB and IL18. This correlation will determine if EDP is having a similar effect to that seen in the mice and if these markers may be useful in predicting and targeting toxicity.Our intervention will determine pharmacokinetics of and test a novel therapy for safety and effectiveness at improving response to chemotherapy, improve quality of life, and reducing cytopenias and peripheral neuropathy in patients. If effective, this therapy will: improve treatment regimens by making current chemotherapy regimens more effective, less toxic, and impact

survival - ie allowing full dose chemotherapy without the side effects of neuropathy, anemia, and other cytopenias.

P3-12-18: NEOMET: A phase II randomized trial to evaluate the impact of “targeted” nutritional support and exercise on modulation of metabolic and immune-related markers in early breast cancer (eBC) patients candidate to neoadjuvant therapy (NAT)

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Background: Metabolic reprogramming is a well-known hallmark of cancer. In breast cancer several metabolic pathways (including fatty acid metabolism) are frequently dysregulated and contribute to enhanced proliferation and resistance to treatments. Metabolomics is an “omics” approach that allows identification of metabolic host-related factors potentially correlated with treatment outcomes. Recent evidence from the SenNeoad trial showed that untargeted metabolic analysis can identify a metabolomic signature associated with a higher probability of achieving pCR after NAT. Among 445 small molecules analyzed, 61 were differentially expressed in patients with pCR vs residual disease (RD) ($p<0.05$); patients with RD showed lower levels of fatty acids, such as 9-hexadecenoic acid ($p <0.001$) and doconexent ($p<0.001$).
Trial Design: NEOMET is an exploratory randomized prospective multicenter study whose primary aim is to evaluate if plasma metabolomic signatures can be modified by lifestyle interventions including dietary supplements and physical exercise in eBC patients candidate to NAT. Eligible patients will be randomized to one of four groups: a) NAT according to molecular subtype; b) NAT plus nutritional supplementation; c) NAT plus supervised physical exercise; d) NAT plus nutritional supplementation plus supervised physical exercise. Nutritional supplementation will consist of two main long-chain polyunsaturated fatty acids omega-3 (n-3 Lc-PUFA), EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), plus a source of palmitoleic acid (hexadecenoic acid).
The primary objectives are: 1) to explore the impact of lifestyle interventions on the modulation of metabolomic signatures in eBC patients candidate to NAT; 2) to assess the Omega-3 Index at baseline and before surgery. Secondary objectives are: to estimate the effect of combined lifestyle intervention on patient metabolic profile and body composition; to correlate immune and metabolic-related gene-expression signatures evaluated on baseline tumor tissue with metabolic profiles identified on plasma and response to NAT; to evaluate potential pharmacokinetic interactions between nutritional supplementation and NAT; to assess pCR rate in different treatment arms; to analyse the impact of the different interventions on Quality of Life (QoL).
Due to the explorative intent of the study no formal sample size calculation is provided. A

total of 160 consecutive patients will be enrolled into the four arms of the study, with an allocation ratio equal to 1:1:1:1 (40 pts/arm). It can be estimated that the accrual will be completed in 20 months. Data collection, molecular assessments and data analysis with final scientific output are expected to extend the study duration to a total of 26 months.
Planned analysis will include: conversion rate from poor to good metabolomic prognostic signature from baseline to end of NAT; change in Omega-3 Index and body composition; correlation between metabolic and immune-related gene-expression signatures and response to therapy (pCR vs RD).
Clinical Trial Identification: not yet assigned
Funding: Support and funding for the study has been provided by MUR-PRIN 2022 and Pharmanutra S.p.A.
Date of registration: June 2024.
First patient enrolled: September 2024

P3-12-19: NRG-BR008: A phase III randomized trial of radiotherapy optimization for low-risk HER2-positive breast cancer (HERO)

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Background: Breast radiotherapy (RT) is the standard of care for patients with early-stage breast cancer (BC) who undergo breast-conserving surgery (BCS). However, the magnitude of benefit of RT is less clear in BCS patients with low-risk disease who receive effective systemic therapy. Among patients with early-stage HER2-positive (HER2+) BC, 10-year locoregional recurrence has been reported as low as 1.5% following BCS, adjuvant chemotherapy and HER2-targeted therapy, and RT. Given these exceedingly favorable outcomes, with the addition of HER2-directed therapy, we seek to evaluate the feasibility of omitting RT among patients with early-stage HER2+ BC following BCS and appropriate systemic therapy.

Methods: This is a phase III randomized trial for patients ≥40 years with early-stage, node-negative, HER2+ (IHC/FISH) BC treated with BCS with negative margins and sentinel lymph node biopsy or axillary dissection. Patients undergoing primary surgery must have pathologic T1 (≤2 cm) N0 disease, whereas patients receiving neoadjuvant therapy must have clinical T1-2 (with radiographically T≤3.0 cm) N0 disease and exhibit a pathologic complete response (ypT0N0) at surgery. All patients must receive cytotoxic chemotherapy and HER2-targeted therapy, either in the adjuvant or neoadjuvant setting. Stratification is by age (<60; ≥60), tumor size (≤1 cm; >1 cm), estrogen-receptor status (positive; negative), and systemic therapy sequencing (adjuvant v neoadjuvant). Patients will be randomized to standard breast RT in addition to continuation of trastuzumab to complete one year of treatment (Arm 1), or trastuzumab alone (Arm 2). Endocrine therapy will be recommended

for patients with hormone-receptor positive tumors. The primary endpoint is the recurrence-free interval (RFI). Secondary endpoints include time to ipsilateral breast recurrence, locoregional recurrence, disease-free survival, and overall survival, in addition to the 7-year ipsilateral breast recurrence rate among those not receiving RT. A health-related quality of life sub-study will assess differences in patient-reported breast pain and worry. We estimate a 7-year RFI of 97.5% with RT and allow for a clinically acceptable decrement of 3.63% without RT (7-year RFI of 93.87%; HR 2.5) to establish omission of RT as non-inferior. NRG-BR008 aims to enroll 1,300 patients over 4.5 years, yielding 80% power to detect the non-inferiority of RT omission with a one-sided α=0.05. We expect to observe the required 38 RFI events within 6 years of additional follow-up.
The NRG-BR008/HERO trial opened to accrual in March 2023.
Accrual is 32/1,300 as of July 9, 2024.
NCT #: NCT05705401.
Support: U10CA180868, -180822, UG1CA189867, U24CA196067.

P3-12-20: NRG-BR009: A phase III trial evaluating addition of adjuvant chemotherapy to Ovarian Function Suppression + Endocrine Therapy in premenopausal women with pN0-1, HR+/HER2- breast cancer (BC) and Oncotype Recurrence Score (RS) ≤25 (OFSET)

Shannon L. Puhalla, Gong Tang, Sandra M. Swain, Patricia A. Ganz, N. Lynn Henry, Reena S. Cecchini, Sonya A. Reid, Priya Rastogi, Charles E. Geyer, Jr., Julia R. White, Amy S. Clark, Tufia C. Haddad, Gregory A. Vidal, Norman Wolmark, Eleftherios P. Mamounas

Background: The TAILORx and RxPONDER trials demonstrated that RS identifies many postmenopausal pts with node-neg and node-pos BC and RS ≤25, who do not benefit from addition of ACT to endocrine therapy (ET). Both trials also showed that certain subsets of premenopausal pts (node-neg/high clinical risk/RS 16-20, node-neg/RS 21-25, and node-pos/RS ≤25) benefited from adding ACT to ET. Most premenopausal pts in these trials did not receive ovarian function suppression (OFS) as part of their ET regimen. Given the observed benefit from OFS in high-risk premenopausal pts with HR+/HER2- BC in the SOFT/TEXT trials, many questioned whether all or part of the observed ACT benefit in the TAILORx/RxPONDER trials may have been the result of chemotherapy-induced OFS. To address this question, we developed OFSET, a phase III, multicenter clinical trial comparing OFS+ET v ACT+OFS+ET.

Methods: We hypothesize that addition of ACT to OFS+ET is superior to OFS+ET in improving invasive breast cancer-free survival (IBCFS) among premenopausal, early-stage BC pts with HR+/HER2- tumors, and a 21-gene RS between 16-25 (for pN0 pts) and 0-25 (for pN1 pts). Secondary objectives include invasive disease-free survival, overall survival, distant recurrence-free interval, breast cancer-free interval, and health-related quality of life (HRQOL). Pts must be node-neg with RS 16-20 (plus high clinical risk), or RS 21-25, or

have 1-3 positive nodes with RS ≤25. Stratification is by nodal status/RS status (pN0 RS 16-25 v pN1 RS 0-15 and pN1 RS 16-25), intent to receive CDK4/6 inhibitor (yes; no), and age (18-39 v ≥40). Pts are randomized after surgery to either OFS+ET or ACT+OFS+ET. ET is an aromatase inhibitor (AI). Choice is per investigator discretion; tamoxifen is allowed if AI is not tolerated or if OFS is incomplete. Radiotherapy will be administered per investigator discretion per protocol guidelines. The HRQOL substudy will assess differences in severe menopausal symptoms, measured by the FACT ESS-19 score between arms, as well as increased pain severity (PROMIS). Blood and tumor specimens will be collected for future research.
Accrual of 3,960 pts is anticipated to be completed in 7 yrs, 7 mos. Per NSABP B-28 and RxPONDER data, 5-yr IBCFS of pN1 pts on the ACT+OFS+ET arm is estimated at 92.3%. Based on TAILORx data, 5-yr IBCFS of pN0 pts on the ACT arm is ~95%. Assuming 56% of pts to be pN0 and 44% pN1, and a 0.5% annual loss-to-follow-up rate, the definitive analyses to detect a hazard ratio: 0.75 with ACT+OFS+ET v OFS+ET, with one-sided α of 0.025 and 80% power, will require 380 IBCFS events, expected to occur ~11 yrs after study initiation. OFSET was activated August 2023.
Accrual is 69/3,960 (as of July 3, 2024).
NCT #: NCT05879926.
Support: U10CA180868, -80822, UG1CA189867, U24CA196067.

P3-12-21: NeoTAILOR: A phase II biomarker-directed approach to guide neoadjuvant therapy for patients with clinical stage II/III ER+, HER2-negative breast cancer

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Background: Although aromatase inhibitors are the mainstay of therapy (tx) in postmenopausal women with early-stage ER-positive (ER+), HER2-negative (HER2-) breast cancer (BC), optimal tx strategies remain to be elucidated in high-risk, early-stage BC, particularly in Black women who have historically been underrepresented in BC clinical trials. Hormone-receptor expression remains the single predictive biomarker guiding endocrine therapy (ET) use, yet ET response remains heterogeneous and ~20-30% of patients (pts) will relapse. Molecular heterogeneity of BC, including molecular BC subtype (via PAM50), likely contributes to the observed differential responses to standard neoadjuvant (neo) ET and chemotherapy (chemo). Several studies have also demonstrated that proliferative changes by Ki67 expression post short-course neo ET (Ki67post) serves as an early pharmacodynamic marker of ET response, correlates with long-term outcomes and thus may guide personalized therapeutic decision-making. Routine genomic assays traditionally utilized in the adjuvant setting have helped identify pts who may be spared chemo toxicity, but are less prognostic in Black women, and significant racial disparity in survival exists. Few studies have had sufficient data on intrinsic subtype and recurrence

outcomes in Black women. An adaptive strategy utilizing dynamic Ki67 changes post neo ET may help to identify pts who will achieve excellent clinical response to neo ET, and others who will require tx escalation in a rapid setting. Prospectively integrating genomic signatures into the neo tx selection paradigm may permit a personalized biomarker-directed approach to better tailor optimal tx strategy to improve clinical response.

Methods: NeoTAILOR (NCT05837455) is a phase 2 clinical trial investigating the use of clinical genomic signatures to guide neo tx for postmenopausal women with clinical stage II/III ER+, HER2- BC using a dynamic Ki67 response-adaptive strategy, with a 33% minority enrollment goal to permit analysis of outcomes in a racially diverse patient population. Eligible pts (n=81) will undergo baseline (BL) breast MRI and tumor/blood collection, followed by a 28-day cycle of anastrozole (ana) (+/- 14 days, C1), during which BC risk category will be determined based on BL tumor Ki67 and study-funded Prosigna(R) PAM50 subtype. Pts with protocol-defined low-risk disease (Luminal A subtype or BL Ki67 ≤10%) will continue to receive 5 additional 28-day cycles of ana (C2-6). Pts who are considered to have high-risk BC (non-Luminal A or non-diagnostic subtype) will undergo breast tumor tissue collection at Week 4 for Ki67post assessment. If Ki67post ≤10% (high-risk endocrine-sensitive (ETsens) group), pts will continue to receive 5 additional 28-day cycles of ana (C2-6), while pts with Ki67post >10% (high-risk endocrine-resistant (ETres) group) will receive escalated tx with SOC chemo (combination anthracycline- and/or taxane-based). All pts will then undergo breast MRI followed by surgery. Adjuvant tx will be at the treating physician's discretion. The primary endpoint is to determine the objective response rate (ORR) by breast MRI in the pooled protocol-defined low-risk plus high-risk ETsens group. Key secondary endpoints include: breast conserving surgery rate, change in need for oncoplastic breast reduction surgery, ORR in the high-risk ETsens and ETres groups. Exploratory endpoints include delineating mechanisms of ET response and resistance by race. A sample size of 77 evaluable pts will be needed to obtain 23 pts with protocol-defined high-risk ETsens BC to test a desired ORR of 75% against a null ORR of 50%, by 1-sided 1-sample proportion test (alpha=5%, 80% power). If responses are observed in ≥16 pts among 23 ORR evaluable pts, the trial claims preliminary efficacy. The study is actively enrolling pts at Siteman Cancer Center.

P3-12-22: Neoadjuvant Therapy with Pyrotinib, Subcutaneous Trastuzumab, and Capecitabine for HER2-Positive Early Breast Cancer: A Prospective, Single-Arm, Multicenter Trial

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Background: Neoadjuvant therapy has become a standard approach for HER2-positive early breast cancer, improving surgical outcomes, pathological complete response (pCR) rates, and survival. Previous studies have demonstrated the efficacy and safety of pyrotinib, an

oral, highly selective tyrosine kinase inhibitor, combined with trastuzumab and chemotherapy in this setting. However, these regimens primarily relied on intravenous administration, which can impact patient quality of life and increase treatment burden. This prospective trial is the first to investigate a fully non-intravenous neoadjuvant regimen consisting of oral pyrotinib, SC trastuzumab, and oral capecitabine in low- to intermediate-risk HER2-positive early breast cancer patients, aiming to evaluate its efficacy and safety.

Methods: This prospective, single-arm, multicenter trial (NCT06483386) will enroll 109 women with low- to intermediate-risk HER2-positive early breast cancer (clinical stage I-II, T1c-2N0-1M0) from May 2024 to June 2026. Eligible patients are aged ≥18 years, have no prior treatment, an ECOG performance status of 0-1, and at least one measurable lesion. HER2 positivity is defined as immunohistochemistry (IHC) 3+ or IHC 2+/fluorescence in situ hybridization (FISH) positive, regardless of hormone receptor status. Patients will receive 6 cycles of neoadjuvant therapy consisting of oral pyrotinib (400 mg daily), SC trastuzumab (600 mg on Day 1 every 3 weeks), and oral capecitabine (1000 mg/m2 twice daily for 14 days followed by a 7-day break). Treatment response will be assessed according to RECIST 1.1 criteria, and subsequent management will be determined by the investigator. The primary endpoint is pCR rate, with secondary endpoints including event-free survival (EFS), overall response rate (ORR), disease control rate (DCR), breast-conserving rate, and safety.

Conclusions: This prospective, single-arm, multicenter trial will provide valuable insight into the efficacy and safety of a novel, fully non-intravenous neoadjuvant regimen combining pyrotinib, SC trastuzumab, and capecitabine for low- to intermediate-risk HER2-positive early breast cancer patients. If proven effective and well-tolerated, this regimen may offer a more convenient and less burdensome treatment option for this patient population, potentially improving quality of life and treatment adherence.

P3-12-23: PATIENT ENGAGEMENT IN RESEARCH: CINDERELLA TRIAL AND LESSONS LEARNT FROM SCREENING FAILURES

André Pfob, Rosa Di Micco, Silvia Paola Corona, Eduard-Alexandru Bonci, Veronica Zuber, Sara Baleri, Giovanni Cisternino, Francesca Calabretto, Mario Rampa, Manuela Morgante, Vitalba Scaduto, Angelica Critelli, Nicole Rotmensz, Maciej Bobowicz, Jörg Heil, Pawel Kabata, Orit Kaidar-Person, Maria-Joao Cardoso, Oreste Davide Gentilini

Background: CINDERELLA is a randomised controlled multicentre clinical trial initiated with the aim of addressing the need for a more patient-centred care, enhancing shared decision-making, satisfaction, and health-related quality of life in breast cancer patients eligible for locoregional treatment. The project focuses on increasing patient awareness and engagement throughout their breast cancer journey, by providing easily accessible, always available, tailored, written and visual information. Despite its patient-centric design, the

trial recorded a significant number of screening failures in its initial year of enrolment. Our objective is to explore the reasons behind screening failures among patients recruited during the initial phase of the trial, aiming to provide valuable insights that can enhance future recruitment efforts or the design of new trials. To achieve this objective, we propose an unplanned analysis conducted in two phases: first, a pilot phase involving data collection from a single recruiting centre, followed by analysis and evaluation by the Trial Scientific Committee; subsequently, based on the findings from the initial phase, a second observational phase involving all recruiting centres.
TRIAL DESIGN: Data regarding screening failures in the randomised controlled CINDERELLA trial (ClinicalTrials.gov ID NCT05196269) from IRCCS San Raffaele Hospital (Milan, Italy) were compiled into a prospective database from October 1st, 2023, to July 3rd, 2024. Reasons for refusal to participate in the trial were categorised into 3 distinct groups as follows:
- Emotional distress: defined as potential patient anxiety arising from "too much information", namely learning more about surgery techniques, potential surgical complications, or visualization of postoperative images;
- Time constraints: defined as time constraints and necessary commitment to understand the project's details, complete informed consent forms and multiple questionnaires;
- Long distance (and other contingent-logistic issues): defined as long distance or other logistical challenges in reaching the hospital, which limit the patient's ability to attend subsequent follow-up appointments for postoperative photographs.
ENDPOINT: The primary endpoint of this analysis is to identify the reasons behind screening failures and potentially propose new strategies for better inclusivity, diversity, and equity by decreasing the number of patients who decline participation in this and other future trials, despite fulfilling inclusion/exclusion criteria.
CURRENT STATUS: During the enrolment period, 144 patients were screened at IRCCS San Raffaele Hospital. Among them, 43 patients (29.8%) declined participation. Reasons for refusal included: emotional distress in 21 cases (14,6%), time constraints in 14 cases (9.72%), and distance from the hospital in 8 cases (5,55%). The mean age of these patients was 57. Out of the 43 who declined, 34 underwent breast conservation (79%) while 9 underwent mastectomy with reconstruction (21%). Breast cancer staging revealed stage I in 36 patients and stage II-III in 7 patients.
FUTURE PLANS: To date, 489 patients have been recruited in the CINDERELLA trial. After examining results of this single-centre preliminary analysis, the Trial Scientific Committee proposed a study protocol amendment to start prospective data collection on the reasons behind screening failures. In this second analysis across all study sites, patients who decline to participate in the trial will be asked to complete a paper questionnaire detailing their reasons for refusal. Data collection for this second phase is scheduled to begin in September 2024.
FUNDING. European Union grant HORIZON-HLTH-2021-DISEASE-04-04 Agreement No. 101057389

P3-12-24: PREMO CNS: PREsentation, Management and Outcomes of patients with CNS disease secondary to breast cancer in England

Talvinder Bhogal, Kukatharmini Tharmaratnam, Christopher Cheyne, Gary Leeming, Marta Garcia-Finana, Carlo Palmieri

Background: Breast cancer is the second most common primary tumour to metastasise to the brain. Improvements in the systemic treatment of extracranial metastatic disease have resulted in patients surviving longer with their metastatic disease, which appears to be contributing to the increase in the incidence of cerebral metastases. There is a need to better understand the number of patients affected, their outcomes and treatments in the context of current modern oncological treatment. Finally, it is recognised that inequality can exist with regard to treatment. This study aims to document the burden treatment and survival from CNS disease secondary to breast cancer in England utilising national cancer registry data.

Study Design Methods: The study is a retrospective, cohort study using population-based registry data from The National Cancer Registration and Analysis Service (NCRAS) which is managed by NHS Digital (NHS England). Individual data on approx. 28,000 consecutive patients with CNS disease treated between 1995 and 2023 within the English Healthcare System will be utilized. Datasets utilised include: Hospital episode statistics (HES), Hospital Admitted Patient Care Activity (HESAPC), Systemic Anti-Cancer Therapy (SACT) and Radiotherapy Data Set (RTDS). Eligibility criteria: Inclusion Criteria Male or female, aged >16 years, Histologically and/or cytologically confirmed breast cancer with CNS involvement, as defined as having one or more of the following: a) Metastases to the brain parenchyma; b) Metastases to the leptomeninges c) Paraneoplastic Neurological Disorders. There were no formal exclusion criteria. This data release was approved by NHS Digital (ODR2021_030).

Study Objectives: The primary objectives are to audit the overall survival from the initial diagnosis of CNS involvement secondary to breast cancer in English centres. With Secondary objectives will include to audit (1) the number of cases of metastatic breast cancer (MBC) involving the CNS presenting per year. (2) the current practice in regarding the diagnosis and management of CNS disease and (3) the outcomes of patients treated for CNS involvement secondary to breast cancer. Dara will be analysed by breast cancer subtype as well as overtime, by geographical location and by deprivation index.

P3-12-25: PRSONAL - Population-based Randomized Study Of a Novel breast cancer risk ALgorithm and stratified screening

Stig Bojesen, Pia R Kamstrup, Bodil Ørkild, Berit Andersen, Ilse Vejborg, Antonis Antoniou, Janne Bigaard

Purpose: To measure short-term safety and efficacy of personalized versus standard biennial mammography screening among 50-67-year aged women.
Method: Randomized clinical trial of 962 consenting Danish women aged 50-67 years attending regular screening mammography. At recruitment all women fill in questionnaires on personal risk factor information, quality of life, anxiety and breast cancer worry. Height, weight and BIRADS density are also measured. Women in the intervention have blood sampled for measuring polygenic risk score for breast cancer.The control group will continue with standard biennial mammography, while women in the intervention arm will be grouped in categories based on their absolute 10-year breast cancer risk using the BOADICEA model. Risk categorization will be communicated on-line together with a suggested future screening program for each group: low risk (mammography every 4th year), intermediate risk (mammography every 2nd year), elevated risk (mammography every year), and high risk (MR mammography every year). Women could call the project telephone for questions.

Outcomes: Primary outcome is the acceptance of prolonged screening interval among low-risk women in the intervention group. Secondary outcomes consist of anxiety, breast cancer worry and quality of life. Tertiary outcomes include health care staff time spent on consultations after communication and suggested screening program.

Status: Recruitment began Feb. 1st 2024 and by June 30th, 851 women had been randomized. Of the 216 women who have received risk estimates, there were 109, 85, 17 and 5 in the low, intermediate, elevated and high risk group, respectively. This distribution was not different from the expected 46%, 44%, 8.5% and 1.5%, respectively (p=0.08). The automated on-line risk communication lead to 10 seconds telephone call per woman.

Conclusion: Recruitment, collection of risk factor information, real-time calculation of risk and automated communication of risk is possible and seems acceptable to the women in this randomized trial of personalized versus standard biennial mammography screening.

P3-12-26: Partial breast re-irradiation using ultra hypofractionation: A Phase 2 multi- institutional study

Danielle Rodin, Fadwa Abdel Rahman, Michelle Audoin, Aisling Barry, Jean-Marc Bourque, Keelan Byrne, Michelle Chan, Hanbo Chen, Eileen Connolly, Marc David, Jane De Rocchis, Frances Duane, Elizabeth Evans, Naamit Gerber, Guilherme Gondim, Revathy Krishnamurthy, Zhihui Liu, Tom Purdie, Valerie Theberge, Timothy Whelan, Martina Wood, Michael Yassa, Eileen Rakovitch, Anne Koch

Background: Most patients with breast cancer are treated with breast conserving surgery (BCS) followed by adjuvant radiation (RT) to reduce the risk of recurrence in the same

breast (local recurrence (LR)) and to maximize long-term breast preservation. Although these treatments are quite effective, a growing number of individuals who receive them are at risk of LR or develop a second primary breast cancer, which is traditionally treated with mastectomy. However, mastectomy has been associated with deleterious effects on quality of life. Advances in screening now allow many LRs to be detected as localized, small tumours amenable to further BCS.
BCS followed by reirradiation with partial breast irradiation (rPBI) has recently been found to be a safe treatment option in women with prior breast RT, but the optimal fractionation is unknown. For women with early-stage breast cancer receiving upfront treatment, a shorter 1-week course of breast RT (ultra-hypofractionation) has been found to be equivalent to longer fractionation schedules. However, this data cannot be directly applied to LR due to the higher cumulative RT doses and to tissue changes from previous treatment. The safety and efficacy of ultra-hypofractionated rPBI for LR are the focus of the proposed study. We hypothesize that ultra-hypofractionated rPBI following BCS for LR or new primary breast cancer in the previously irradiated breast will be associated with acceptable toxicity at 1 year (<13% grade >3 toxicity).

Methods: This study is a phase II, prospective, multi-center, international trial of ultra-hypofractionated rPBI following repeat BCS for LR or new primary breast cancer in the previously irradiated breast. rPBI will be delivered at a dose of 26 Gy in 5 daily fractions over a period of 1-week (excluding weekends and statutory holidays). Boost RT is not permitted. The primary endpoint is the risk of grade >3 adverse events (AEs) occurring at 1-year from rPBI completion (CTCAE v5.0). Secondary endpoints include local, regional and distant recurrence, invasive breast cancer-free survival, mastectomy-free survival, overall survival, financial toxicity, and patient-reported satisfaction. To achieve 80% power with a Type I error rate of 0.05 and 3% lost to follow up or unevaluable, 171 patients will be accrued.
Eligible patients are status post BCS for ductal carcinoma in situ or invasive cancer <3 cm in greatest diameter (invasive and non-invasive components) with negative margins (no tumor on ink) who are clinically node negative and who completed treatment >5 years earlier for breast cancer in the ipsilateral breast treated with BCS and adjuvant whole or partial breast RT. Patients with grade >2 late skin toxicity from prior radiation are excluded, as are those with T4 or multicentric disease or the presence of an extensive intraductal component. Final eligibility is determined at the time of RT planning based on ability to clearly define the surgical cavity. The RT planning target volume must be <50% of the whole breast.
The study is open to accrual 7 sites in Canada, the US, and Jordan, with an additional 9 international centers in the process of opening; 17 patients have been enrolled and treated as of 10-07-2024. Patients will be followed for a total of 5 years. Clinical trial information: NCT05592938. Supported by the Canadian Cancer Society and the Princess Margaret Cancer Foundation.

P3-12-27: Phase 1 Analysis from the PYNNACLE Phase 1/2 Study of Rezatapopt in the Subgroup of Patients with Advanced Breast Cancer Harboring a TP53 Y220C Mutation

Ecaterina Dumbrava, Shivaani Kummar, Melissa Johnson, Kim Le Duke, Yajuan G Qin, Marc Fellous, Alison M Schram

Background: Mutations in the TP53 gene occur in ~51% of breast cancers and result in loss of p53 tumor suppressor function and tumor progression. Reactivation of wild-type p53 is an attractive therapeutic approach for breast cancers with a TP53 mutation including the more aggressive triple-negative breast cancer (TNBC), where treatment options are limited. Rezatapopt (also known as PC14586) is an investigational first-in-class, p53 reactivator that selectively binds to the mutated p53 Y220C protein and stabilizes the structure in wild-type conformation, thereby restoring p53 wild-type activity. PYNNACLE (NCT04585750) is a Phase 1/2 clinical trial of rezatapopt in patients with locally advanced or metastatic solid tumors harboring the TP53 Y220C mutation. In Phase 1, rezatapopt demonstrated favorable safety and anti-tumor activity in heavily pre-treated patients (n=67 treated at the efficacious dose range of 1150 mg once daily [QD] to 1500 mg twice daily [BID]). This subgroup analysis assessed rezatapopt in patients with advanced breast cancer treated across the efficacious dose range.

Methods: Patients with locally advanced or metastatic breast cancer with a TP53 Y220C mutation were eligible to receive rezatapopt orally on a continuous schedule across the efficacious dose range (1150 mg QD to 1500 mg BID). Safety and preliminary efficacy, as evaluated by the investigator using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, were assessed. Tumor next-generation sequencing was used to determine TP53 Y220C, BRCA, PIK3CA, and KRAS tumor mutation status.

Results: As of September 5, 2023, nine patients with breast cancer (HR+/HER2- n=3; HR+/HER2+ n=1; HR-/HER2+ n=1; TNBC n=4) received rezatapopt in the PYNNACLE Phase 1 trial. The median age was 53 years (range 32–65 years) and Eastern Cooperative Oncology Group (ECOG) performance score was 0 (n=3) or 1 (n=6). One patient had a somatic BRCA2 mutation, no patient had a BRCA1 mutation, two patients had a PIK3CA mutation, and all patients were KRAS wild-type. The median number of prior lines of systemic therapy was 4 (range 2–9). Of the eight patients who had measurable disease at baseline, three (37.5%) achieved a confirmed partial response (PR), four had stable disease (SD), and one had progressive disease (PD) as the best objective response. Reductions in target lesions were reported in all patients, with a maximum reduction in tumor volume from baseline ranging from -2.4% to -55.2%. The most frequent treatment-related adverse events in patients with breast cancer were consistent with the overall Phase 1 PYNNACLE population with solid tumors treated at the efficacious dose range (n=67), and included nausea (51%), vomiting (43%), and increased blood creatinine (27%), which were mostly

grade 1/2. Administration of rezatapopt with food led to an improvement in gastrointestinal adverse events including nausea and vomiting.

Conclusions: In this Phase 1 PYNNACLE trial, rezatapopt demonstrated promising preliminary single-agent efficacy in heavily pre-treated patients with advanced breast cancer, including TNBC, harboring a TP53 Y220C mutation. Rezatapopt had a favorable safety profile in the efficacious dose range with improvements in gastrointestinal adverse events observed when administered with food. The PYNNACLE tumor-agnostic registrational Phase 2 trial, which includes a breast cancer cohort, will assess rezatapopt as monotherapy at the recommended Phase 2 dose of 2000 mg QD with food in patients with TP53 Y220C-mutated and KRAS wild-type advanced solid tumors.

P3-12-28: Phase 1b study of EZH1/2 inhibitor valemetostat in combination with trastuzumab deruxtecan in subjects with HER2 low/ultra-low/null metastatic breast cancer

Toshiaki Iwase, Senthil Damodaran, Angela Marx, Funda Meric-Bernstam, Debu Tripathy, Carlos Hernando Barcenas, Jangsoon Lee, Naoto T Ueno

Background: Low HER2-expressing breast cancers have traditionally been classified as HER2-negative and treated as TNBC or hormone receptor (HR)-positive. Trastuzumab deruxtecan (T-DXd), an antibody-drug conjugate composed of an anti-HER2 antibody conjugated to topoisomerase I payload, is approved for clinical use in HER2-low (IHC 1+ or IHC 2+/ISH negative) metastatic breast cancer (MBC). In the DAISY (NCT04132960) and DESTINY-Breast06 (NCT04494425) studies, meaningful clinical response and survival benefit were observed with T-DXd in HER2 IHC 0 MBC. Valemetostat is an oral, selective dual inhibitor of enhancer of zeste homolog 1 and 2 (EZH1/2, methyltransferases that specifically methylate histone H3 lysine 27. It is approved for patients with relapsed or refractory adult T-cell leukemia/lymphoma in Japan. EZH2-mediated PP2A inactivation has been shown to confer resistance to HER2-targeted therapy. Additionally, valemetostat has been shown to upregulate Schlafen11 (SLFN11), a putative DNA/RNA helicase that regulates the sensitivity to DNA-damaging agents such as topoisomerase I inhibitors. Accordingly, this study examines the safety and anti-tumor activity of valemetostat in combination with T-DXd in subjects with HER2 low/ultra-low/null MBC.
Trial Design
This is a single-arm, phase-1b study to evaluate the safety and clinical activity of T-DXd in combination with valemetostat in patients with HER2 low/ultra-low/null MBC. The dosing for T-DXd is 5.4 mg/kg Q3W administered intravenously as indicated for current clinical use. Valemetostat will be evaluated in up to five doses (50mg, 75mg, 100 mg, 150 mg, and 200 mg) with a starting dose (level 1) at 100 mg QD. The dose-limiting toxicity (DLT) evaluation period will be the first 2 treatment cycles (42 days).
Eligibility criteria

Pathologically confirmed HER2 low/ultra-low/null breast cancer
ECOG performance status ≤1
Measurable disease (for dose-expansion cohort)
Received at least one line of chemotherapy in the metastatic setting
Progressed and would no longer benefit from endocrine therapy (HR-positive)
Normal organ and marrow function
Exclusion: symptomatic brain metastases, interstitial lung disease, cord compression, prior treatment with any anti-HER2 therapy

Specific aims: To evaluate the safety and determine the maximum tolerated dose (MTD)/recommended dose for expansion (RDE) of valemetostat in combination with T-DXd.
To evaluate the objective response rate (ORR) of valemetostat at the RDE in combination with T-DXd
To determine the duration of response, clinical benefit rate, progression-free survival, and overall survival of valemetostat at the RDE in combination with T-DXd.
To evaluate the pharmacokinetics and pharmacodynamic markers of valemetostat and T-DXd combination
To evaluate the immunogenicity of T-DXd when co-administered with valemetostat

Statistical methods: Approximately 15 evaluable patients will be enrolled for the dose-escalation portion based on the Bayesian optimal interval design with a target DLT rate of 25%. Patients enrolled and treated in cohorts of 3. The expansion will be performed at the RDE using the 2-stage Bayesian optimal dose-expansion design. In the first stage, 13 evaluable patients (including those treated at the RDE in the dose-escalation part) will be enrolled. If <5 patients respond in the first stage, the study will be stopped for futility. If ≥5 responses are observed, 13 additional evaluable patients will be enrolled. If 11 or more responses are observed among 26 patients, the treatment will be considered promising. This two-stage design yields 78% power under the alternative hypothesis of ORR = 50% (null ORR = 30%) while controlling the one-sided type I error at 10%.

P3-12-29: Phase 1b/2 Study of Naxitamab (Danyelza), Gemcitabine and Ex Vivo Expanded Allogenic Universal Donor, TGFβi Natural Killer (NK) cells in Advanced GD2-expressing Breast Cancers (DiG NKs)

Margaret Gatti-Mays, Marcelo de Souza Fernandes Pereira, Sumithira Vasu, Lynn O'Donnell, Marcos DeLima, Zihai Li, Daniel Stover, Robert Wesolowski, Sagar Sardesai, Gilbert Bader, Mathew Cherian, Kai Johnson, Ashley Pariser Davenport, Dionisia Quiroga, Nicole Williams, Joanne Kim, Dean Lee

Background: Intrinsic resistance to immunotherapy observed in breast cancer is attributed to low neoantigen levels, defective antigen presentation, low mutational burden, reduced

programmed death ligand 1 (PD-L1) expression and the presence of immunosuppressive signals like transforming growth factor-beta (TGFβ) in the tumor microenvironment (TME). These collectively attenuate the effector functions of T cells and natural killer cells (NK cells). NK cells are an important component of innate immunity.

We designed a clinical trial to evaluate the stepwise addition of (1) gemcitabine, (2) an off-the-shelf, TGFβ imprinted, IL-21 expanded allogenic universal donor NK cells (TGFβi NKs), and (3) naxitamab (FDA-approved humanized IgG1 monoclonal antibody targeting the tumor-associated antigen GD2) to overcome intrinsic resistance to immunotherapy in breast cancer. While generally TGFβ dampens the NK cell response in the TME, chronic stimulation of donor cells ex vivo with TGFβ during the expansion process produces TGFβi NKs which exhibit high cytotoxicity and resistance to suppression by TGFβ. Furthermore, the anti-tumor activity of monoclonal antibodies, like naxitamab, is enhanced by NK cells. Given high GD2 expression in aggressive breast cancer subtypes, there is a strong rationale for evaluating an anti-GD2 antibody along with NK cells in breast cancer. Finally, systemic chemotherapies like gemcitabine exhibit both cytotoxic effects as well as immunomodulatory effects including lymphodepletion and improved NK cell recruitment through upregulation of NKG2DL. In our preclinical assessment of this combination in an MDA-MB-231 mouse model, we found longer survival and no evidence of toxicity with gemcitabine + anti-GD2 antibody + TGFβi NKs.

Methods: In this phase Ib/II trial, the safety and efficacy of the doublet (gemcitabine + TGFβi NKs) and the triplet (gemcitabine + TGFβi NKs + naxitamab) is being assessed. . Patients with advanced/metastatic, HER2 negative, breast cancer that is measurable and have received ≥1 line in the metastatic setting are eligible for the trial. Patients must be ≥ 18 years old with a good functional status and adequate organ and bone marrow function. Key exclusion criteria include symptomatic/recently treated brain metastases (defined as < 6 months), leptomeningeal disease, cardiomyopathy, history of myocarditis, or immunocompromised status.
Patients receive gemcitabine 800mg/m2 on days 1, 8. 15 of a 28-day cycle. TGFβi NKs 3x107 cells are given on day 16 of each cycle and NK cell persistence is assessed on day 21. Naxitamab 2.4mg/kg (max 150mg/day per prescribing information) is given on day 1, 4, and 8 per the current recommend phase 2 dose along with GM-CSF. Dose limiting toxicities are evaluated in a modified 3+3 design. Clinical response is evaluated every 8 weeks. Co-primary objectives are response rate (ORR) per RECISTv1.1 and safety. Exploratory analyses include changes in immune cells and cytokines in the peripheral blood. While GD2 expression is not required for eligibility, we will assess clinical and immune outcomes by GD2 expression of archival tissue. Other exploratory outcomes including progression free survival and ORR per iRECIST. If there is adequate safety of the combination, subsequent patients will be enrolled to determine initial clinical efficacy of the combination using a Simon-two step design.
We plan to recruit up to 39 evaluable patients, with an accrual ceiling set at 45 patients.

This study is open and enrolling at The Ohio State University Comprehensive Cancer Center (NCT06026657, Columbus OH).

P3-12-30: Phase I trial of pegylated liposomal doxorubicin chemotherapy in combination with CD40 agonist and Flt3 ligand in metastatic HER2 negative breast cancer

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Background: Only a subset of patients with metastatic breast cancer respond to FDA approved immune checkpoint blockade, and few have durable responses. Data suggest that breast cancers have defects in antigen presentation and that antigen presenting dendritic cells (DCs) are required for response to T cell directed immunotherapies such as immune checkpoint blockade. CD40 agonists activate antigen presenting cells, including DCs and B cells, and repolarize macrophages to an anti-tumor phenotype. Flt3 ligand is a growth factor that increases differentiation and expansion of DCs. We recently demonstrated in pre-clinical breast cancer models that the combination of liposomal doxorubicin chemotherapy, a CD40 agonist, and a Flt3 ligand improves outcomes compared to chemotherapy alone. Methods: This is a single arm phase I pilot study of the combination of liposomal doxorubicin, CDX-1140 (CD40 agonist monoclonal antibody), and CDX-301 (recombinant Flt3 ligand) in patients with metastatic or unresectable locally advanced HER2 negative breast cancer. Patients are randomized to 2 lead-in arms in 2:1 ratio (triplet therapy or liposomal doxorubicin only) for one cycle prior to receiving triplet therapy with tumor biopsies done before and after the lead-in treatment. A recent protocol amendment expanded eligibility from triple negative breast cancer (TNBC) to also include hormone receptor positive (HR+) breast cancer and closed a lead-in cohort of immunotherapy only. CDX-301 will be given for two cycles; liposomal-doxorubicin and CDX-1140 will be continued until disease progression or clinically limiting toxicities. Primary endpoint is determination of a recommended phase II dose of CDX-1140 based on treatment-related adverse events and dose-limiting toxicities. Secondary endpoints include anti-tumor immune response after triplet therapy and after liposomal doxorubicin alone, median progression-free survival, overall response rate, duration of response, and clinical benefit rate. Key eligibility criteria are unresectable stage III or stage IV HER2 negative breast cancer, for TNBC up to 3 prior therapies for metastatic disease allowed, for HR+ disease prior cyclin dependent kinase 4/6 inhibitors required and up to 3 prior lines of chemotherapy and/or antibody drug conjugates for metastatic disease allowed, measurable disease by RECIST 1.1 criteria, consent for pre- and on-treatment biopsies of tumor lesions, no prior treatment with an anti-CD40 antibody or a Flt3 ligand, no anthracycline treatment in the metastatic setting, no prior progression while on anthracycline-based therapy or within 6 months of completing (neo)adjuvant anthracycline-based therapy, and no history

of non-infectious pneumonitis or current pneumonitis. This trial will enroll up to 30 evaluable patients across multiple sites (NCT05029999) and is currently open at University of Texas Southwestern Simmons Cancer Center, Texas Oncology, University of Chicago, University of Texas San Antonio, and Johns Hopkins.

P4-01-01: An Innovative Approach to Alleviating Fatigue in Metastatic Breast Cancer Patients

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Background: Fatigue is debilitating for patients with metastatic breast cancer (MBC), impacting quality of life (QoL), pain, sleep, depression, and anxiety. Slow, whole-body vibration in the 0.01-0.3 Hz range may increase parasympathetic tone. Vibration at 100 Hz may activate the posterior insula, associated with increased attention to interception as seen in many meditative practices. This proof-of-concept study explores the potential of Apollo (Pittsburgh, PA), a wearable vibration delivery device, to mitigate fatigue in MBC patients.

Methods: In an 8-week single-arm trial, 27 women with MBC scoring $^{3}4$ on a Likert scale of 0-10 for fatigue self-applied the Apollo bracelet to the wrist or ankle. After informed consent and teaching, the device was used per manufacturer's instructions to deliver vibration on a user-determined schedule and intensity. Fatigue and QoL were measured at baseline, 4 weeks, and 8 weeks. Measures of efficacy included Patient-Reported Outcomes Measurement Information System (PROMIS) – fatigue, PROMIS – pain interference, PROMIS – sleep disturbance, Functional Assessment of Chronic Illness Therapy (FACIT) – Fatigue, Functional Assessment of Cancer Therapy – General (FACT-G), and Hospital Anxiety and Depression Scale (HADS). A repeated measures ANOVA was conducted at 4 weeks and 8 weeks.

Results: Of 27 patients enrolled, 23 completed the study and 4 withdrew (unrelated hospitalization (2), inconvenience of wearing the device (2)). The mean usage time of Apollo was 6575 minutes (range 216-26715). PROMIS-fatigue scale improved from 13.96 at baseline to 10.95 at 8 weeks ($p<0.001$). FACIT-fatigue score improved from 17.07 to 24.58 at 8 weeks ($p<0.001$). PROMIS-pain interference scale decreased from 10.04 to 7.19 at 8 weeks ($p=0.005$), and PROMIS-sleep disturbance declined from 11.44 to 9.29 ($p=0.014$). HADS-depression scores declined from 5.93 to 4.67 at 8 weeks, ($p=0.046$) while HADS-anxiety did not show a significant change ($p=0.160$).

Conclusions: MBC-related fatigue is challenging to treat and adversely affects QoL. Therapeutic vibration via Apollo may be a non-invasive way to alleviate fatigue and related outcomes in MBC. Placebo-controlled randomized trials are needed to confirm its efficacy.

P4-01-02: QUALITY OF LIFE IN BREAST CANCER PATIENTS UNDERGOING NEOADJUVANT CHEMOTHERAPY IN A BRAZILIAN CANCER CENTER: THE IMPACT ON YOUNG WOMEN

Fabiana Makdissi, Isabela Rodrigues Neves, Silvana Soares Dos Santos, Solange Moraes Sanches

Introduction: Quality of life/QoL is a subjective concept assessed from the patient's perspective, which encompasses physical, social and emotional functions of individuals. In recent decades, this concept has become an important outcome measure in breast cancer/BC patients, as it is the most frequent malignant neoplasm in women worldwide and due to the evolution in treatments, enabling an increase in the cure and survival rates of these patients.
Objectives: To evaluate the quality of life of patients undergoing neoadjuvant chemotherapy at a Brazilian Cancer Center.
Methods: Prospective cohort study carried out between 2022 and 2023 at A.C. Camargo Cancer Center. The study included 149 participants with non-metastatic breast cancer undergoing chemoradiotherapy; all of them signed an informed consent form approved by the local Research Ethics Committee. The study participants filled in assessment instruments, the "Psychosocial Well-being/PWB", "Sexual Well-being/SWB" and "Breast Satisfaction/BS" scales, translated into Portuguese, from the BREAST_Q Portfolio (https://qportfolio.org/breast-q/). Data was collected at M0 (pre-CT), M1 (halfway through the protocol) and M2 (end of CT). For the analysis of the scale scores, we considered $p < 0.05$. Results: Of the 149 women included, around 74.% were between 45-59 years of age; almost 70.0% were married or had a partner; 72.0% reported having some religion and 82.6% had at least a university degree. As for BC, almost half were Stage II (47.0%), of which 59.7% were T2 and 41.6% had a positive axilla (N+); 94.0% were invasive ductal carcinoma; 50.3% had B luminal tumors and 42.2% were triple negative. The median score on the "PWB" scale fell from 80 to 60 over the course of treatment ($p<0.05$); on the "SWB" scale, the reduction was from 62.5 to 45 ($p<0.05$). On the "BS" scale, there was a reduction from 81 to 68, but $p>0.05$. We checked whether education, marital status, religion and immunophenotype had an impact on the various scales, but all the analyses showed $p>0.05$. For the "SWB" scale, age was not significant either. For the "PWB" scale, there was a significant reduction for all ages ($p<0.05$); the greatest impact on QoL was between 45-59 years, with a 19-point reduction in the score over the course of treatment. In addition, we noted that on this scale, the final median score after treatment remained higher among women aged 60+ compared to younger women, up to 44 years old ($p<0.05$). The same happened with the "BS" scale. Discussion and Conclusion: In this study, we were able to observe the impact of CT on the QoL of women with BC. The reduction was already noticeable in the first assessment after starting treatment (M1), and became more pronounced at the end of treatment (M2). Chemotherapy treatment is responsible for changes in a woman's external image, but it is also responsible for transforming the perception of her internal image, i.e. the image a woman has of herself, which is subjective and corresponds to her identity. These bodily changes remind women of their illness,

affecting their sense of femininity and self-esteem. This has been demonstrated especially in younger women, who seem to be more affected by image-related symptoms. The impact on quality of life can even reduce the effectiveness of self-care and symptom management. Thus, identifying early on and taking on board the subjective and individual aspects of each woman will enable personalized care for the needs of the cancer journey.

P4-01-03: Beyond Aesthetics: Cold Cap Hair Preservation Correlates with Lower Chemotherapy Symptom Burden in Patients with Breast Cancer

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Introduction: Hair loss is one of the most feared treatment side effects for women starting chemotherapy. Treatment-induced alopecia creates feelings of increased social stigma and isolation, emotional states correlated with poorer overall disease prognosis. Supporting quality of life during cancer treatment, including successful hair preservation via scalp cooling, has been shown to correlate with higher ratings of health-related well-being. Currently, the only option for reducing hair loss during chemotherapy is scalp cooling with devices like the Paxman and Dignicap, which are usually well-tolerated. This study aims to investigate the difference in reported symptoms and total symptom burden in women with breast cancer receiving scalp cooling interventions and matched controls during chemotherapy.

Methods: In this ongoing IRB-approved study at Lankenau Medical Center, 25 patients have chosen to use scalp cooling during breast cancer treatment. These patients were matched by age and chemotherapy regimen to those who did not use scalp cooling. Our patient base typically has a high proportion of African Americans given that our medical center is located adjacent to West Philadelphia, a community with a 90 - 95% African American population. However, only 8% of patients in the cold cap group were African American compared to 36% of African American patients in the control group. With a high up-front co-pay, many patients of all races forego this supportive care. The average age was approximately 50 in both groups. At treatment visits participants completed the Modified Rotterdam Symptom Checklist; a self-report questionnaire rating 29 chemotherapy side effects on a scale of zero to three. Data from the first 50 patients was analyzed in GraphPad Prism 8.4.3 for relationships between factors and the patient's total symptom burden, calculated as the total symptom checklist score at each visit. Wilcoxon matched pairs signed rank test (CI 95%) was used to assess the difference between total symptom burden in cold cap users and their matched controls.

Results: Most patients who chose scalp cooling were younger and predominantly Caucasian. The total symptom burden was significantly lower ($p<0.0002$) among patients using scalp cooling compared to the matched controls. Of individual symptoms, there was a moderate reduction in reported tiredness even though the Paxman-using patients reported a

moderately significantly higher level of difficulty sleeping. Patients using scalp cooling reported a significant reduction in appetite compared to their controls but had no significant weight loss. There was no significant difference in sexual interest between the two groups.

Discussion and Conclusion: Cold cap systems have benefits beyond just aesthetics, use of scalp cooling interventions provide significant improvement in patient's symptom ratings. Maintaining hair can contribute to a patient's sense of normalcy and self-esteem during a challenging time which can positively impact their overall quality of life and well-being. Even though scalp cooling had a positive effect by decreasing the overall symptom burden, individual symptoms that can be associated with emotionally related symptoms such as appetite, sleep, and sexual interest were not necessarily improved by the use of scalp cooling. The current co-pay system prevents many of our patients from the West Philadelphia community from taking advantage of this supportive car. We are currently in the process of reducing the economic barrier to access to scalp cooling to increase the diversity of the cohort and include other hair textures, a factor known to affect the efficiency of scalp cooling.

P4-01-04: Adaptation of the EORTC Quality of Life Breast Cancer Module for male Breast Cancer – Results of the Phase I-II module

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Background: Approximately 1% of all new cases of breast cancer (BC) cases occur in men. Treatment and symptom management for male BC patients are largely based on strategies developed for women. While the impact of BC diagnosis and treatment on the quality of life (QoL) in women with BC is well documented, there is a paucity of comprehensive, prospective data on QoL in men treated for BC. Due to absence of a validated QoL questionnaire, QoL in male BC patients has recently been assessed using the EORTC QLQ-C30 and the breast module QLQ-BR23 with female-specific items replaced by male-specific items from the EORTC QoL prostate module (EORTC QLQ-PR25). The development of the first validated worldwide questionnaire for male BC is sponsored by the EORTC QoL Group and is conducted in collaboration with the EORTC BCG and the International Male BC Program (a collaboration of EORTC-BCG, TBCRC, within the BIG and NABCG networks). The trial design was presented at SABCS 2000 (abstract OT-14-01). This report presents the results from phase 1 and 2 of the EORTC module development study to assess QoL in male BC patients.

Methods: Phase I and II of the EORTC module development study focused on identifying QoL issues relevant to male BC and translating these issues into a questionnaire for a comprehensive QoL assessment. The development process included a systematic literature review, interviews with patients and healthcare professionals (HCPs) and consultations with experts in oncology and QoL. According to the recommendations of the EORTC module development manual, an issue was included to the issue list if it had a patient and/or HCP relevance rating ≥ 2 (on a scale of 1 to 4) and there was a patient and/or HCP priority rating ≥ 30 %. The relevant issues were translated into items with the help of the EORTC item library and the experts, resulting in a preliminary questionnaire. This questionnaire has been reviewed by the EORTC Quality of Life Department translation unit and will be tested in the phase III.

Results: The mixed methods approach (systematic literature search, questionnaires review, investigator brochures, focus group, research group meetings) identified 86 issues that appear to be related to QoL in male BC patients. An issue list was created and used for interviews with patients and HCPs, who rated each issue according to relevance for male BC patients (using a four-point EORTC response format: not at all, a little, quite a bit, very much) and priority (yes/no response format). Interviews were conducted with male patients (N=64) with histologically confirmed diagnosis of BC from a total of eleven centres. A total of 29 HCPs from seven centres representing different specialities participated in this study. Centres were located in northern, southern and eastern Europe, USA, Egypt, and Asia. Of the initial 86 issues, 27 (rated by patients) and 46 (rated by HCPs) fulfilled both inclusion criteria. Additionally, 24 issues are covered by the BR-45 (updated BR-23 module Phase III) and/or the PR-25, and 25 are new issues not covered by these modules. In phase II, these issues were translated into items and a preliminary Phase II module/questionnaire with 68 items to assess QoL in male BC patients was created and will be presented at SABCS 2024.

Conclusion: The results of the phases I-II highlight that the current practice of using combining parts of the EORTC QLG breast module and prostate module does not sufficiently cover all the relevant QoL issues for male BC patients. Ten items commonly used in the assessment may not add value, and 25 potentially valuable new items are not included in existing modules. Therefore, a new, male BC dedicated QoL questionnaire should be developed. A preliminary phase II questionnaire will be tested in phase III of the EORTC module development.

Note. This document represents preliminary findings of the module development and is subject to revision; subsequent modifications of the module in the next stages may reflect significant changes.

P4-01-05: Impact of treatment for early-stage breast cancer on patient quality of life: a large single-institution experience in Brazil

Adriana Kahn, José Roberto Rossari, Arthur Pille, Juliana Sena de Souza, Kim RM Blenman, Maryam Lustberg

Background: Early-stage breast cancer local and systemic treatments may affect patients' quality of life (QoL) in many aspects. Depending on age, role, culture and beliefs, QoL may be impacted differently among women. The purpose of this study is to describe and compare QoL scores before and after initial treatment of patients with early-stage breast cancer from a single institution in Brazil.

Methods: Between 2019 and 2023, patients with stage 0-III breast cancer treated at Hospital Moinhos de Vento, Brazil, were included. QoL data were collected using two questionnaires of The European Organisation for Research and Treatment of Cancer (EORTC): the Cancer Quality of Life Questionnaire (EORTC QLQ-C30); and the Breast Cancer-Specific Quality of Life Questionnaire (EORTC QLQ-BR23). Functional and symptom scales were dichotomized according to patient responses. The associations between age groups (<60y [younger] vs. ≥60y [older]) and QoL scales were defined to include only patients who completed all the QoL questionnaires at each comparison segment (baseline, 6m and 1y). Both the Bhapkar test and the Wilcoxon signed-rank test were conducted, depending on the nature of the variable. Bonferroni correction was applied to adjust p-values due to multiple tests. The level of statistical significance was set at 5%.

Results: 745 patients completed QoL questionnaires at baseline. Answers were collected at follow up (FUp) of 6m (708 pts, 95%) and 1y (633 pts, 85%) post-diagnosis. At baseline, most patients were <60 years-old (419 pts, 56.2%) with a postmenopausal status (397 pts, 73%), and were treated for invasive disease (664 pts, 89%). Mastectomy was performed in 262 patients (35%), and breast-conserving surgery in 417 (56%). By the time of the 6m FUp, 305 patients had radiotherapy (41%), 316 chemotherapy (42%) and 306 endocrine therapy (41%). Regarding baseline vs. 6m FUp (296 pts): Emotional functioning at 6m improved from baseline for both younger ($p<0.0001$) and older women ($p=0.0011$). Physical functioning worsened for younger patients ($p=0.0097$) and future perspective improved for both young ($p<0.0001$) and older women ($p=0.024$). For older women, the function score increased significantly ($p<0.0001$). Regarding baseline vs. 1y FUp (185 pts): emotional functioning ($p <0.0001$), sexual enjoyment ($p=0.003$) and future perspective ($p<0.0001$) improved in younger women. Function score improved in older women ($p<0.0001$). Regarding 6m FUp vs. 1y FUp (236 pts): physical functioning continued to improve in older women ($p=0.013$). Breast-related symptoms worsened for the whole group of patients ($p=0.037$), but the difference was not statistically significant for age groups ($p=0.375$).

Conclusions: Treatment of early-stage breast cancer affects younger and older women in different ways and domains of QoL. In this single-institutional dataset, emotional functioning and future perspectives tended to improve over time for younger and older women. For younger women, physical functioning tended to worsen, and sexual enjoyment tended to improve. These results may aid in development of strategies to mitigate specific needs of patients based on their age groups.

P4-01-06: Evaluation of the results of fully implantable catheters for chemotherapy (cervical and brachial region) in cancer patients

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Introduction: Totally implantable venous devices (DVTIs) or ports, are essential for patients with malignant neoplasia who require chemotherapy and other long-term parenteral treatments. Because they were totally implantable, they enabled better quality of life, longer durability, lower infection rate and other complications when compared to other devices. Implantation through the internal jugular vein has been the preferred route of insertion, however it has been debated in recent years due to its association with severe complications such as pneumothorax, hemothorax, puncture of the subclavian artery and carotid artery. An alternative to avoid these complications is the use of totally implantable venous devices for peripheral insertion in the arm, less invasive procedure and with better aesthetic results.

Methods: It is a prospective study in which 36 patients with neoplastic disease from the vascular surgery outpatient clinic of the Ipiranga Hospital - UGA II (public) and Santa Catarina – Paulista Hospital (private), submitted to port implantation through cervical or brachial access, according to medical indications and patient preference. These patients were followed up from June 2018 to December 2023, and the primary outcomes were: time of the procedures, complications and discomfort of the patient in the intraoperative period, 10 days, 1 month and 6 months after the procedure. The satisfaction assessment was performed at the end of the study based on the application of a specific questionnaire.

Results: In all cases the procedure was successfully completed and the proper functioning of the catheters was confirmed. Complications observed in patients with brachial access (nineteen cases) included three cases of local bruise, one case of asymptomatic thrombophlebitis, one case of subcutaneous infection and one case of pain when stretching the arm. All of these complications were resolved without the need for surgical procedures. There was one case of extrusion due to a probable allergic reaction to the chemotherapy drug requiring early removal of the port. In patients with cervical access (seventeen cases), two cases of malfunctioning catheters were observed, one of which required early removal of the port, and another case of subcutaneous pocket infection unresponsive to clinical treatment, with early removal of the port also performed. Most patients preferred catheter implantation in the arm, but cervical access was more practical due to the larger venous caliber in this topography and greater availability of appropriate material. Of the thirty-

three patients who maintained the port for 6 months and underwent the quality of life questionnaire (fifteen patients with cervical access and eighteen patients with brachial access), thirty-two would recommend to another person. Conclusion: The implantation of the port through the brachial and cervical access did not present serious complications, and the patients demonstrated high general satisfaction.

Keywords: Catheters; Fully implantable; Chemotherapy; Brachial; Port

P4-01-07: The contribution of Acceptedance, awareness of illness, social contexts and economic status to mental health among women with breast cancer

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Background: In 2023 the Lancet Commission evaluated the three main asymmetries in relation to cancer and gender differences: decisional processes, awareness of illness and economic domains. It highlighted the necessity of considering a complex frame, in which women live with cancer and beyond it, and fill the role of caregivers for family members and friends, job employers and members of a social community. METHODS: In the attempt to contribute to fill this gap, the aim of the study was to explore a) acceptance and awareness of illness, social contexts (family and relationships), economic difficulties, anxious/depressive symptoms, and mental health in cancer patients (pts) undergoing active therapy with breast cancer (BC); b) the relationship of acceptance and awareness of illness, social contexts, and economic difficulties with anxious/depressive symptoms and positive mental health among BC pts. A monocentric cross-sectional study was conducted. Pts were enrolled during the first session of a psychological support path. Patients' cancer diagnosis and stage, as well as presence of family and friendships, and economic difficulties were explored. The Revised Illness Perception Questionnaire (IPQ-R), Hospital and Depression Anxiety Scale (HADS), Benefit Finding short form (BF-SF) and Mental Health Continuum Short-Form (MHC-SF) were administered. Descriptive, parametric and non-parametric statistics, and regression models were conducted.RESULTS: Out of the 70 BC pts (median age =54.5, mean=54.5, SD=11), 78.8% of them had a localized cancer and 21.2% a metastatic/advanced one. As concern socio-demographic characteristics, 65.7% of the BC pts was married or cohabiting, 72.7% reported having structured friendships, 17.1% economic difficulties, and 7.1% of them reported housing difficulties. Participants reported moderate levels of beliefs, showing positive awareness of illness. As for HADS, pts mean score exceeded anxiety and depression clinical cutoff (depressive symptoms mean score=8.29, SD=4.39, and anxiety symptoms mean score=9.06, SD=4.78). Overall, pts reported moderate values of acceptance of illness at BF-SF, and moderate values of mental health at MHC-SF, scoring higher in psychological than in hedonic and social well-being, with 28.6% of BC pts flourishing, 25.7% languishing and of 45.7% of pts with moderate mental health. Finally, multiple linear regression showed that acceptance of illness and

perceived illness control promoted high mental health ($F=3.82$, $p<.05$). No significant contribution emerged for cancer stage, perceived consequences of illness, having a family, structured friendships and absence of economic difficulties. On the opposite, when depressive and anxiety symptoms were tested, multiple linear regression founded that low illness control and perceived consequences of illness was associated with high levels of anxiety and depressive symptoms ($F=2.84$, $p<.05$). No significant contribution emerged for cancer stage, low acceptance of illness, family and friendships contexts, and economic difficulties.

Conclusions : Findings supported the literature highlighting that acceptance and perceived control of illness promote better quality of life in BC pts, fostering mental health and defending from anxiety and depressive symptoms. In line with scientific litterature in heallth psychology, it is, nevertheless, interesting notice that in this group of participants cancer stage, the presence of a partner and solid friendships, and no economic difficulties did not emerge as a predictor of well-being and mental health. Tailored interventions for BC women should consider psychological needs and individuals' potentials, and social opportunities for the empowerment or maintenance of a good quality of life.

P4-01-08: PYNK at 20: From SABCS Inspiration to Multisite Clinical, Research, and Educational Program for Young Women with Breast Cancer

Sandy Vuong, Katarzyna Jerzak, Brooke Macdonald, Yonina Juni, Alexandra Landsberg, Karen Fergus, Judith Weinroth, Ellen Warner

Introduction: The last session of the 2004 San Antonio Breast Cancer Symposium focused on the unique biological, fertility, and psychosocial issues faced by women diagnosed with breast cancer at age 40 or younger (YWBC). Attendees EW and JW, for whom this was a startling eye-opener, returned to Sunnybrook Health Sciences Centre in Toronto determined to create an interdisciplinary program for YWBC. Their vision was to ensure these patients received: 1) State-of-the-art clinical care, 2) A continuum of support for themselves and their families, and 3) Educational resources. Additional priorities would be: 4) Clinical and translational research focused on YWBC, and 5) YWBC-focused education of health care professionals. Today's PYNK program - its origins and operations (including challenges and successes) - are described in this presentation.

Methods: From January 2005 through January 2008 the program infrastructure was built. Steps included: Review of the literature on unmet needs of and existing programs for YWBC; Monthly meetings of a multidisciplinary executive from medical, radiation, and surgical oncology, nursing, social work, psychology plus 2 young breast cancer survivors; Presentations to skeptical colleagues and to "management" who liked the concept but could not offer financial support; Establishment of a seamless fertility preservation referral process; Fundraising to pay a part-time nurse for the 1st year; Creation of a prospective

research database, serial patient questionnaires, and a mechanism for storing baseline blood; and Selection of a name for the program: PYNK.

Results: Since the enrollment of the first patient in Feb. 2008, there have been 40 to 50 newly diagnosed patients per year who remain in the program until discharged from our centre. Within 2 years a full-time nurse navigator became necessary. Since Jan. 2023 the navigator role has been taken over by a Physician Assistant (SV). Program objectives: 1 & 2) The navigator, along with the social worker (BM), meets the patient +/- accompanying family shortly after the 1st visit post-diagnosis, helps facilitate referrals/appointments, provides intensive support through active treatment, and medical/psychosocial support as needed thereafter. The navigator and social worker also co-lead 2 monthly support groups, one for non-metastatic and one for metastatic patients. A child life specialist is available when necessary for home visits to children. 3) In addition to brochures, books, and educational events, an online multimedia virtual library focusing on YWBC issues is maintained. 4) Consenting patients were enrolled in the prospective clinical database including family history, lifestyle factors, and serial psychological measures until 2015 when PYNK became one of the biggest contributors to the pan-Canadian RUBY (Reducing the bUrden of Breast cancer in Young women) prospective cohort. We have also enabled our patients to join pivotal trials such as the Dana Farber's Young Women's Breast Cancer Study and the Breast International Group's POSITIVE study (NCT02308085). 5) Trainees of all levels and health disciplines rotate through PYNK clinics. In addition, we host formal CME events.

Conclusions/Discussion: Since 2008, PYNK has helped hundreds of YWBC and their families manage their complex medical and psychosocial issues. PYNK research has resulted in numerous presentations and publications. Trainees learn unique skills and perspectives about YWBC management. In 2023 PYNK expanded to 2 additional donor-funded sites in the Greater Toronto Area. PYNK has not only been a model for other YWBC programs internationally but has sparked the creation of special programs for young patients in other disease sites. The greatest strength of PYNK has been the continuous input of the YWBC themselves on its executive.

P4-01-09: REAL-LIFE OUTCOMES OF A 10-YEAR ONCOFERTILITY PROGRAM IN EARLY BREAST CANCER PATIENTS

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Introduction: Fertility preservation (FP) is a critical aspect of care for young breast cancer (BC) patients. Guidelines on FP from cancer societies recommend that patients in their

reproductive years should receive fertility counselling and, if interested, should be timely referred to a FP specialist.
There is, however, limited information on real-life follow-up of BC patients who underwent FP.
We aimed to assess the outcomes of an Oncofertility program in young women with early breast cancer (EBC) proposed for curative treatment with chemotherapy (CT).

Methods: Retrospective analysis of EBC patients under 40 years proposed for FP before curative CT in an EBC clinic of a tertiary cancer center from January 2012 to December 2021. FP procedures were carried out in 3 different fertility units.
Collected data covered patient demographics, BC characteristics, genetic risk, treatment, survival outcomes and FP methods and complications, number of oocytes retrieved, oocyte utilization, pregnancies, live births, ongoing pregnancies, miscarriages and elective terminations.
Disease-free survival (DFS) and overall survival (OS) were estimated by Kaplan Meyer method.

Results: In the study period 88 women were referred to FP and 70 (80%) accepted to proceed with FP after specialist appointment. Median follow-up time was 6.9 years. Median age was 32 years (interquartile range [IQR] 29-36). Before diagnosis 38% of women had been pregnant and 30% had children. Patient BC was staged as II in 55% and III in 24% of cases and 48% had node positive disease. Hormone receptor expression was found in 77% of tumors, HER2 in 25% and 16% were “triple negative”.
Genetic testing was done in 93% of patients and germline pathogenic variants were identified in 16%, including BRCA in 13%.
Neoadjuvant CT was performed in 67% of patients and adjuvant CT in 32%. CT protocol included anthracyclines in 87% of treatments. Median time from FP specialist referral to CT start was 3.7 weeks (IQR 3.0-5.5).
The methods used for FP were oocyte (91%) (median number of oocytes retrieved: 10 [IQR 6-16.3]), both oocyte and embryo (2%) and ovarian tissue (4%) cryopreservation. There were complications in 2 patients (3%): ovarian hyperstimulation syndrome and hemorrhagic corpus luteum.
Adjuvant endocrine therapy (ET) was administered to 76% of patients. Interruption to attempt pregnancy happened in 40% with a median ET exposure time of 4 years (IQR 2.8-5.4).
After FP, 38 pregnancies occurred in 24 patients (34%). We observed 18 live births (47%), 11 miscarriages (29%), 4 elective terminations (11%) and 5 pregnancies are ongoing (13%). Artificial reproductive technologies were used in 29% of pregnancies, all with oocytes retrieved pre-CT. Median time to pregnancy was 4.3 years (IQR 2.9-6.5).
Oocyte utilization rate was 19% (13 patients). Among oocyte users 11 pregnancies (29% of all pregnancies) occurred in 10 women (77%). We observed 6 live births (55%), 4 miscarriages (36%) and 1 pregnancy is ongoing (10%).
Overall, FP was performed in 7 patients to achieve one pregnancy via cryopreserved

material and in 12 patients to achieve a live birth.
DFS and OS at 5 years was 82.1% and 92.0%, respectively.

Conclusion: In our cohort FP outcomes were encouraging.
FP appeared to be effective and complications were rare and manageable, with no worrisome trends regarding DFS or OS.
Clinician awareness and ongoing research in FP will provide more opportunities for BC patients to achieve their reproductive goals.

P4-01-10: Sexual well-being and its association with breast surgery indications: an exploratory study

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Purpose: Although breast surgery can have a negative impact on a woman's body image, we are not aware of studies measuring the impact of surgery for benign breast disease on sexuality or body image. There are studies on the impact of breast surgery on sexuality and body image for breast cancer (BC) survivors. We want to know if breast surgery has an impact on sexual wellbeing after breast surgery and/or if other BC specific treatments weigh on the decline in sexual functioning after breast surgery. Therefore, we compared sexual functioning in patients who underwent breast surgery for invasive BC versus patient who underwent breast surgery for a benign, prophylactic or premalignant indication.

Methods: Between October 2021 and January 2024, we recruited female patients older than 18 years with a history of breast surgery (self-report) via: 1) consultation site of the breast clinic of the University Hospitals Leuven; 2) social media platforms of Think Pink vzw (https://www.think-pink.be/nl); and 3) patients support groups. After giving eConsent, participants completed an online survey, which included validated questionnaires: Female Sexual Function Index (FSFI), Female Sexual Distress Scale (FSDS-R), World Health Organization – Five Well-being Index (WHO-5), and Dyadic Adjustment Scale (DAS).

Results: We recruited 86 participants who underwent surgery for invasive BC (n=74; mean age: 50,1y; age range: 25-69y) and non-invasive indication (n=12; mean age: 45,5y; age range: 21-72y). Both groups had a comparable outcome for DAS (BC – group mean score: 117,9 versus non-BC group mean score: 119,9) indicating a good partner relation in both groups and WHO-5 (BC-group: 51,17 versus non BC-group: 61,45) indicating a good well-being in both groups (using the cutoff score of 50).
Combing the cutoff score of the FSFI (i.e., <25) and the FSDS-R (i.e., >11), we found that in the BC-group 41/74 (55,4%) had a sexual dysfunction compared to 2/12 (16,7%) in the non BC-group. When considering the use of endocrine therapy, we noticed that a sexual disfunction was present in 52,4% of BC survivors who never took endocrine therapy, in

55,9% of those still taking endocrine therapy and in 60,0% of those who stopped endocrine therapy.
We found poorer sexual function after breast surgery in the invasive BC group (mean total FSFI score: 18.83) compared to the non-BC group (mean total FSFI score: 23,47). While participants with invasive BC who never took endocrine therapy (n=21) had a mean FSFI-score of 21,4, those still taking endocrine therapy (n=43) had a mean FSFI-score of 17,67 and those who stopped endocrine therapy (n=10) had a mean FSFI-score of 15,51.
We found more sexual distress after breast surgery in the invasive BC group (mean FSDS-R score: 24,0) compared to the non-BC group (mean FSDS-R = 10,9). While participants with invasive BC who never took endocrine therapy (n=21) had a mean FSDR-R-score of 18,14, those still taking endocrine therapy (n=43) had a mean FSDS-R-score of 24,65 and those who stopped endocrine therapy had a mean FSDS-R score of 34,89.

Conclusions: This study shows that a higher percentage of women who underwent breast surgery due to a malignancy (52.4%) reported a sexual dysfunction compared to the non-BC group (16,7%).
Moreover, women using endocrine therapy reported lower sexual function and higher sexual distress compared to women who never used endocrine therapy. Women who stopped endocrine therapy reported the lowest scores on sexual function and the highest scores on sexual distress.
These results highlight the importance of addressing sexual side effects during BC treatment and follow-up.

P4-01-11: Patient-reported outcomes assessment using mobile applications in the HR+/Her2- breast cancer adjuvant setting.

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Introduction: Adjuvant treatments for HR+/Her2- breast cancer include various options such as tamoxifen, aromatase inhibitors, CDK4/6 inhibitors, and ovarian suppression. These agents are associated with specific adverse events, which may impair quality of life and treatment adherence. Numerous studies have shown that adverse events are often underreported in clinical records. This study aimed to evaluate the adherence to online questionnaires for patient-reported outcomes (PROs) using a mobile application based on the Spanish version of the PRO-CTCAE tool.

Methods: An online questionnaire was designed using 39 questions from the Spanish version of the PRO-CTCAE tool. Patients were instructed to complete the questionnaire after the first month of adjuvant treatment and then every six months, with email reminders. The questionnaire was available for completion at the patient's request. The primary objective was to evaluate adherence to online questionnaires, with the expectation that at least 73%

of a sample size of 42 patients would answer two questionnaires after one year of follow-up. Secondary objectives included evaluating the incidence of self-reported adverse events across different treatment types. Fisher tests and longitudinal-analysis models using generalized estimating equations were performed to evaluate the incidence of adverse events throughout the patients' follow-up. A p-value of <0.05 was defined for statistical significance. This pilot experience only included patients from an Argentinean private center, Instituto Alexander Fleming.

Results: Forty-two patients were included between December 2022 and June 2023, and a total of 123 questionnaires were recorded. The median age was 51.5 years (range 32-73), 59.5% were premenopausal, 14.3% received ovarian suppression, and 16.7% received abemaciclib. 95.2% of patients were followed up for more than one year. The median number of completed questionnaires was three (IQR 1-5). Additionally, 85.7% (95% CI: 71-97%) and 66.6% (95% CI: 50-80%) of the cohort answered one and two questionnaires, respectively. The most reported adverse events were decreased libido (81%), headache (78.6%), and insomnia (78.6%). Treatment with tamoxifen was associated with higher intensity of specific adverse events, including decreased libido (p=0.021) and insomnia (p=0.049). Higher intensity of diarrhea was more frequently reported among patients receiving abemaciclib (p=0.024). Longitudinal analysis showed that the incidence and intensity of hot flushes increased during the first year of follow-up (p=0.024).

Conclusions: This study represents the first experience of PRO assessment using online and mobile applications in Argentina. Strategies tailored to our population are required to promote adherence to online questionnaires, which may include WhatsApp text messages and patient education materials. As expected, this study allowed the recognition of certain treatment-related adverse events that are often overlooked during medical visits.

P4-01-12: Advanced Breast Cancer, Comorbidities, and Overall Survival: An Integrated Review

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Purpose: Patients living with advanced breast cancer (ABC) and comorbidities are at a greater risk for decreased overall survival. The survivorship needs of ABC patients are an emerging science and little is known about how to best treat ABC patients living with one or more comorbidities. Understanding the impacts of comorbid diagnoses in addition to a diagnosis of ABC may influence treatment and long-term survival. Therefore, the purpose of this review was to determine the state of the science regarding the impact of comorbidities on overall survival among patients with ABC.

Methods: Following PRISMA reporting guidelines, we conducted a comprehensive search of MEDLINE Ovid, EMBASE, and Web of Science databases using defined inclusion and exclusion criteria. The Joanna Briggs Institute Critical Appraisal Tool was used to evaluate article quality. The process of member checking was completed. Covidence electronic software was utilized to manage and streamline data review and extraction.
Results: A total of 876 articles were identified and imported into Covidence electronic software. 172 duplicated citations were removed; 704 studies title and abstracts were manually screened. A total of 691 studies were excluded as they did not meet the review's inclusion criteria. Thirteen full text articles were reviewed for inclusion and exclusion criteria. Ultimately, seven articles were included in the review. Study designs included retrospective chart reviews (n=4), prospective chart reviews (n=2), and a longitudinal cohort study (n=1). The sample sizes ranged from 100 patients (n=1), 250 patients (n=2), 500 patients (n=3), and 1000 patients (n=1). All studies (n=7) were conducted in large medical centers. Three studies were completed in the United States of America, two in Mexico, one in the Netherlands, and one in Germany. Data collection time frames ranged from four to 25 years. Articles were published in the past two decades. Patient setting characteristics included patients with ABC admitted to the hospital, patients with ABC receiving palliative chemotherapy, those living four years after an ABC diagnosis, those with ABC and brain metastasis, and those diagnosed with denovo ABC. Results of included studies are reported in the context of Sarfati's "Cancer and Comorbidity Construct" framework. None of the included studies were grounded to a theoretical framework. A frequency count of comorbidities reported in included studies was preformed: previous cancer (1), hyperuricemia (1), endocrine/other metabolic disorders (1), primary chronic polyarthritis (1), depression/psychosis (2), lung disease (3), dyslipidemia (4), cardiovascular disease (5), obesity (5), and diabetes (6).

Conclusion: There remains limited literature available on the comorbidities experienced by patients living with ABC. Further quantitative and qualitative studies are needed with the inclusion of men, rural communities, and minority populations to explore the impact of comorbidities on outcomes among persons with ABC. The long-term goal of this work is to develop comorbidity management guidelines specific to the oncology and ABC population to positively impact their overall survival.
Keywords: advanced breast cancer, metastasis, comorbidity, overall survival.

P4-01-13: Are we tired of talking about sleep? Insomnia amongst breast cancer patients

Emily Scott, Amani Chowdhury, Amy Guppy, Andreas Makris

Background: Sleep disturbance is one of the most common symptoms experienced by cancer patients at all stages of the disease and treatment.1 Studies indicate that up to 61% of these patients face sleep difficulties, which negatively impacts fatigue, mood, pain sensitivity, cognitive function, and physical activity.2,3 A systematic review found that

addressing insomnia in breast cancer patients can reduce depressive symptoms and anxiety, improve therapy adherence, and enhance quality of life.3
Significant disruption to sleep, of more than 3 months duration is classified as insomnia. In 2023, the European Society for Medical Oncology released guidelines for assessing and managing insomnia in adult cancer patients.4 They recognise insomnia is often underdiagnosed and poorly treated in this population. The Insomnia Severity Index, validated for cancer patients, is recommended for screening all cancer patients, with a further detailed evaluation for those scoring 15 or higher.
Cognitive behavioural therapy (CBT) is the recommended standard of care for treating insomnia. While hypnotics are the most widely used treatment for insomnia, they are associated with side effects and adverse effects with long-term use. Melatonin can be considered when there is circadian rhythm disruption. Some evidence suggests exercise, either alone or as a supplementary measure, can provide benefits.

Methods: We conducted a study using a custom-designed survey to gather information from patients about their sleeping patterns, duration of insomnia, and management they have undertaken. Following the survey, we carried out a service evaluation to assess the knowledge of breast oncology staff regarding the assessment and management of sleep disturbance at our centre.

Results: From our patient cohort, 63% had metastatic breast cancer, and all were female. The median age was 58 years (range 37-79). Among the respondents, 52% reported sleep disturbance, with the majority (22 out of 27) experiencing symptoms for over three months. Of those with insomnia, 30% expressed a desire to discuss treatment options, while 63% were either unsure or did not want to discuss treatment with their oncology team. Patients with insomnia reported higher incidences of fatigue (81%), poor concentration (52%), and mood disturbance (37%) compared to those without insomnia. Among those with insomnia, 86% had not been offered treatment, and the remaining 14% had been offered treatment by their General Practitioner. No reasons were provided for their reluctance to discuss insomnia with their oncology team.
Our staff survey revealed staff felt moderately comfortable discussing insomnia with patients (mean Likert score of 2.7/5, mode 2/5). Only 15.8% were aware of validated screening tools, and 11% felt comfortable managing insomnia (mean Likert score of 2.8/5, mode 3/5). Additionally, 26.3% were aware of insomnia management options available at our centre. The preferred treatment options among the staff included mindfulness therapy, drug therapy (excluding melatonin), and exercise.

Conclusions: The analysis corroborates previous studies indicating that insomnia is prevalent among breast cancer patients and is associated with other symptoms such as fatigue, mood disturbances, and poor concentration. Our cohort expressed unease in discussing insomnia with their oncology team, and all treatment offers were made by primary care providers. Our staff lacked awareness of validated assessment tools for insomnia and ESMO guidelines. To address this gap, it is crucial to enhance staff education

and direct patients to the available resources at our centre.

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P4-01-14: The impact of early breast cancer on psychological resilience, distress levels and perception of health, a longitudinal study

Anuska Budisavljevic, Renata Kelemenic Drazin, Marina Letica Crepulja

Background: Confronting early breast cancer (EBC) diagnosis, along with complex and challenging treatment procedures is an extremely stressful experience. Psychological resilience (PR) is the ability to maintain or restore normal functioning while facing adversity. The aim of this study was to explore the impact of EBC on PR, distress, and perception of health throughout the first year of EBC diagnosis.

Study design: The longitudinal case-control study was conducted at the GH Pula, Croatia and approved by the Ethics Committee. Study included 50 patients newly-diagnosed with EBC and 67 healthy women with screening mammogram graded 1 or 2 using a Breast Imaging-Reporting&Data System.
Levels of distress, perception of health, and PR were assessed using the Depression, Anxiety, and Stress scale (DASS-21); the SF 36-Item Health Survey 1.0 (SF-36), and the Connor-Davidson RISC-25 scale (CD-RISC-25). Differences between variables were examined using the t-test and Chi-Square test, for interval and categorial variables. We made adjustment for age by using age as covariate. The significance level was set at $p < 0.05$. The first measurement was conducted within four weeks after EBC diagnosis and the second after 12 months.

Results: Participant age ranges were 27–70 (mean = 57.94, SD = 10.24) years and 40–70 (mean = 57.01, SD = 9.62) years in the EBC and control group, respectively (t = -0.501, df =

115, p = 0.618). The groups were well balanced considering all the demographic characteristics. In the EBC group 70% of participants had stage I, 24 % stage II, and 6% stage III EBC. Around 36% of EBC patients were premenopausal. Breast conserving surgery was obtained in 84% of patients. Regarding systemic treatment, 66% were treated with adjuvant/neoadjuvant chemotherapy, 90% were treated with antihormonal therapy, and 12% with antiHER2 therapy.
First measurement showed that EBC group had significantly higher CD-RISC-25 scores than the control group, indicating higher levels of PR (t(df) 2.530, $p < 0.05$). Also, patients in the EBC group had lower scores on the single SF-36 item evaluating perceived change in general health compared to 1 year prior than those in the control group (t (df) 3.835, $p < 0.01$) while no significant differences in the four domains of physical and emotional health perception were detected between the EBC and control group.
After one year of follow up EBC group showed lower scores on SF-36 subscale of physical functioning (t(df)2,472, $p < 0.05$), while at the same time they perceived better general health compared to 1 year prior than the control group (t(df) -4,656, $p < 0.01$). Moreover, participants from EBC group achieved higher results on the CD-RISC-25 scale than healthy control (t(df) -2.801, $p < 0.01$). Furthermore, when comparing the first and second measurement for EBC group, 12 months after being diagnosed the EBC group showed lower scores for depression (t(df) 2,744, $p < 0.01$) and stress (t(df) 2,158, $p < 0.05$), better social functioning (t(df) -2,169, $p < 0.05$), and better perception of general health compared to 1 year prior (t(df) -7,713, $p < 0.01$).

Conclusion: To the best of our knowledge, this is the only longitudinal study comparing PR levels in EBC patients and healthy controls and we found EBC patients to be more resilient even though EBC group underwent first year of EBC treatment. This altogether suggests that being diagnosed with EBC might induce effective adaptation to stress, leading to higher PR and consequently better perception of change in general health in EBC patients compared to healthy controls.

P4-01-15: Treatment tolerability and adherence in people living with metastatic and non-metastatic breast cancer: Findings from the Cancer Experience Registry

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Background: Treatment adherence, an essential component of effective cancer care, can be affected by treatment tolerability. The widely accepted definition of tolerability, based on how well patients tolerate adverse events, does not fully address patient perspective and their quality of life. This study aims to further understand breast cancer patients' perceptions of treatment tolerability by metastatic status and association with adherence.

Methods: A total of 244 participants (242 women, 2 men) with a primary diagnosis of breast cancer enrolled in the Cancer Experience Registry, an online survey research study, between November 2023 and June 2024. For tolerability, participants were asked to rate the level of importance (1=Not at all to 5=Very much) for 11 factors when deciding if a treatment (past or current) is tolerable or not. For adherence, participants indicated if they ever deviated from their cancer care plan and rated the contribution of 8 factors to their non-adherence (1=Not at all to 5=Very much). Chi-square analysis was utilized to assess group differences among tolerability items based on metastatic status and adherence.

Results: The sample was 84% Non-Hispanic (NH) White, 9% NH Black. Participants varied in age (range: 28-88; mean=63 yrs; SD=12), time since diagnosis (range: 2-41; median=9 yrs), and socioeconomic status (13% household income <$40K); 34% currently receiving cancer treatment, with 72% in remission, 17% metastatic, and 18% with history of recurrence. In the full sample, the three most important factors determining whether a treatment was tolerable (as reported by participants who rated these factors as Somewhat to Very much important) were: ability to slow disease progression (99%), symptom relief (96%), and side effects of treatment (95%). Conversely, the least endorsed factors were related to treatment administration: frequency of treatment (71%), home vs. in-clinic (64%), and oral vs. injectable (60%). When looking at top factors by metastatic status, the top three remain unchanged for those who are metastatic (n=41). For those who are non-metastatic (n=200), while ability to slow disease progression and symptom relief remain important, potential cure was deemed more important than side effects; 98% of non-metastatic patients rated this as Somewhat to Very much important compared to 75% of ever metastatic patients (X^2=28.8, p<.001). A total of 56 individuals (23% of total sample; 10 of these ever metastatic) indicated they had not always adhered to their treatment plan. The most frequently endorsed reasons (as reported by participants who rated these factors as Somewhat to Very much contributing to non-adherence) included avoiding side effects (80%), interference with day-to-day activities (67%), and interference with personal life (62%). Those who reported non-adherence were significantly more likely to report high importance on the following tolerability items: cost/affordability of treatment (X^2=5.77, p<.05), impact of treatment on daily life (X^2=5.88, p<.05), and impact on emotional well-being (X^2=6.06, p<.05).

Conclusion: Slowing disease progression, symptom relief, and side effects of treatment are among the most important factors for breast cancer patients when determining tolerability of treatment. Curative potential is also highly rated for non-metastatic patients. Non-adherence was specifically associated with some tolerability factors including financial cost of treatment, treatment impact on daily life, and emotional well-being. There are multiple drivers of treatment intolerance and multiple reasons for treatment non-adherence in breast cancer. Individual priorities, preferences, and expectations are important to consider to maximize treatment acceptability and adherence.

P4-01-16: Comprehensive Genomic Characterization of ILC Organoids: A BCRF Legacy Project Initiative

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Background: Invasive lobular carcinoma (ILC) is a distinct histologic type of breast cancer with unique features. The lack of well-characterized ILC organoid models has limited research and therapy development. We aim to establish a well-characterized ILC organoid biobank under the BCRF Legacy Project, creating a valuable resource for ILC research.

Methods: We performed whole-genome (WGS) and RNA-sequencing (seq) on patient FFPE tumor tissues. ILC organoids are subjected to whole-exome (WES), bulk RNA-seq, single cell (sc)RNA-seq, scATAC-seq, and MSK-IMPACT targeted sequencing. STR profiling confirmed specimen relatedness. Data were analyzed using validated bioinformatics tools. Immunofluorescence (IF) analysis of E-cadherin and p120 was performed.

Results: After establishing a total of 9 ILC organoids, WES was performed on all 9 organoids while WGS was performed on 2 patient samples corresponding to 2 of these organoids. WGS analysis of bilateral ILCs (TP19-M179), corresponding to LIO-046 organoid, revealed that both tumors shared a CDH1 p.L333Wfs23 frameshift mutation coupled with loss-of-heterozygosity (LOH), concurrent 1q gain and 16q loss, and dominant aging/clock mutational signatures. The left ILC was found to have fewer copy number alterations and somatic mutations than the right ILC. LIO-046 organoid was generated from right breast tumor tissue. Organoid IPM-BO-084 was generated from left ovarian metastasis of a mixed ILC (TP20-M130). Based on WGS analysis, the primary invasive carcinoma and ovarian metastases were found to be characterized by a somatic BRCA2 p.Q2157Ifs18 mutation associated with LOH, and displayed genomic features of homologous recombination deficiency (HRD). The levels of chromosomal instability were higher in ovarian metastases compared to the primary lesion. WES analysis of ILC organoid models identified genetic alterations affecting CDH1, which correlated with IF data where frameshift or nonsense mutations were associated with loss of E-cadherin and cytoplasmic p120 expression.

Conclusions: We successfully established long-term ILC organoids. Our comprehensive genomic analysis of ILC organoids and patient samples underscores the genomic heterogeneity of ILC. Advanced sequencing techniques provide a framework for understanding ILC's molecular landscape. As we expand our biobank, these organoids and their data will be invaluable for developing targeted therapies and advancing ILC research. We are committed to making the genomic data and organoid models available to the research community. Our future goal is to foster collaboration and accelerate advancements in ILC research through this shared resource.

P4-01-17: Uncovering of NRAGE/JNK and PI3K/AKT pathways through serial WGS of breast cancer patients

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Purpose: Cancer genomic sequencing has fundamentally advanced our understanding of the basic biology of this disease, and more recently, has offered method to guide and evaluate treatment in clinic. We performed whole-genome sequencing (WGS) on three consecutive tissue and blood samples from breast cancer patients: diagnosis, post-neoadjuvant chemotherapy, recurrence after curative resection.

Methods: We performed WGS to compared the genetic profiles of the primary, surgical and recurrent samples (tissue and blood) to determine: (1) how closely related the genetics are among the primary , post- neoadjuvant chemotherapy, and the recurrent tumors, (2) whether there are variations in mutational processes among the primary, post- neoadjuvant chemotherapy, and recurrent tumor. WGS was performed on three patients for whom both tissue and blood were available at diagnosis, at surgery after neoadjuvant chemotherapy and at a recurrence.

Results: As a result of somatic protein coding variant analysis, PIK3CA mutation was confirmed at the time of diagnosis tissue in all cases. Mutations were found at p.E545K in patient 14, p.R916C in patient 18, and p.R916C in patient 3. Among these, patients 14 and 18 had PIK3CA mutations even at the time of recurrence. In patient 14, the same PIK3CA mutation was found in 3 consecutive samples, but in patient 18, it changed from p.R916C at diagnosis to p.G480E at relapse. Furthermore, functional enrichment analysis identified a common NRAGE signaling system in the cfDNA. The NRAG pathway was found in the blood at diagnosis, surgery, and recurrence in all three patients.

Conclusion: This study was a proof-of-concept study, in which we hypothesized that NRAGE/JNK and PI3K/AKT signaling pathways are involved in breast cancer recurrence and treatment response through WGS of consecutive samples. Further research is ongoing with larger numbers of patients.

P4-01-18: NEDD9 expression as an indicator for pathological complete response (pCR) in early-stage HER2-positive breast cancer patients undergoing neoadjuvant therapy

Bei Jiang, Elena Pugacheva, Stell Patadji Santiago, Sijin Wen, Jordan Hill, Michelle Hartzell, Hiba Khan, Maria Hafez

Background: Neural Precursor Cell Expressed Developmentally Downregulated Protein (NEDD9) is a scaffolding protein implicated in various oncogenic signaling pathways, contributing to cancer progression and metastasis. Our preclinical study has highlighted the crucial role of NEDD9 in the tumorigenesis of HER2-positive breast cancer. This study aims to assess the potential of NEDD9 expression as an indicator for pathological complete response (pCR) in early-stage HER2-positive breast cancer patients undergoing neoadjuvant therapy, especially those with low estrogen receptor (ER) expression (ER <10%).

Methods: NEDD9 expression was assessed by tissue microarray in a cohort of 134 early-stage HER2-positive breast cancer patients. The correlations of NEDD9 expression with clinical/biological characteristics, treatment, and pCR rate were analyzed by Fisher's exact test.

Results: Significant associations were found between NEDD9 expression and tumor stage (cT) (p=0.018), clinical stage (Cstage) (p=0.035), lymph node surgery (p=0.001), adjuvant radiotherapy (p=0.017), and Ki67 index (p=0.002). In the overall HER2-positive cohort who received neoadjuvant chemotherapy (n=67), no significant association was found between NEDD9 expression and pCR (p=0.554). However, in the subgroup of ER low (<10%) HER2-positive patients (n=27), high NEDD9 expression was significantly associated with an increased pCR rate of 93.8% compared to 27.3% in those with low NEDD9 expression (p=0.0006). NEDD9 expression did not correlate with pCR rate in the ER/PR-positive subgroup.

Conclusions: Consistent with our previous preclinical study, high NEDD9 expression is significantly associated with aggressive tumor characteristics such as advanced tumor stage and high proliferation rates in early-stage HER2-positive breast cancer. High NEDD9 expression is significantly associated with a higher pCR rate in early-stage HER2-positive breast cancer patients with low ER expression (<10%). This suggests that NEDD9 could serve as a predictive marker for pCR in this specific patient population. Further research is ongoing to explore the mechanisms underlying this association and the potential therapeutic implications of targeting NEDD9 in HER2-positive breast cancer.

P4-01-19: Clinical impact of MRD detection via ctDNA tumor-agnostic assay in early-stage breast cancer patients: a real-world experience

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Background: Detecting minimal residual disease (MRD) in the adjuvant setting can help identify patients (pts) with early breast cancer (EBC) at a higher risk of recurrence. Circulating tumor DNA (ctDNA)-based MRD detection is strongly associated with recurrence. Typically, MRD tests require prior knowledge of the tissue genetic alterations. However, obtaining tissue samples can be challenging. This multi-institution retrospective analysis investigates the impact of a plasma-only genetic and epigenetic ctDNA testing on the care of pts with EBC.

Methods: Our retrospective study included 73 pts with stage I-III EBC who had MRD testing after curative-intent treatment between 09/2022 - 06/2024 at Weill Cornell Medicine (New York) and University of California Los Angeles (Los Angeles, CA). ctDNA evaluation was performed using the Guardant Reveal® (GR) tissue-free assay (Guardant Health, Redwood City, CA), a next-generation sequencing panel. ctDNA presence was determined by a custom bioinformatics classifier identifying tumor-derived variants and epigenetic methylation profiles. All tests were ordered in the real-world clinical setting, and data were gathered through a retrospective review of electronic medical records with appropriate IRB approval. A descriptive analysis was performed.

Results: By 06/2024, 73 EBC pts had plasma-only ctDNA testing for MRD monitoring post-surgery. At diagnosis 22% were stage I, 53% were stage II and 21% of pts were stage III. Tumor subtypes included HR+/HER2- (44; 60%), HR-/HER2- (11; 15%), HER2+ (17; 24%), 1 case was unknown. Fifty-five pts (75%) had received neo/adjuvant chemotherapy. Among these, 10 pts had pathological complete response. At the time of the analysis, 49 pts were still receiving adjuvant therapy. The median recurrence free survival (RFS) since surgery was 22.5 months (interquartile range [IQR] 13.3-36.8). With a median follow-up of 2.2 years (IQR, 1.6-3.2) since diagnosis, 6 pts had a distant recurrence. Among them, 3 were HR+/HER2-, 2 were HR-/HER2- and 1 was HER2+ EBC. From 09/2022 to 06/2024, 124 GR tests were performed, with a median of one test (IQR 1-2) per patient. Thirty-nine pts (53%) had one GR test, while 47% had ≥2 tests. The median time between subsequent tests was 119 days (IQR, 93-200). 16 tests (13%) were positive, with 10 pts having at least one GR+ result over a median time of 7 months (IQR 2.7-12.1) from first testing to last follow-up. The first GR test was ordered after a median time from surgery of 13.8 months (IQR 7.0-50.5). Five out of 6 relapses were preceded by MRD+ testing, except for one patient that developed a solitary brain lesion. Pts with ctDNA+ had a primary EBC stage II or III (7 and 2, respectively, 1 unknown). In 7/10 pts, after a first ctDNA+ result, an imaging scan was planned with detection of distant asymptomatic disease recurrence in 3 cases. In 3/10 patients, a follow-up test after 3 months led to further scans and detection of 2 additional relapses. At the time of the analysis 5 patients MRD+ were free of detectable metastases (clinically or radiologically). Overall, ctDNA detection was the only prognostic factor for RFS ($p < .001$). These data estimate a negative predictive value of 98.4% and a positive predictive value of 50% for this GR test, with a related sensitivity and specificity of 83.3% and 92.5%, respectively.

Conclusions: Our analysis suggests the utility of a tissue-free ctDNA assay in diagnosing minimal residual disease. This could have clinical implications by enabling earlier diagnosis and possibly intervention to delay or prevent metastatic recurrence. We confirm prior studies showing high specificity for recurrence detection with multi-omic plasma-only MRD testing. Sensitivity limitations may be due to the small sample size and short follow-up. These findings warrant further study in larger cohorts and future clinical trials.

P4-01-20: Impact of neoadjuvant chemotherapy on PD-L1 status in patients with high-risk early-stage triple-negative breast cancer

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Triple negative breast cancer (TNBC) stands out as one of the most lethal breast cancer subtypes, primarily due to the lack of targeted therapies, being chemotherapy the standard treatment. Thus, the development of new targeted therapies for this aggressive subtype are required, given its high rates of tumor recurrence and metastasis. Immunotherapy is emerging as a promising therapeutic approach for TNBC, driven by its greater genomic instability, higher mutation rate, and increased production of neoantigens, all of which contribute to heightened immunogenicity. Therefore, the main objective of this study is to analyze the PD-L1 status in paired tumor samples from high-risk TNBC patients undergoing neoadjuvant chemotherapy (NACT).
The retrospective study included a total of 25 patients diagnosed with early-stage TNBC who were treated at the Catalan Institute of Oncology of Girona between 2010 and 2019. Clinicopathological variables and tumor characteristics were collected from clinical records. For each participant, paired pre- and post-NACT samples (core biopsy and tumor from the surgical specimen after NACT, respectively) were obtained from Institutional Tumor Biobank. The expression of PD-L1 was assessed by immunohistochemistry.
The median age of the participants was 47 years, 79% of the tumors were classified as invasive ductal carcinomas and 62% had positive node involvement at baseline. Moreover, 79.4% of the patients received both anthracyclines and taxanes as neoadjuvant chemotherapy, followed by surgery and 75.9% also received adjuvant radiotherapy. At the time of data cut-off, 48.3% of participants were alive, with a median follow-up of 59 months. At baseline, 92% (23/25) of our population exhibited PD-L1-negative (<1%). Following NACT, 52% (13/25) of these patients positivized PD-L1 status (≥1%), being statistically significant the association between PD-L1 status and NACT (p=0.002). Additionally, the patient cohort whose tumors positivized PD-L1 after NACT showed higher median progression-free survival (PFS) (44.3 m vs. 21.0 m) and overall survival (OS) (56.5 m. vs. 34.1 m.), with time to progression (TTP) not reached (NA vs. 21.0 m), compared to the cohort that remained PD-L1 negative. However, the differences between the two cohorts were not statistically significant.
In conclusion, in patients with early-stage TNBC and PD-L1 negative tumors, NACT can

induce the positivization of PD-L1 status. This conversion may correlate with improved response to NACT and better clinical outcomes. Furthermore, PD-L1 positivization suggests potential suitability for immunotherapy in these high-risk patients. Nevertheless, further studies involving a larger cohort of patients are necessary to validate these findings in a more robust manner.

P4-01-21: The clinical significance of disialoganglioside GD2 synthases in breast cancer

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Introduction: Disialoganglioside GD2 is a glycosphingolipid found in the plasma membrane, consisting of a hydrophobic ceramide linked to a hydrophilic sialic acid-containing glycan chain. Its synthesis involves sequential glycosylation and sialylation steps facilitated by multiple enzymes. Recent studies have identified GD2 as being overexpressed in certain cancers and cancer cells, potentially influencing their survival, motility, and invasive properties. This study aimed to investigate the clinical significance of GD2 synthases in breast cancer by examining their clinical, pathological, and prognostic associations.
Materials and Methods: A cohort of human breast cancer and background tissues were assessed for expression of a group of disialoganglioside GD2 synthases using q-PCR and statistically analysed using Mann Whitney U tests for comparisons, ROC, logistic regression Kaplan-Meier and Baysian methods for survival analyses. The relationship between the expression of the enzymes and patient's responses to drug therapies were also explored.
Results: We tested eight key enzymes involved in GD2 synthesis and explored them as a coherent group of synthesis regulators. Excessive expression of B4GalT5 and, notably, ST8Sia1, two enzymes involved in GD2 synthesis, significantly influenced patients' clinical outcomes ($p=0.023$ and $p=0.006$, respectively). When treatment response was assessed from the public database (TCGA), patients with high levels of B4GalNT1 in breast cancer did not respond to chemotherapies ($p<0.00001$) but showed response to endocrine and anti-Her2 therapies. This association was more pronounced in Luminal-B subtypes patients but had little connection with ER or Her2 alone. Conversely, patients with high levels of B3GalT4 were more sensitive to endocrine therapies ($p=0.001$) but showed no connection with chemotherapies. Further analysis using a combination of the three synthases provided a significantly effective prognostic indicator for patient survival, identifying two distinct divisions for the synthases as favourable and non-favourable for survival ($p=0.0009$, HR=2.02 (95%CI 1.33-3.08), a finding further supported by Bayesian analysis. The combination indicator, when stratified by ER, Her2 and TNBC subgrouping, revealed that the signature identified from the synthases is strongly associated with hormone status and the molecular subtype.

Conclusions: These data indicate that GD2 synthases have potential as a significant indicators for prognosis and therapeutic responses in human breast cancer, particularly in

association with hormone receptor status. Further work is warranted to unpick the potential mechanism/s underlying this phenomenon.

P4-01-22: Serum Thymidine Kinase as a Biomarker for Response Beyond Progression in First-line CDK 4/6 Inhibitors: Insights from the Randomized Phase II MAINTAIN Trial

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Background: There are multiple therapeutic strategies for patients (pts) with hormone receptor positive (HR+), HER2 negative (HER2-) metastatic breast cancer (MBC) following progression on a first-line cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) and endocrine therapy (ET). There is an unmet need for predictive and prognostic biomarkers to identify HR+, HER2- MBC pts likely to benefit from continued CDK4/6i therapy beyond progression. Thymidine kinase (TK), an enzyme involved in DNA synthesis, represents a promising biomarker due to the association of elevated serum TK activity (sTKa) with greater tumor cell division and poorer clinical outcomes. We investigated sTKa as a potential biomarker of response to continued CDK4/6i therapy beyond progression in the MAINTAIN trial.

Methods: MAINTAIN was the first randomized controlled trial to demonstrate a significant progression-free survival (PFS) benefit for HR+, HER2- MBC patients who switched ET (fulvestrant or exemestane) and received the CDK4/6i ribociclib compared to ET plus placebo after progressing on prior CDK4/6i and ET. We evaluated the association between sTKa and PFS. In this exploratory analysis, sTKa levels were analyzed from blood samples collected at baseline and cycle 2 day 1 (C2D1) by Biovica using the Divitum® TKa assay. Patients were categorized into low (<250 Divitum® unit of Activity [DuA]) and high (≥250 DuA) sTKa groups for PFS analysis. The cut-off point of 250 DuA is a clinically validated and FDA 510(k) cleared reference value for prognosis in HR+ MBC. Log-rank test and Cox regression models were used to assess the association between sTKa levels and PFS as well as calculate hazard ratios (HR) and 95% confidence intervals (CI). The database was locked on Jan 4, 2022.

Results: Of the 119 MAINTAIN participants, 90 had samples available for analysis. Forty (44%) in the placebo+ET group and 50 (56%) in the ribociclib+ET group. Eighty-one pts had baseline sTKa, 73 had C2D1 sTKa, and 69 had sTKa at both timepoints. Fifty-three percent (43/81) of pts had high baseline TKa and 37% (27/73) of pts had high C2D1 TKa. Pts with low baseline sTKa exhibited a non-significant trend towards improved PFS compared to those with high baseline sTKa (median PFS [mPFS] 5.6 vs 3.0 mo; HR 0.73,

95% CI 0.45-1.18, p=0.198). In contrast, pts with low C2D1 sTKa demonstrated a statistically significant PFS benefit compared to those with high C2D1 sTKa (mPFS 5.7 vs 2.7 mo; HR 0.30, 95% CI 0.17-0.52, p<0.001). Notably, the mPFS in the ribociclib+ET group was 10.9 mo for pts with low C2D1 sTKa compared to 2.7 mo for those with high C2D1 sTKa (HR 0.15, 95% CI 0.06-0.37, p<0.001). The placebo+ET group did not show a statistically significant PFS benefit for those with low C2D1 sTKa versus high C2D1 sTKa (mPFS 4.8 vs 2.5 mo; HR 0.51, 95% CI 0.23-1.13, p=0.098). Among 69 pts evaluated for sTKa dynamics, the longest PFS was seen in 13 pts (19%) who transitioned from high baseline to low C2D1 sTKa (mPFS 8.2 mo; 95% CI 5.6-16.6). Twenty-one (30%) pts who maintained high sTka levels from baseline to C2D1 had the shortest PFS (mPFS 2.7 mo; 95% CI 2.1-3.0).

Conclusions: In the phase II MAINTAIN trial, sTKa was a predictive biomarker for response to switching ET + continuing CDK4/6i in HR+, HER2- MBC pts following progression on CDK4/6i and ET. Low (<250 DuA) C2D1 sTKa levels predicted a positive treatment response, especially among those receiving ribociclib+ET. sTKa dynamics from baseline to C2D1 were prognostic, providing insights into PFS outcomes. Our analysis supports C2D1 as a critical timepoint for sTKa in assessing continued benefit of CDK4/6i and risk of progression. Further research in a larger trial evaluating sTKa dynamics and clinical outcomes is warranted.

P4-01-23: Potential prognostic Value of HER2/CEP17 FISH ratio in HER2-positive non-metastatic breast cancer

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Background: HER2-positive breast cancer (BC) requires anti-HER2 therapy. We aimed to determine whether the expression of the HER2/centromeric probe for chromosome 17 (CEP17) ratio is associated with prognosis in patients with HER2-positive non-metastatic BC.
Methods: 267 HER2-positive BC were enrolled between January 2010 and December 2011. Stabilized inverse probability treatment weighting (sIPTW) was used to balance baseline characteristics. Real-world disease-free survival (DFS) and overall survival (OS) was analyzed.
Results: The median follow-up time was 10.3 years (interquartile range: 9.4-10.8 years). HER2/CEP17 ratio of > 7.0 was defined as the HER2 ultra-positive group; a HER2/CEP17 ratio of ≤ 7.0 was defined as the HER2 normal-positive group. After sIPTW adjustment, no differences were observed in DFS and OS when anti-HER2 therapy was unclear, and so was in the patients who did not receive trastuzumab (all p > 0.05). Interestingly, HER2 ultra-positive group had a worse DFS than the normal-positive group (hazard ratio [HR] = 2.72, p = 0.02), but there was no difference in OS (p = 0.30) in patients receiving trastuzumab. The multivariate Cox models also showed that the HER2 ultra-positive have worse DFS than HER2 normal-positive patients (HR = 3.71; p < 0.01).
Conclusion: For non-metastatic HER2-positive BC with or without trastuzumab treatment,

the HER2/CEP17 ratio did not predict the survival time. However, our study supports that HER2 ultra-positive group had a worse DFS than the normal-positive group among non-metastatic HER2-positive BC patients receiving trastuzumab; therefore, this could be a potential predictor of DFS in these patients.

P4-01-24: Common determinants of CDK4/6 inhibitor-based therapy in metastatic HR+/HER2- breast cancer

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CDK4/6 inhibitors in combination with endocrine therapy are widely used to treat HR+/HER2- metastatic breast cancer. Generally, CDK4/6 inhibitors lead to a doubling of progression-free survival compared to single agent endocrine therapy. Despite a known mechanism of action, features that define the duration of response to CDK4/6 inhibitors with either aromatase inhibitors or fulvestrant remain lacking. Over 300 patients receiving standard-of-care CDK4/6 inhibitor combination therapy for metastatic disease were enrolled in an ongoing observational study (NCT04526587) and an IRB-approved retrospective analysis. Clinical, pathological, and gene expression data were employed to define determinants for the duration of progression-free survival (PFS). Visceral disease (HR 1.55, p=0.0013), prior endocrine therapy (HR 2.34, p<0.001), and the type of endocrine therapy (HR 2.16, p<0.001) were associated with duration of progression-free survival. Multiple gene expression signatures associated with prognosis of HR+/HER2- breast cancer were evaluated for association with PFS with variable statistical significance.
Random survival forest was applied to combined prognostic signatures and clinical variables to identify independent features associated PFS. The resultant final model resulted in robust association with PFS (HR: 6.9, p<e-10) using all samples, as well as selectively in a validation subset (HR: 2.2, p<e-04). This tool was effective at predicting intrinsic resistance in metastatic and neoadjuvant samples. Additionally, the mortality predicted by this model tracked with the median PFS observed in PALOMA-2/3 and PEARL studies. Interrogating significant genes across all studies indicate common enrichment of genes associated with cell cycle and estrogen receptor signaling. These findings indicate that there are common features from real-world use of CDK4/6 inhibitors and clinical studies that could be used to infer time-to progression.

P4-01-25: THE ROLE OF SERUM THYMIDINE KINASA 1 ACTIVITY (TK1) AS A PROGNOSTIC FACTOR Y LUMINAL HER2 NEGATIVE BREAST CANCER

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TKI plays an important role in DNA synthesis and cellular proliferation so has been studied a prognostic marker and as an early indicator of response in luminal HER2 negative.

We analyze the function of TK1 in a cohort of 30 cases with early luminal HER2 negative breast cancer. Serum sampled were collected at baseline and after surgical resection. The median level of TK1 was 704 DuA (172 -1759) in the baseline and 903 (299 – 1977) after surgical resection. This association is not significative but in patients with high risk ONCOTYPE (>25 in 10 cases) the median level of TK1 was superior in this point, 1154 DuA vs 800 p:0,02.
We also analyze the dynamics change of TK1 in a series of 55 metastatic luminal HER2 breast cancer to compare the level of TK1 in the initial treatment with standart ET plus iCDK 4/6 and patients with durable response of this treatment. The median level of TK1 for initial treatment was 1315 DuA (215 – 18751) and for durable response 416 Dua (147 – 1004). In 27 patients we had 2 TK1 samples, at the beginning and 3-6 months after treatment. The median value in the initial sample was 512 (215 – 18751) and the second sample was 622 (182 – 1320). 12 patients had in progression at the time of the analysis, of which 10 had an increase in TK1 levels while in the 15 patients who remained in response, only 3 had an increase in TK1 levels.
The kinase activity has an important role in the evolution of HER2-negative luminal breast cancer. In early breast cancer there is an increase in cases with high-risk genomic testing, which are cases that intrinsically carry a worse prognosis. In advanced breast cancer, the decrease in its expression is associated with a good response.

The expression of kinase activity should be considered in large prospective studies to validate its role as a prognostic biomarker.

P4-01-26: The Xpert® Breast Cancer Insight test predicts distant recurrence and overall survival in estrogen receptor-positive, HER2-negative early breast cancer: A validation study in ABCSG Trial 8

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Purpose: To validate the clinical performance of the Xpert® Breast Cancer Insight (BC Insight) (IUO*) test in patients of the prospectively randomized Trial ABCSG 08. BC Insight is a prognostic signature designed to be run in local pathology laboratories on the GeneXpert in-vitro diagnostic platform in a closed-cartridge RT-qPCR format with a turnaround time of approximately 3 hours using formalin-fixed, paraffin-embedded (FFPE) samples.
Patients and Methods: We studied the BC Insight test in 1278 surgical FFPE specimens from postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative primary breast cancer with 0–3 involved lymph nodes who received at least 5 years of endocrine therapy. Time to distant recurrence (DR) and

overall survival (OS) were analyzed using Cox models.
Results: The BC Insight test categorized 764 of 1089 (70.2%) evaluable patients as “low risk”. The Xpert BC Insight Risk Score (XRS) was highly prognostic for prediction of DR (hazard ratio (HR) 1.93, 95% confidence interval (CI) 1.58 to 2.35, P<0.0001; C-index 0.73) and OS (HR 1.45, 95% CI 1.26 to 1.67, P<0.0001; C-index 0.65). At 10 years of follow-up, 95.3% of Xpert BC Insight Risk Category (XRC) low-risk patients were metastasis-free and 89.6% were alive, compared with 85.6% and 77.1% of XRC high-risk patients, respectively (absolute DR risk difference 9.7%, P<0.0001; absolute risk of death difference 12.5%, P<0.0001). Furthermore, XRS was highly prognostic in node-negative patients (HR for DR 1.59, 95% CI 1.25 to 2.03, P=0.0003, C-index 0.70; HR for death 1.26, 95% CI 1.06 to 1.50, P=0.0077, C-index 0.62), and in patients with 1–3 involved lymph nodes (HR for DR 3.01, 95% CI 2.01 to 4.52, P<0.0001, C-index 0.77; HR for death 2.07, 95% CI 1.54 to 2.79, P<0.0001, C-index 0.71).
Conclusions: XRS and XRC are highly prognostic for 10-year DR in postmenopausal women with ER-positive, HER2-negative primary breast cancer treated with endocrine therapy. Furthermore, the BC Insight test accurately predicted OS in these patients, particularly in patients with 1–3 involved lymph nodes.
*For Investigational Use Only. The performance characteristics of this product have not been established.

P4-01-27: Detection, isolation, and genetic characterization of circulating tumor cells from the cerebrospinal fluid of breast cancer patients with leptomeningeal metastasis

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The gold standard to diagnose leptomeningeal metastasis (LM) is to identify malignant cells in the cerebrospinal fluid (CSF) or in a leptomeningeal biopsy. Using conventional cytology its sensitivity remains low with approximately 50% and 85% for the first and the second lumbar puncture, respectively. While rare cell capture technologies like the CellSearch system reach higher sensitivity than conventional cytology, they have limitations, including capturing only cells with sufficient EpCAM antigen expression, limited antibody customization, and high costs. Once diagnosed with LMs, to date most patients are still treated with untargeted therapies with limited effectiveness and significant side effects.
To address this, we developed a workflow using Sievewell 370 K microwell chips. Each chip contains 370,000 hexagonal microwells (20 µm in diameter, 25 µm deep) with two 2 µm pores at the bottom. On the chip, individual cells are separated, identified with cytokeratin antibodies, and isolated as single cells via micromanipulation for molecular analysis. 15 CSF samples from 9 LM-patients were analyzed on the microwell chips and by conventional cytology in parallel. The isolated cells’ DNA was amplified by whole genome amplification and sequenced by low pass whole genome sequencing and panel sequencing on single cell

level and compared to extracranial tissue biopsies.
On-chip immunostaining achieved less than 5% cell loss and over 95% single cell isolation for the MCF7 breast cancer cell line. For 10 of the 15 CSF samples, concordant results were obtained by analysis on the microwell chips and conventional cytology: 1 sample was tumor cell-negative, 9 samples were tumor cell-positive. In the other 5 samples, tumor cells were detected by analysis on the microwell chip with either inconclusive (3 samples) or negative (2 samples) results obtained by cytological analysis. With the microwell chips, 5 to 10,000 (mean 1,218; median 116) CSF-CTCs were found per mL CSF. The detection of chromosomal aberrations confirmed the malignant origin of detected tumor cells, including those from samples that were tumor cell-negative in conventional cytology. A high level of clonality was observed among the tumor cells in the CSF of each patient, yet these CSF tumor cells often exhibited an aberration pattern with distinct differences compared to the corresponding extracranial metastases or primary tumors. In addition to mutations shared by corresponding tissue biopsies, panel sequencing revealed private mutations of CSF-CTCs in resistance related genes and tumor driver genes.
In conclusion, the microwell-based CSF-CTC detection appears superior to conventional cytology. The high level of clonality indicates a very close evolutionary relationship within the CSF-CTCs and a monoclonal origin of LMs. Private mutations in resistance related genes and tumor driver genes could impact drug susceptibility and therapy resistance. Therefore, the CSF-based liquid biopsy should be considered for genetic profiling of LMs, potentially improving diagnosis, treatment monitoring, and targeted therapy selection.

P4-01-28: The role of ctDNA clearance in the adjuvant setting in triple negative breast cancer patients with residual tumors after neoadjuvant chemotherapy in the TRICIA trial.

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Triple negative breast cancer patients with incomplete response to neoadjuvant chemotherapy (NAC) have a poor prognosis. The CREATE-X clinical trial showed that the addition of adjuvant capecitabine resulted in a 42% decrease in disease relapse (Hazard Ratio 0.58). The TRICIA trial is a multicentre trial for TNBC patients with residual disease after NAC, in which plasma was collected after NAC pre-operatively (T1), immediately after surgery (T2, median 1.1 months), 3 months (T3, median 3.8 months) and at least 6 months after surgery (T4, median 8.7 months). The median follow up of these patients is 32 months. We assayed for circulating tumor DNA (ctDNA) in these samples using a tumor-informed academic digital droplet PCR approach (Cavallone L et al, Sci Reports 2019). We recently reported that the lack of ctDNAdetection in plasma collected at the post-NAC pre-operative timepoint is highly prognostic with 95% of patients with undetectable ctDNA at this T1 time point not presenting distant disease relapse. We now focus on the ctDNA clearance during the post-surgical period in 42 patients who had detectable ctDNA after surgery (T2) and a

blood collection at T4. 32 of these patients received capecitabine while 10 did not (before the CREATE-X report). This is the largest series of TNBC patients with residual disease at surgery with serial ctDNA measurements before (T2), during (T3) and immediately after capecitabine treatment (T4). In the 10 non-capecitabine patients, only 1 cleared and that patient did not show relapse. Of 32 patients receiving adjuvant capecitabine, 13 cleared their ctDNA by the T4 time point (42%, similar to the benefit of capecitabine in the CREATE-X study), significantly higher than in the cohort without capecitabine (p=0.03). Only one of these patients (8%) showed disease relapse. This patient had detectable ctDNA 8 months after T4 and relapsed 33 months after surgery. Four of these 13 with ctDNA clearance had become negative by the T3 time point, while the remainder still had detectable ctDNA at the T3 time point. The relapse-free survival for patients on capecitabine who cleared their ctDNA at T4 was 86% while that for those who did not was 55%, with a HR of 0.16 (95% CI 0.04-.66, p=0.05). These results show that ctDNA clearance in the short-term post-operative period has prognostic value and that the addition of capecitabine increases ctDNA clearance throughout the immediate adjuvant period. The clearance of ctDNA after surgery in these high-risk TNBC patients may be predictive of response to adjuvant capecitabine.

P4-01-29: Identification of Urinary microRNA as Potential Biomarkers in Breast Cancer Patients

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Backgrounds and Aims: MicroRNAs (miRNAs) are small functional nucleic acids consisting of 20-25 nucleotides. They maintain homeostasis in the body by balancing regulatory functions within cells, while abnormalities in their expression levels have been associated with various diseases, including the transformation of cells into cancer. Over 2000 miRNAs have been identified in humans, and it is known that there are specific expression patterns of miRNAs depending on the type of cancer. miRNAs are encapsulated in exosomes and circulate in the blood, with some being excreted into the urine. Therefore, urinary miRNAs are garnering attention as potential biomarkers in cancer diagnostics. Yasui and colleagues have developed a method for analyzing exosomal miRNAs in urine using a zinc oxide nanowire device, successfully achieving comprehensive analysis of miRNAs from urine. Urinary miRNAs are expected to be applied as non-invasive diagnostic biomarkers for early cancer detection, assessment of treatment efficacy, and monitoring post-treatment for recurrence. In SABCS 2023, we reported that 28 differentially expressed miRNAs (DEMs), defined as miRNAs with adjusted p-values < 0.05 and Log2FC > 0.5, were identified between the primary breast cancer group and the control group, suggesting their potential as biomarkers. In this presentation, we advanced our research by analyzing the time-course in these miRNAs along with treatment to their clinical significance.
Patients: Patients who were diagnosed with breast cancer from October 2021 to May 2022

in our hospital were recruited. The eligibility included female, 20 years or older, no prior treatment for breast cancer. The protocol was approved by the institutional review board and informed consent was obtained from all patients.

Methods: Samples were collected before starting the treatment, after surgery, and 3 to 6 months after surgery. Small RNA seq was performed using a NextSeq 550 System (Illumina, San Diego, CA, USA), and urinary miRNA profiles were obtained and analyzed. Differential expression analysis was conducted using DESeq2 between breast cancer group and the control group, to identify DEMs. The analysis of time-course in DEMs was performed by calculating p-values using the Wilcoxon rank sum test, followed by multiple comparison correction using the Benjamini-Hochberg method.

Results: Two hundred patients with stage 0-III primary breast cancer treated in our hospital (breast cancer group) and 105 healthy volunteers (control group) were included in the study. Among the 200 cases in the primary breast cancer group, the initial treatment was surgery for 170 cases and chemotherapy for 30 cases. Comparing the primary breast cancer group with the control group, 15 of the 28 identified DEMs were elevated in the primary breast cancer group compared to the control group, while 13 were decreased. When examining the time-course in the 170 cases which the initial treatment was surgery, it was found that among upregulated 15 DEMs, miR-22-3p, miR-374a-5p, miR-450b-5p, miR-500a-5p/500b-5p, miR-57b-5p, and miR-92b-3b significantly decreased after surgery. On the other hand, we found variable alteration of downregulated 13 DEMs after surgery. miR-125a-3p, miR-151a-5p/151b, miR-30a-3p, miR-331-3p, and miR-574-5p significantly increased after surgery, while miR-12136 and miR-619-5p significantly decreased. Similar trend was also observed in the 30 cases where the initial treatment was chemotherapy.

Conclusions: We identified urinary miRNAs that could serve as biomarkers for breast cancer. Notably, the upregulated 15 DEMs showed declining trend after surgery or chemotherapy.

P4-01-30: Proteogenomic profiles of HER2-positive breast cancer and response to neoadjuvant anti-HER2 treatment

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Background: The prospective and randomized PREDIX HER2 (n=202) compared neoadjuvant treatments of docetaxel, trastuzumab, and pertuzumab (DHP) against trastuzumab emtansine (T-DM1) in HER2-positive breast cancer, demonstrating similar pathologic complete response (pCR) rates and disease-free survival between the two

groups (Hatschek, JAMA Oncology 2021). In this study, we aimed to elucidate the proteogenomic (PG) characteristics of pre-treatment HER2-positive breast cancer biopsies from the PREDIX HER2 trial, and identified predictive biomarkers for DHP or T-DM1.

Methods: We performed high quality shallow whole-genome sequencing (CUTseq), RNA-sequencing (RNA-seq), and mass spectrometry-based (MS) proteomics profiles of pre-treatment core biopsies. Imputed and normalized protein abundance data were used as an input for differential protein abundance analysis using DEqMS. Correlations between mRNA expression (trimmed mean of M-values (TMM) normalized transcript per million reads (TPM)), somatic copy number alterations (SCNA) and normalized protein abundance for each gene-protein pair were measured using Spearman correlation (rho), and a false discovery rate (FDR) lower than 0.05 was considered statistically significant. Furthermore, we assessed the association of biomarkers with pathologic complete response (pCR) in each treatment arm using logistic regression, and evaluated their predictive value by adding the interaction term between biomarker and treatment arm.

Results: In total, we identified 9,550 proteins (median (interquartile range): 7,424 (6,655-7,884)), of which 7099 proteins were finally quantified in each of the 137 tumors (70 samples from DHP and 67 samples from T-DM1 arm). We conducted differential protein abundance analysis adjusting for estrogen receptor (ER) status between responders (pCR) and non-responders (i.e., residual disease (RD)) within DHP and T-DM1 arm, respectively. We identified 112 upregulated (e.g., ERBB2, GRB7, CDK12, HLA-B) and 83 downregulated proteins (e.g., MAST4, FBN1, COX11) (nominal P values<0.05, log2 fold-change>0.5) within patients who attained pCR from DHP arm compared to counterparts without reaching pCR. Furthermore, GSEA demonstrated that proliferation (E2F, MYC) and inflammation and immune (IL2-STAT5, inflammatory response, IFN-γ response) pathways were enriched in pCR cases, whereas the epithelial–mesenchymal transition (EMT) pathway was enriched in RD patients. Similarly, we revealed 103 upregulated (e.g., ERBB2, ZNF652, MAPK11, CD3E) and 107 downregulated (e.g., ECM2, COL2A1, PDCD2L) proteins that were enriched in EMT and immune pathways (IFN-γ and IFN-α response, IL6-JAK-STAT3) in T-DM1 arm. We identified significant cis-correlations (rho > 0, FDR < 0.05) for 4706 (71.8%) SCNA–mRNA and 603 (9.2%) SCNA–protein pairs, while trans-effects (FDR < 0.05, for at least 50 genes) were identified in 4,636 (70.8%) and 493 (7.5%) genes at the transcriptional and translational level, respectively. We prioritized 43 putative drivers based on correlated SCNA-mRNA-protein genes, which were located on 1q32.2, 6q21, 8p11.23, 8q23.3, 11q13.3 and 17q12. Amplification of 8p11.23 (ERLIN2, RAB11FIP1) (Pfor interaction=0.02) was associated with pCR in the T-DM1 arm (odds ratio (OR)=1.95, p=0.1), but exhibited a trend towards RD in the DHP arm (OR=0.67, P=0.2). Interestingly, 36 pseudo-HER2+ cases were clustered with 9 HER2-low tumors that were re-evaluated using the 2018 ASCO guidelines, based on protein level of HER2 and its adjacent genes PNMT, MIEN1, GRB7. Patients with pseudo-HER2+ subtype were less likely to attain pCR compared to those with HER2-

positive breast cancer (DHP arm, OR=0.16, p=0.001; T-DM1 arm, OR=0.27, p=0.03).

Conclusion: This study underscores the importance of PG analysis in predicting the treatment response to chemotherapy, dual blockade or antibody drug conjugate in HER2-positive breast cancer.

P4-02-01: Clinical and biological markers to improve the identification of frailty in patients with early breast cancer

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Background: Aging is associated with deficits accumulation and increased vulnerability to adverse outcomes, ultimately resulting in frailty. Comprehensive geriatric assessment (CGA) is considered the gold standard to assess aging. Using the information gathered as part of the CGA in elderly breast cancer (BC) patients (pts), our group has designed a 43 item-frailty index (43-FI). Over the last few years, the analysis of cellular senescence through senescence-associated secretory phenotype (SASP) has been suggested as alternative biomarker of aging. In this study, we identified a panel of 23 SASP-related factors(RF) that was correlated with the 43-FI, and both with prognosis, resulting in new indicators for aging assessment.

Methods: 164 pts aged ≥65 years, with early BC included in 3 onco-geriatric trials, were evaluated by the means of a CGA. Geriatric assessments and blood sampling were performed after breast surgery, and before the initiation of systemic treatment (adjuvant endocrine therapy or chemotherapy). Serum samples were available for 140 pts, and were analyzed to identify the 23 SASP-RF. Pts were classified as fit, vulnerable or frail according to the Balducci criteria (Balducci-c) and the 43-FI. The 23 SASP-RF were correlated with the 43-FI and with survival. For each SASP-RF, Area Under the Receiver Operating Characteristic Curve (AUC-ROC) analysis on the fit and frail pts was perfomed to identify the best performing markers (AUC > 0.65) and optimal cut-points. Marker-specific optimal cut points were then used to classify vulnerable pts into high- and low-risk groups. A senescence score (SenS) was developed by training a Random Forest model on fit and frail pts considering the best-performing SASP-RF, defined as those showing relative importance > 5%. The SenS was used to classify vulnerable pts into high- and low-risk groups. Overall survival (OS) was computed from study entry to death from any cause.

Results: The 43-FI identified 20% of pts as fit, 71% as vulnerable, and 9% as frail. Vulnerable and frail pts were older than fit (77 and 78 yrs respectively vs 73 yrs), and had less aggressive tumors (62% and 60% of vulnerable and frail pts had Ki67 < 20%; 71% and

80% had G1-2). Hormone receptor-positive HER2-negative tumors were predominant in all three categories. Chemotherapy was administered more often to fit (63%) than vulnerable (33%) and frail (7%) pts. 43-FI and Balducci-c provide markedly concordant classification of pts (frail pts identified by Balducci-c had higher 43-FI score, overall percent agreement= 47%). The 43-FI identified pts with different prognosis: median(m)-OS was 5.5 months (mo) in frail vs 13 mo in fit (HR=4.39, 1.88-10.23 95% CI, p=0.001), and 9.8 mo in vulnerable pts. This association was independent from age. A total of 8 SASP-RF were found associated with the 43-FI, including GDF15, ICAM1, TIMP1, HUIL9, HUGCSF, HUMIP1A, HUMIP1B, HU TNFA. Among these, GDF15 was the best associated with frailty (AUC=0.88, fit vs. vulnerable p=0.001; Fit vs. Frail p=0.001; Vulnerable vs. Frail p=0.038). Applying GDF15 optimal cut-point to the vulnerable cohort, we find that higher levels of GDF15 were correlated with worse prognosis (mOS 11.6 mo low-GDF15 vs 7.8 mo high-GDF15, HR 2.23, 1.29-3.85 95% CI, p=0.003). The SenS model, including 7 SASP-related markers (GDF15, TIMP1, ICAM 1, HU IL9, HU MIP1A, HU MIP 1B, TNF a), was positively associated with prognosis (mOS 10.9 mo low-SenS vs 9.3 mo high-SenS, HR 1.97, 1.14-3.41 95% CI, p=0.013).

Conclusions: Our findings suggest that the 43-FI may serve as a potential clinical marker of aging in pts with early BC. GDF15 and SenS, could be used to dissect the heterogeneity of vulnerable pts, identifying subgroups with different prognoses within this cohort. Further analyses in a wider population are needed to confirm these results.

P4-02-02: Hospitalizations and emergency visits for Ontario breast cancer patients undergoing neoadjuvant chemotherapy with pembrolizumab: A population-based analysis

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Background: Immunotherapy-containing chemotherapy regimens are increasingly used for the neoadjuvant treatment of triple negative breast cancer (BC). However, these regimens can be associated with immune-related adverse events, which may lead to increased hospitalizations and emergency department (ED) visits. The objective of this study was to assess the rate of hospitalization and ED visits for BC patients undergoing neoadjuvant chemotherapy (NAC) with and without immunotherapy in Ontario, Canada.

Methods: Breast cancer patients who underwent NAC and surgery between April 1, 2022 and August 31, 2023 were included. Neoadjuvant chemotherapy regimens were classified into three groups containing either pembrolizumab, an anti-HER2 drug, or chemotherapy alone. The proportion of patients who had ED visits or were hospitalized during the neoadjuvant period (first date of chemotherapy to the day of surgery) and the post-

operative period (day of surgery to 30 days after surgery) were calculated. Rates and 95% CI of ED visits and hospitalizations during each time period per patient per year were analyzed.

Results: The study cohort consisted of 1301 newly diagnosed BC patients who had systemic therapy before surgery with a median age of 52 (44 - 62); 94.7% of patients had no previous comorbidities. A total of 229 patients (17.6%) received a regimen with pembrolizumab, 532 (41%) had an anti-Her 2 agent, and 540 (41.4%) had chemotherapy alone. The proportion of patients on pembrolizumab who had one or more ED visits during the neoadjuvant period was significantly higher (54.6%) than patients on anti-HER2 (42.3%) or chemotherapy alone (40.0%; $p < 0.001$). The rate of ED visits was also higher for the pembrolizumab cohort at 2.81 visits per patient per year (95% CI: 2.52-3.15) compared to anti-HER2(1.95 [95% CI: 1.77-2.15]) or chemotherapy alone groups (1.74 [95% CI 1.57-1.93]). However, there was no significant difference in ED visits in the post-operative period between the groups (pembrolizumab: 18.3%, anti-HER2: 14.5%, chemotherapy alone: 14.4%, $p=0.33$). The rate of ED visits in the post-operative period was also similar: pembrolizumab: 2.67 visits per patient per year (95% CI: 2.04 – 3.51), anti-HER2: 2.10 (95% CI: 1.72 – 2.77), chemotherapy alone: 2.35 (95% CI 1.95 – 2.85). Hospitalization was more frequent among patients receiving pembrolizumab pre-operatively (pembrolizumab: 32.3%, anti-Her2: 15.2%, chemotherapy alone: 11.1%; $p < 0.01$), but there were no significant differences in hospitalization after surgery.

Conclusion : Breast cancer patients undergoing neoadjuvant therapy including pembrolizumab had higher rates of ED visits and hospitalizations before surgery compared to those receiving anti-HER2 therapy or chemotherapy alone. However, differences in these outcomes were not seen in the post-operative period. This study provides confirmatory population-level data regarding the integration of immunotherapy into NAC. Further work to understand the etiology and mitigate unanticipated health care utilization for BC patients undergoing NAC is needed.

P4-02-03: Transparency for patient assistance and compassionate use programs for high-cost intravenous and oral therapies in breast cancer patients at an NCI-designated cancer center

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Background: Financial toxicity (FT) is a growing concern for cancer patients and affects overall survival, treatment adherence and quality of life. 1 Approaches to address FT include incorporating cost information into treatment decision making, which relies on accurate cost information as well as transparency into the process and timeline for treatment initiation. 1 Objectives: the primary objective is to identify the rates of patient assistance program (PAP) approval for oral anticancer medications (OAMs) and approvals

for compassionate use high-cost intravenous (IV) therapies in breast cancer patients. The OAMs and high-cost IV therapies of interest include palbociclib, ribociclib, abemaciclib, fam-trastuzumab deruxtecan, trastuzumab, pertuzumab, pembrolizumab, goserelin, leuprolide, liposomal doxorubicin, and sacituzumab govitecan. The secondary objectives include characterization of insurance approval, copay information, time to manufacturer PAP or EAP approval, clinical decision making (switching or discontinuing therapeutic agents) due to lack of PAP or EAP approval for OAMs and high-cost IV therapies in breast cancer patients.

Methods: A retrospective chart review was conducted at Fred Hutchinson Cancer Center (FHCC) for breast cancer patients referred to the Patient Assistance (PA) team for OAMs and high-cost IV therapies of interest within the study period of 1/1/2023 to 6/31/2024. Data was collected for therapy of interest, insurance type, insurance approval, copay information (if available), date of PAP initiation and determination, other patient assistance, off-label indication, and clinical decisions made based on PAP denial.

Results: For OAMs, 79 referrals to the PA team for 74 distinct patients were evaluated. For high-cost IV therapies, 17 referrals to the PA team for 15 distinct patients were evaluated. For OAMs, 48 were approved by insurance and 8 were not. Copay information was available for 49 patients and the median copay was $2520 (range $0 to $15,680). The median time from PA initiation to notification of PA determination was 19 days (range 0 to 200 days). Patients referred to the PA teams were insured by a Medicare Part D plan (53.2%), Medicaid (2.5%), private insurance (21.5%), or had no insurance (8.9%). Most patients identified as white (83.5%) with English as their primary language (88.6%). Of the 8 patients denied PA, one patient declined therapy, one patient switched to another agent in that class. For high-cost IV therapies, 1 out of 15 patients pursued PA due to insurance denial while 8 out of 15 patients pursued PA as they had no insurance. The median time from PA initiation to notification of PA determination was 17 days (range 4 to 221 days).

Conclusion: Mitigation of financial toxicity should include transparency and metrics for time to drug approval for patients. Our review showed that the median time to patient assistance approval for OAMs and high-cost IV therapies was 19 and 17 days, respectively, and this can be in addition to the time to initial insurance denial or determination. This review also identifies patients with Medicare as a population that may benefit from upfront financial navigation to expedite paperwork around the drug approval and acquisition process, as their median copay was $2520. The scope of this review should be broadened across all OAMs and high-cost IV therapies to provide patients and clinicians clear expectations and opportunities for patients and clinicians to identify and participate in quality improvement efforts and patient advocacy with manufacturer programs. References: Khan HM, Ramsey S, Shankaran V. Financial toxicity in cancer care: implications for clinical care and potential practice solutions. J Clin Oncol 2023; 41: 3051-3058.

P4-02-04: Development of ASSESS, a breast cancer decision support tool using SEER data and treatment benefit estimates from randomized adjuvant therapy trials.

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Background: Shared decision-making about adjuvant therapies requires accurate and personalized predictions of prognosis and treatment benefit. Multiple independent clinical characteristics influence the prognosis, and the most accurate individualized prediction can only be made by integrating these variables into a statistically valid multivariate model. The absolute improvements from each additional layer of systemic adjuvant therapy are also difficult to estimate. Our goal was to develop a multivariate prognostic model for breast cancer that also predicts absolute improvements in outcomes with various systemic adjuvant treatment modalities.

Methods: SEER incidence data including 394,503 breast cancer patients diagnosed in the years 1975-2020 were used to develop four distinct subtype-specific baseline (no-adjuvant chemotherapy) Fine-Gray subdistribution hazard prognosis models for overall survival. Multivariable fractional polynomials were used to model the non-linear effect of continuous variables on with a custom method for searching for best data transformations. Baseline model validation was done using 4,485 patients without chemotherapy from TCGA, Metabric, and GSE96058 cohorts. Hazard ratios (HR) from randomized adjuvant therapy trials were taken from the literature, 18 treatment regimens were grouped into 6 categories: (i) CMF, AC, and EC; (ii) CEF, TC, (iii) AC->T, FEC+Docetaxel, FEC+Paclitaxel, TAC, ddAC->T, A->T->C, AC->Docetaxel; (iv) AC_T+Olaparib, AC_T+Pembrolizumab; (v) TCH, ACTH, AC->T+Trastuzumab; (vi) TCHP. The final prognostic prediction included adjusting the baseline model with pooled HRs corresponding to each of the 6 treatment categories. Adjuvant chemotherapy validation in hormone receptor (ER) positive/HER2 negative patients was performed on data from the PACCT-1 clinical trial (NCT00310180) obtained from the NCTN/NCORP Data Archive (Request ID – 1843). The models were evaluated for overall survival in terms of calibration (a ratio between predicted and observed survival with 95% confidence intervals) and predictive ability (area under ROC curve (AUC) for alive/dead classification) 5 years after diagnosis.

Results: The baseline model built on 4 explanatory variables (age, tumor size, tumor stage, and number of positive lymph nodes) on SEER data showed good calibration in all BC subtypes: 1.02 (1.02;1.03) for ER+/HER2-, 1.03 (1.02;1.04) for ER+/HER2+, 1.04 (1.02;1.06) for ER-/HER2+, and 1.07 (1.05;1.08) for TNBC. Similar results were obtained on the independent PACCT-1 dataset: 1.05 (1.02;1.09) for ER+/HER2-, 1.10 (0.93;1.29) for ER+/HER2+, 1.18 (0.85;1.63) for ER-/HER2+, and 1.19 (1.06;1.34) for TNBC. Visual inspection of the calibration curves did not show major deviations from the diagonal, demonstrating good model performance for patients with varying survival. The model efficiently predicted whether the patient would still be alive after 5 years, giving an AUC

equal to 0.760 for ER+/HER2-, 0.762 for ER+/HER2+, 0.751 for ER-/HER2+, and 0.747 for TNBC subtypes. Similar results were obtained on the PACCT-1 dataset: 0.722 for ER+/HER2-, 0.750 for ER+/HER2+, 0.547 for ER-/HER2+, and 0.728 for TNBC subtypes. The chemotherapy model also gave an accurate survival estimate (1 (0.95;1.05) for CMF or AC, 1 (0.96;1.05) for TC, and 1.01 (0.91;1.11) for AC->T) and a prediction of vital status (AUC equal to 0.636 for CMF or AC, 0.619 for TC, and 0.648 for AC->T).

Conclusions: We developed and successfully validated a multivariable risk prediction model to estimate patient-specific survival and incremental benefits from systemic adjuvant therapies. The model is being used to develop ASSESS, a web-based decision support tool that can be used to inform shared decision-making.

P4-02-05: Real-world treatment patterns of neoadjuvant chemotherapy delays in operable breast cancer

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Background: Delays in initiation of neoadjuvant chemotherapy (NACT) after breast cancer (BC) diagnosis are associated with worse outcomes including increased risk of death. Previous studies have shown that Black patients are more likely to receive NACT compared to White patients. Currently there are limited studies that have evaluated delays in NACT initiation (NACTi), which could contribute to worse survival. Our previous work using the National Cancer Database showed that patients who were Black, Hispanic, non-commercially insured, or treated at an academic facility were more likely to be delayed. We used an electronic medical record (EMR) based database to evaluate incidence and predictors of delays of NACTi among high clinical risk patients in the real-world setting.

Methods: We conducted a retrospective cohort study using the ConcertAI Patient 360TM Database, an EMR-based breast cancer dataset comprised of both structured and curated data derived from over 900 US oncologic community and academic entities and linked with ASCO CancerLinQ data. Eligibility included age ≥18 years, diagnosis of cT2-4 or cN1+, M0 BC between 1/1/2010 – 12/31/2022, treated with NACT within 180 days of BC diagnosis. Patients who received neoadjuvant endocrine therapy were excluded. Time to chemotherapy (TTC) in days was recorded. Multivariable logistic regression, adjusted for demographic, socioeconomic and tumor characteristics, was used to identify characteristics associated with delays > 60 days from diagnosis to NACTi. Multiple imputation was used for missing data.

Results: We identified 2107 eligible patients, 213 (10.1%) of which experienced a delay greater than 60 days from diagnosis. Of the entire cohort, 70.8% were White, and 18.8% were Black; across all racial categories, 30.4% were Hispanic. Median age at diagnosis was

53 years (range 22-85) and median TTC was 31 days (range 0-178). By tumor type, 35.1% (n=740) were hormone receptor positive/HER2-negative, 35.7% (n=752) were HER2-postive, and 29.2% (n=615) were triple negative BC; 11.4%, 9.2%, and 9.8% (p = 0.4) had a delay in NACTi, respectively. In a multivariable analysis across all tumor types, patients who were Black (OR 2.31 95%CI 1.46-3.63, p <0.001) or Hispanic (OR 2.20 95%CI 1.23-3.94, p 0.011) were more likely to be delayed compared to White and non-Hispanic patients, respectively. Patients with Medicaid were more likely to be delayed compared to those with commercial insurance (OR 2.0 95%CI 1.30-3.07, p 0.002). Patients treated at community facilities were less likely to be delayed (OR 0.50 91%CI 0.34-0.74, p <0.001) compared to those treated at academic facilities.

Conclusions: Among patients with operable breast cancer who received NACT in this real-world EMR-based database using both structured and curated data, patients who were Black, Hispanic, had Medicaid insurance, or received treatment at an academic facility had a significant increase in odds of a delay in NACTi. These racial, socioeconomic, and access disparities warrant targeted interventions to ensure equitable care.

P4-02-06: Assessing the True Cost of Breast Cancer Screening: Chargemaster, Payer, and Cash Price Discrepancies for Mammography

Mason Alford-Holloway, Austin Triana, Katherine Baker, Samyukta Mullangi

Introduction: Patients in the United States (US) often lack information about the price of breast cancer screening services across hospitals and payers. Recent legislation in the US, including the 2021 Hospital Price Transparency Final Rule, requires hospitals to publish standard chargemaster rates, payer-specific negotiated prices, and discounted cash prices for all services, creating a unique opportunity to understand variation in hospital pricing. While uninsured or underinsured patients may be exposed to high chargemaster rates for screening services, those who are able to pay upfront in cash or have negotiated rates through insurance face significantly discounted prices. More research is needed to better understand the relationship between the distinct screening prices facing patients in the United States.

Methods: We extracted standard chargemaster prices, payer-specific negotiated prices, and self-pay cash prices for screening mammography (CPT code 77067) from machine-readable files published by ten top US hospitals. For each hospital, we compared the standard chargemaster rate for screening mammography with the average payer negotiated price and determined the chargemaster-payer price ratio. Additionally, we compared the standard chargemaster rates and discounted cash prices for screening mammography, and determined the chargemaster-cash price ratio for each hospital.

Results: Across ten top US hospitals, the average chargemaster rate for screening mammography was $855. The average cash price for the same service was $305, with an average chargemaster-cash price ratio of 3.7. The average payer-negotiated price for screening mammography was $411, and chargemaster rates were greater than payer-negotiated prices by an average factor of 2.2. The average cash price for breast cancer screening was 74.2% of the average payer negotiated rate for the same service. We observed wide variation across institutions for chargemaster rates, payer-negotiated rates, and discounted cash prices for screening mammography, with interquartile ranges of $198, $292, and $203, respectively.

Conclusion: Across ten top US hospitals, the chargemaster price for screening mammography was greater than the average payer negotiated rates and discounted cash price for the same service by a factor of 2.2 and 3.7, respectively. This price discrepancy has important consequences for individuals seeking essential breast cancer screening services, with uninsured patients facing prohibitive costs. As curable breast cancers go undetected, the burden on the healthcare system escalates. Policies should aim to improve price transparency, equity, and access to lifesaving breast cancer screening for the most vulnerable populations in the United States.

P4-02-07: Uptake of Ovarian Function Suppression Over Time and Endocrine Therapy Adherence Among Premenopausal Women with Breast Cancer

Dawn Hershman, Ling Chen, Melissa Accordino, Claire Sathe, N. Lynn Henry, Jason D Wright

Purpose: About 50,000 women under the age of 50 are diagnosed each year with invasive breast cancer. In 2017 the SOFT and TEXT trials demonstrated that endocrine therapy (ET) in combination with ovarian function suppression or ablation (referred to as OFS) improves disease-free survival by about 35% in premenopausal women with hormone receptor-positive breast cancer compared to tamoxifen without OFS. However, the addition of OFS increases the frequency of adverse events and symptoms resulting in reluctance to initiate this treatment. Factors influencing the rates of uptake are unknown.

Methods: We used the MarketScan health care claims database to identify women <50 years of age, diagnosed with breast cancer between the years of 2008-2022 using a standard algorithm. Patients with a prior history of a gynecologic cancer or prior oophorectomy were excluded. The analysis was limited to patients with insurance coverage one year prior and one year following first diagnostic claim. Non-adherence to ET was defined as proportion of days covered (PDC) < 80%. Logistic regression was used to determine factors associated with OFS in the overall cohort and then stratified by chemotherapy use and age category.

Results: We identified 36,670 women who met inclusion criteria, of whom 4,344 (11.8%) received OFS. Among the 18,409 women who underwent chemotherapy, 19.3% received OFS. OFS use increased over time from rates as low as 6.6% prior to 2016, up to 24.8% in 2021 (OR= 3.80 (3.19-4.52)). Oophorectomy remained constant at 5% and therefore the increases in OFS resulted from increased use of GNRH agonists. Among women who underwent chemotherapy, OFS rates increased over time from 9.4% in 2008 to 45.9% in 2022, OR=6.19 (5.05-7.59). Higher rates of OFS use were associated with younger age (22.4% for 18-34 yo vs. 8.7% for 45-49 yo; OR=2.42, 95% CI 2.13-2.76) and chemotherapy use (OR=4.89 (4.50-5.33)), By 2021, about 50% of women undergoing chemotherapy under the age of 45 underwent OFS. Among women who initiated OFS, only 30.8% underwent bone mineral density testing within the first year. Among women who initiated OFS, 39.8% were on antidepressant medications within the first year compared to 33.0% among those who did not initiate OFS ($p<0.001$); however, new initiation of antidepressant medications did not differ between group (12.6% vs 12.7%). Non-adherence to endocrine therapy in the first year was higher among women undergoing OFS compared to those on endocrine therapy alone (19.2 vs 16.9%, $p=0.001$).

Conclusion: Use of OFS has dramatically increased over time, with a quarter of all premenopausal women, and half of women <45 who underwent chemotherapy, receiving OFS by 2021. It is important to understand how clinicians are selecting patients for the addition of OFS, in order to optimize ET and reduce breast cancer recurrence. Of concern, in just the first year, OFS was associated with higher ET non-adherence rates. Efforts to improve adherence should focus on these high-risk pre-menopausal women to better understand how overall adherence and care quality is affected overtime.

P4-02-08: Comparative budget impact of CDK 4/6 inhibitors at a Mexican public healthcare institution

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Background: Metastatic or advanced breast cancer (ABC) management has experienced significant improvements since the advent of CDK 4/6 inhibitors (iCDK4/6). Abemaciclib, palbociclib, and ribociclib are iCDK 4/6 medications approved for use in the Mexican public healthcare system to treat HR+ HER2-ABC in postmenopausal women as first and second line treatments. All competitors have equal cost per pack but portray different survival and economic evidence. ISSSTE, a Mexican public healthcare institution caring for government officials, introduced palbociclib and ribociclib to treat these patients. Currently, the number of patients treated with each of these therapies is similar at ISSSTE. Therefore, it is important to understand the economic implications of this decision.

Objective: To compare the economic impact of having palbociclib and ribociclib available instead of palbociclib as the only available iCDK4/6 at a Mexican public healthcare institution.

Methods: We conducted a budget impact analysis (BIA) with a 5-year horizon using MS Excel to compare the current scenario (i.e., palbociclib + ribociclib) to a hypothetical scenario considering palblociclib as the only available iCDK 4/6. Target population were postmenopausal women with HR+/HER2- ABC without previous treatment for advanced disease treated at ISSSTE. The BIA accounted for the historical and projected market shares and costs for drug acquisition. Market shares of current scenario were based on historical data from two previous years and assumed a static participation of 50% ribociclib and 50% palbociclib, from the year 3 onwards. Market share of hypothetical scenario assumed a 100% use of palbociclib. Number of eligible patients were estimated based on epidemiological and population data. The analysis considered the cost of medication and assumed dose reductions for every comparator. Dose reductions were based on MONALEESA-2 and PALOMA-2; a sensitivity analysis was also performed using consumption data. Unit costs were obtained from the latest available published data and were reported in 2024 Mexican Pesos. Model outputs included annual and cumulative budget impact (absolute and as a percentage of the Institution's budget in medicines).

Results: The average annual cost per patient treated with ribociclib was $64,430.87 MXN (-20.9%) less costly than that of palbociclib. On average, 122 patients are diagnosed with ABC every year. As a result, current scenario with ribociclib and palbociclib has resulted in estimated savings of $8,955,710 MXN since its introduction two years ago. In a 5-year timeframe, savings could reach $40,512,962 MXN if market share is maintained. On average, the Institute could save up to $8.1 million MXN annually (0.0005% of 2022 ISSSTE medicines budget).

Discussion: Ribociclib's presentation allows patients to decrease dose using the same pack; this is translated into savings to the institutional budget. Despite portraying equal cost per pack, on average, patients treated with ribociclib consume a smaller number of packs than palbociclib patients due to dose reductions. Our analysis estimated that at current market shares, the Institution could save $40.5 million MXN in a 5-year time horizon; until today, the use of ribociclib has allowed to save $9.0 million MXN. Due to its cost-saving profile, the use of ribociclib in a larger proportion of patients could bring further savings to the institution.

Conclusion: Our analysis demonstrated that current scenario could save $40.5 million MXN in a 5-year timeframe. As compared to other iCDK 4/6, ribociclib is a cost-saving strategy that could maximize institutional resources. Additionally, it has shown a statistically and

clinically significant impact on overall survival and quality of life for HR+ HER2- ABC patients.

P4-02-09: Community Socio-geographic Distress Predicts Metastatic Breast Cancer at Diagnosis and Delays in Treatment Initiation

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Background: Detection of breast cancer at later stages results in higher mortality rates and stark contrasts in 5-year survival: 99% for localized early-stage, 84% for regional stage, and 27% for metastatic-stage disease. Despite advancements in screening and treatment, late-stage diagnoses persist due to various factors. In the US, the proportion of women diagnosed with breast cancer at later stages is higher among those of lower socioeconomic status and among Black women. Socioeconomic factors such as income, education level, and insurance coverage have individually been studied and shown to contribute to delayed diagnoses and poorer outcomes. However, more nuanced measures beyond these traditional proxies are needed to better understand a patient's socio-geographic environment and its cumulative effect on patient health. The Distressed Communities Index (DCI) is a validated socio-geographic measure of economic deprivation derived from the American Community Survey, providing a comprehensive assessment of economic distress at the zip code level. It incorporates factors such as the percentage of people without a high school diploma, unemployment rates, housing vacancy rates, poverty rates, median income, and changes in employment and business within a community. A high DCI score, typically greater than 3, identifies a distressed community. This study aims to determine whether higher community-level socioeconomic distress is predictive of metastatic-stage breast cancer at diagnosis, delays in treatment, and decreased 5-year survival.

Methods: A retrospective study was conducted among women aged 18 years or older who were either diagnosed and/or treated with first primary invasive breast cancer between 2012-2022 at Montefiore/Einstein Comprehensive Cancer Center. The study population was extracted by the tumor registry, and each patient's zip code was linked to the corresponding DCI score. Multinominal logistic regression, adjusting for age, race, ethnicity, and insurance status was used to evaluate the association between breast cancer stage at diagnosis (localized, regional, and metastatic stage) and DCI score. Additionally, an ANCOVA analysis was conducted to compare time to treatment between patients from high versus low DCI areas, controlling for stage.

Results: This study included 3,102 localized (N0), 1,042 regional (N1-3), and 122 metastatic stage (M1) breast cancer cases. After adjusting for age, race, ethnicity, and insurance status, women from zip codes with high DCI scores were twice as likely to be diagnosed with

metastatic disease compared to those from lower DCI areas (OR= 2.02, 95% CI 1.19-3.45, p = 0.009). Additionally, patients from higher DCI zip codes experienced significant delays in initiating treatment in neoadjuvant chemo (33 vs 49 days, p=0.024), adjuvant chemo (85 vs 105 days, p = 0.01), surgery (56 vs 76 days, p < 0.001), and radiation therapy (119 vs 169 days, p = <0.001), compared to those from lower DCI areas. Furthermore, the 5-year survival rates showed a decreasing trend among patients from higher DCI zip codes compared to lower DCI groups: 86.5% vs. 87.2% in localized, 75.4% vs. 82.0% in regional, and 22.1% vs. 30.8% in distant-stage cancer.

Conclusion: Patients residing in higher-distressed communities exhibited a twofold higher likelihood of being diagnosed with metastatic breast cancer and experienced delays in treatment initiation compared to those from lower-distressed communities. These results underscore the influence of socio-geographic factors on breast cancer disparities, emphasizing the need for tailored interventions to enhance outcomes in underserved populations. Leveraging the DCI database enables identification of high-risk zip codes, facilitating targeted screening initiatives aimed at mitigating disparities in breast cancer outcomes.

P4-02-10: Validating Navya Earthshot: An AI Driven Point of Care Solution for Guideline Adherent Treatment Planning in a Decentralized Cancer Care Model.

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Background: Adherence to breast cancer guidelines is associated with increased overall survival and disease-free survival globally (Ricci-Cabello et. al., BMC Health Serv Res. 2020;20(1):920). In resource-constrained settings, approximately 30% of patients receive undertreatment that is not evidence-based, or overtreatment that is potentially harmful (C.S Pramesh et al., J Clin Oncol 38: 2020). Despite significant investment in physical infrastructure for decentralized cancer care—with the goal of tertiary hub centers providing support to multiple non-specialized spoke centers—shortage of oncologists in resource constrained settings creates a knowledge gap at the point of care, which may be partially filled with digital AI solutions.
Navya is a clinically validated digital AI solution for cancer patients in use since 2014 which matches patient-specific evidence and guidelines to generate treatment options for a cancer patient vetted via asynchronous expert review (Nita Nair et. al., Cancer Res (2015) 75). Navya Earthshot is a new, provider facing solution for cancer treatment planning for non-specialized providers at the point of care, and is built as a search interface on Navya's validated domain model supporting subspecialized expert opinions in oncology (Tiffany A

Traina et. al, J Clin Oncol 39, 2021). Navya earthshot was customized to analyze the NCG guidelines for this use case.

Methods: In this multicenter, prospective validation in India, patients with a complete diagnostic workup and confirmed diagnosis of breast, oral and lung cancer at a decision point of treatment planning between January and June 2024 at participating centers were included. All decision points (curative and palliative; surgery, radiation and systemic therapies) addressed by India's standard of care National Cancer Grid (NCG) guidelines were included. At each decision point, Navya Earthshot first prompted a clinical navigator to complete a case specific short set of questions; then matched input case data with NCG guidelines, and output evidence based treatment plans at the point of care. Navya Earthshot output was given to providers at each of the participating centers, and scored as concordant or discordant by the tumor board/treating oncologists, as well as by a group of domain experts experienced in analyzing NCG guidelines. Results for the breast cancer cohort are presented below.

Results: 643 breast cancer patients met eligibility criteria at participating sites, which included 5 centers in the state of Andhra Pradesh, 1 center in Assam, 2 centers in Maharashtra, and a national organization of experts supporting care of underprivileged patients in several cancer hospitals across India. 70 patients (11%) were excluded from analysis due to the presence of rare histologies and bilateral cancers not covered by the NCG guidelines. Patients were well represented with respect to age (<45 years (35%) and >45years (65%), and early stage (45%) and advanced stage (54%). In 96% (553/573) of cases, Navya Earthshot output was concordant with the NCG guidelines as assessed by the domain experts, and validated by the tumor board/treating oncologists. Of these, in 93/553 cases (17%), treating oncologists/tumor board added additional treatment related guidance beyond NCG concordant Navya Earthshot. These cases did not require any further subspecialized expert review. The remaining 4% (20/573) cases were referred to a tertiary center for treatment planning.

Conclusion: A point of care solution used by clinical navigators that outputs guideline concordant treatment plans for consideration by treating providers can improve capacity in resource constrained settings that lack subspecialized organ specific multidisciplinary expertise. Navya Earthshot can enhance adherence to guideline driven care across spoke centers without overreliance on tertiary hub centers influencing treatment outcomes at scale.

P4-02-11: Outcomes of Susan G. Komen's "Navigating People with Metastatic Breast Cancer" Patient Navigation Training Course

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Background: Susan G. Komen's Patient Care Center patient navigators (PNs) reported a need for additional training to meet the unique needs of individuals with metastatic breast cancer (MBC) and a landscape analysis revealed a lack of systematic work done to understand the navigational needs of those with MBC and efforts to equip PNs to specifically address them. Komen's Patient Navigation Training Program provides interactive, virtual training to equip trainees to provide high-quality services addressing the needs of underserved communities. To build a training to fill this identified gap, a study was conducted to identify the needs of people with MBC and the training requirements of their PNs, leading to a targeted training course, "Navigating People with Metastatic Breast Cancer".
Focus groups with MBC patients and PNs identified key themes, including health care experiences, diagnosis challenges, professional and system obstacles, insurance issues, health inequities, coping mechanisms, ideal PN traits, and support needs. People with MBC emphasized the importance of access to PNs, while PNs expressed a need for training to address these challenges, underscoring the need for comprehensive PN training focused on MBC.

Methods: Focus group findings were used to develop the course learning objectives: 1) Highlight strategies and resources PNs can use to support the unique needs of those living with MBC, 2) Define MBC and identify barriers people with MBC may face, 3) Provide tools for PNs to better support people with MBC, and 4) Connect PNs to ongoing MBC training opportunities for continued learning and professional peer support. Komen's Patient Care Center PNs and experts validated content alignment with learning objectives through a survey instrument. Instructional design incorporated interactive, scenario-based learning approaches, including a self-assessment to tailor additional training to each learner's needs. The free course is available in Komen's online learning system, where demographic information and pre- and post-program survey instruments assess content, method of content delivery, and intent to apply content, using a Likert scale.

Results: From May to June 2024, 28 learners completed the course. Participant demographics included 21% Black/African American, 36% White, and 29% Hispanic/Latino (14% preferred not to answer). Common roles were PN (26%) and social worker (14%), with 11% having prior navigation training or certification.
All participants reported increased confidence in describing and navigating barriers and meeting the needs of people with MBC (100%), identified opportunities for continued learning to meet the needs of people with MBC (100%), and most participants intended to use the information professionally (96%). All respondents reported the information was

useful and relevant (100%), presented in an understandable way (100%) and that the level of interaction was helpful (100%).

Conclusion: The course was developed to fill a PN workforce reported need for training to address the unique needs of people living with MBC, informed by focus groups with people living with MBC and PNs who support them. The course attracted a diverse group of learners, including PNs and social workers, with significant representation from Black, White, and Hispanic/Latino communities. The virtual format proved accessible and effective in improving the learners' capacity to meet the needs of people living with MBC and met the need for interactive learning delivered virtually. Participants reported substantial improvements in confidence and ability to navigate MBC-related barriers. The training was universally rated as useful, relevant, and easy to understand, with a high level of engagement. These positive outcomes underscore the program's success in equipping PNs with the necessary skills and knowledge to better support individuals with MBC, addressing a crucial gap in patient navigation training.

P4-02-12: Incidence and Survival Trends in Young Women (Aged 20-49) with Breast Cancer: SEER analysis.

Nikhila Aimalla, Lekha Yadukumar, Rachel Gabor, Meghana Kesireddy

Background: Breast cancer (BC) is the most common cancer worldwide, classified based on the hormone receptor (HRc) and human epidermal growth factor receptor (HER2) status into 3 sub-types: HRc+ & HER2-, HER2+, and HRc- & HER2-. Its incidence, especially in advanced stages, is rising among young women, leading to it being the primary cause of cancer-related deaths for those aged 20 to 49.

Methods: A retrospective study using the Surveillance, Epidemiology, and End Results (SEER) database analyzed women aged 20 to 49 diagnosed with BC from 2010 to 2020. Cox proportional regression analysis was used to assess the impact of various variables on overall survival (OS) across the three BC subtypes.

Results: A total of 123,690 women aged 20-49 were diagnosed with BC: 81,006 (65.5%) HRc+ &HER2-, 25,253 (20.5%) HER2+, and 17,431 (14%) HRc- & HER2-. Incidence was higher in ages 40-49 (75.4%) than in 30-39 (21.9%) and 20-29 years (2.7%). Incidence was higher in 2015-2020 (55.85%) than in 2010-2015 (44.2%). Most were caucasian (72.7%), earned < 75,000$ (53.6%), lived in urban areas (92.3%), and were married (64.8%).
The study examined OS hazard ratios (HRs) across all three BC subtypes. OS HRs for ages 20-29 and 30-39 years indicated poorer survival than ages 40-49 across all subtypes, except for ages 20-29 in HRc- & HER2-, likely due to aggressive biology and advanced stage in the very young population. For ages 20-29 (vs. 40-49), the HRs were 2.4 for HRc+ & HER2-, 1.36 for HER2+, and 1.1* for HRc- & HER2-. For ages 30-39 (vs. 40-49), the HRs were 1.74 for HRc+ & HER2-, 1.11 for HER2+, and 1.08 for HRc- & HER2-.

Although the incidence rose in 2015-2020 across all subtypes, OS was worse for HRc+ & HER2- from 2010-2014 compared to 2015-2020, likely due to newer treatments, with no significant OS difference in the other two subtypes. Specifically, the OS hazard ratios for 2010-2014 (vs. 2015-2020) were 1.08 for HRc+ & HER2-, 1.07* for HER2+, and 1.06* for HRc- & HER2-.
Higher-income, Asian race, urban residence, marriage, receipt of radiation, and surgery were linked to better OS in all subtypes. Higher-income ($75,000+ vs. < $75,000) was associated with OS HRs of 0.69 for HRc+ & HER2-, 0.64 for HER2+, and 0.81 for HRc- & HER2-. Compared to White patients, Asians or Natives had lower OS HRs of 0.82 for HRc+ & HER2-, 0.86 for HER2+, and 0.88 for HRc- & HER2-, while Black patients had higher OS hazard ratios of 2.17, 1.94, and 1.47, respectively. Rural residence was linked to higher OS HRs of 1.31 for HRc+ & HER2-, 1.34 for HER2+, and 1.01 for HRc- & HER2-. Being married improved OS with HRs of 0.61 for HRc+ & HER2-, 0.57 for HER2+, and 0.7 for HRc- & HER2-. Surgery shows substantial protective effects across all subtypes (HRs of 0.1 for HRc+ & HER2-, 0.14 for HER2+, and 0.22 for HRc- & HER2-), radiation also correlates with improved outcomes (HRs of 0.81 for HRc+ & HER2-, 0.83 for HER2+, and 0.93 for HRc- & HER2-). Chemotherapy improved OS, except in HRc+ & HER2-, where it is reserved for high-risk cases (HRs of 2.25 for HRc+ & HER2-, 0.63 for HER2+, and 0.84 for HRc- & HER2-).
The advanced cancer stage was associated with poor OS in all subtypes.
(* indicates not statistically significant.)

Conclusion: Our study highlights trends in young women with BC, emphasizing the necessity for tailored interventions to improve outcomes.

P4-02-13: The global failure of mammography screening accessibility – a systematic literature review

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Background: Breast cancer is the most diagnosed cancer among women and a leading cause of cancer mortality worldwide. In high-resource healthcare settings, screening mammography combined with early treatment has consistently shown a reduction in breast cancer mortality. However, globally, most breast cancers are diagnosed based on clinical symptoms because screening programs are not available, or the participation rate seems to be low. Understanding and addressing the barriers women face in accessing breast cancer care, from screening to treatment, is crucial for improving patients' clinical outcomes.
Objective: This systematic literature review (SLR) aims to estimate the number of women affected by barriers to breast cancer screening mammography worldwide.

Methods: This SLR followed PRISMA guidelines (registered on PROSPERO; CRD42023423823) and included a comprehensive search in bibliographic databases and

grey literature. The number of women potentially eligible and participating in screening mammography was estimated from the UN population data and considering region-specific information on screening guidelines and programs. Extrapolations were made for countries without data, using gross national income level per capita and linear regression for missing values.

Results: A total of 20,434 citations were identified, resulting in 256 extracted sources that fulfilled the inclusion criteria focusing on quantitative data about the number of patients hindered by barriers from participating in breast cancer screening. The review identified living in rural areas, low education level, advanced age, low frequency of gynecologic visits, and concerns about radiation as important barriers to breast cancer screening. The estimated proportions of women aged 50 to 75 eligible for breast cancer screening programs but not participating in screening mammography varied considerably between regions, from 95% in Asia, 93% in Africa, and 52% in Latin America to 45% and 20% in Europe and North America, respectively. The total number of women not participating in mammography screening worldwide amounts to approximately 700 million, or 79% of all breast cancer screening eligible population.

Conclusion: This comprehensive SLR identified major access barriers to breast cancer screening for women worldwide. These hurdles are particularly prevalent in low-resource healthcare settings. Our estimates suggest that globally, most women eligible for screening based on their age do not participate in screening mammography programs, either because there is no screening program in place or because existing programs have low uptake rates for various reasons. This finding supports the need for the urgent dissemination and implementation of the WHO Global Breast Cancer Initiative 2023 guidelines, which focus on establishing early-detection programs to promote favorable stage shifting (earlier tumor stage at diagnosis) to achieve a reduction in breast cancer mortality, especially in low- and medium-income countries.

P4-02-14: A Multi-Practice Quality Initiative Focused on HER2+ and HER2low Metastatic Breast Cancer

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Background: Given the results of both DESTINY-03 and DESTINY-04 trials and the practice changing nature of such studies, Integra Connect (IC) PrecisionQ partnered with several oncology practices, including Regional Cancer Care Associates (RCCA), University of Pittsburgh Medical Center (UPMC), and South Carolina Oncology Associates (SCOA) to conduct a quality initiative to evaluate treatment against the standard of care, identify any potential gaps in care, and address those gaps. We aim to show the improvement in

treatment of HER2low breast cancer patients following chemotherapy in the metastatic setting (HER2low eligible) and HER2 positive (HER2+) breast cancer patients on second line of therapy or greater (LOT 2+).
Methodology: Using the IntegraConnect PrecisionQ de-identified database, a baseline cohort of patients were evaluated and determined as HER2low eligible on or after 6/1/2022 through 9/30/2023 (RCCA – 70, UPMC – 85, SCOA – 28) and HER2+ who started LOT2+ on or after 1/1/2022 through 9/30/2023 (RCCA – 94, UPMC – 92, SCOA – 25). Results of the initial analysis were shared with practice leaders and were also shared among oncologists at the practices. Following those interventions, over the next 6 months, the following number of patients were analyzed by practice to determine whether an improvement occurred among HER2low eligible patients (RCCA – 18, UPMC – 18, SCOA – 13) and HER2+ patients who started LOT2+ (RCCA – 23, UPMC – 25, SCOA – 11). Descriptive analyses were performed, and proportions were compared using a chi-squared test.
Results: The HERlow eligible patients treated with fam-trastuzumab deruxtecan-nxki increased from 51% to 61% at RCCA, from 33% to 56% at UPMC, and 18% to 39% at SCOA. Across all three practices, HERlow eligible patients treated with fam-trastuzumab deruxtecan-nxki increased from 38% to 53% ($p = .05$). The HER2+ LOT 2+ patients treated with fam-trastuzumab deruxtecan-nxki increased from 42% to 74% at RCCA, from 49% to 60% at UPMC, and 16% to 46% at SCOA. Across all three practices, HER2+ LOT2+ patients treated with fam-trastuzumab deruxtecan-nxki increased from 42% to 63% ($P < 0.01$).
Conclusion: This multi-practice quality initiative helped to improve treatment against standard of care in both HER2low and HER2+ breast cancer patients eligible for fam-trastuzumab deruxtecan-nxki across multiple oncology practices. This suggests even after considerable time passes following a new standard of care being established, oncology practices stand to benefit from assessing current treatment practices to ensure patients are receiving the highest standard of care.

P4-02-15: Potential Drug-Drug Interactions and Related Safety Considerations in Patients with Metastatic Breast Cancer Treated with Ribociclib

Claire Sathe, Rohit Raghunathan, Melissa Accordino, Jason Wright, Dawn Hershman

Background: Ribociclib, a cyclin dependent kinase 4/6 inhibitor (CDK4/6i), is part of the preferred first-line treatment of hormone receptor-positive (HR+) metastatic breast cancer (MBC). Polypharmacy is common among all BC survivors, and concomitant medication use is an under-researched safety issue for the growing number of patients on ribociclib. All CDK4/6i are metabolized through CYP3A4 and thus have potential for drug-drug interactions (DDI) when prescribed concurrently with CYP3A4 inhibitors (toxicity) and inducers (reduced efficacy). Furthermore, ribociclib use is associated with QTc interval prolongation, and requires electrocardiogram (ECG) monitoring and avoidance of antiarrhythmics and other agents with QTc prolongation potential.

Methods: We used the MarketScan health care claims database to identify patients who were prescribed ribociclib from 3/2017 (FDA approval) until 12/2022. We identified medications with DDI potential based on FDA labeling and categorized them as follows: CYP3A4 inhibitors (strong or moderate); CYP3A4 inducers (strong or moderate); antiarrhythmics; and QTc prolonging medications. We examined claims for medications with potential for DDI with ribociclib between each patient's first and last claim for ribociclib, or, if only one ribociclib claim was found, in the 180-day period after that claim. We also assessed whether an ECG was obtained within 60 days prior to initiating ribociclib (baseline ECG), and whether 2 ECGs were obtained within 60 days after starting treatment, based on Current Procedural Terminology (CPT) and ICD-10 procedure codes. These timeframes are longer than the FDA label recommendations for ECG monitoring to account for delays between claims and treatment initiation.

Results: We identified a total of 1,010 patients who were prescribed ribociclib between 3/2017 and 12/2022 (97.7% female; 63.4% <60 yo; 58.3% commercially insured, 14.6% Medicare, 27.4% Medicaid). Of these, 323 patients (32.0%) were prescribed a medication with potential for DDI with ribociclib while treated with ribociclib based on claim data. Specifically, 11 patients (2.1%) were prescribed a CYP3A4 inhibitor, 7 (0.7%) a CYP3A4 inducer, 6 (0.6%) an antiarrhythmic, and 289 (28.6%) a QTc prolonging medication. Among all medications with potential for DDI with ribociclib, the most commonly co-prescribed agents were ondansetron (80.8% of claims) and hydroxychloroquine (4.5% of claims), both of which have QTc prolonging potential, followed by the antiarrhythmic sotalol (2.4% of claims), methadone (2.4% of claims), which has QTc prolonging potential, and verapamil (2.1% of claims), an antiarrhythmic and CYP3A4 inhibitor. Of our total study population, 590 (58.4%) did not have a procedure code for a baseline ECG before their first ribociclib order. In addition, 595 (58.9%) were missing procedure codes for two additional ECGs after ribociclib initiation. There was no statistically significant difference between the cohort of patients co-prescribed a medication with DDI potential vs those who were not in terms of baseline ECG prevalence (42.5% vs 41.2%, p=0.7) or post-treatment initiation ECG prevalence (45.1% vs 39.4%, p=0.09).

Conclusions: Concomitant use of medications with DDI potential is common among patients with MBC treated with ribociclib. The antiemetic ondansetron is the most commonly co-prescribed medication with DDI potential as both agents are associated with QTc prolongation. Of concern, safety recommendations regarding ECG monitoring are inconsistently followed. As indications for ribociclib may expand to the adjuvant setting based on recent trial data, future research should identify strategies to improve patient safety, which may involve pharmacist-led interventions and improved dissemination of safety guidelines.

P4-02-16: Genetic Spectrum and Prevalence of Hereditary Breast and Ovarian Cancer Genes in Ceará's Families, Brazil: A Retrospective Study on Germline Mutations and Regional Variants

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Introduction: Approximately 10% of all neoplasms are influenced by germline mutations in pathogenic or likely pathogenic (P/LP) genes, with a majority of these mutations impacting the development of Hereditary Breast and Ovarian Cancer (HBOC) syndrome. The most common genes involved in HBOC are related to homologous recombination (HR) DNA repair, such as BRCA1, BRCA2, PALB2, CHEK2, ATM, RAD51, and BRIP1. In breast cancer, other genes like PTEN, TP53, CDH1, NF1, STK11, MUTYH, and BARD1 also play a crucial role in developing this neoplasm. Furthermore, the specific type of gene variation is important for understanding the spectrum and phenotype of hereditary cancer itself. Most studies on germline mutations and lifetime cancer risk have been conducted in developed countries. Therefore, the regional impact of specific variants in low and middle-income countries remains under investigation. Thus, we present the spectrum of germline mutations observed in Ceará, a northeastern state of Brazil. Methodology: This retrospective, cross-sectional study was conducted between 2018 and 2024 with patients from a private oncology clinic in Brazil, who met clinical criteria for Hereditary Predisposition Syndromes, specifically Hereditary Breast and Ovarian Cancer, according to the National Comprehensive Cancer Network (NCCN) guidelines. Molecular analyses were performed using a commercial multi-gene cancer panel with next-generation sequencing (NGS) capture panels, which included 27 to 84 genes depending on clinical suspension. These panels were validated for copy number variation and were designed to cover at least each exon and 20 base pairs of the intronic sequences adjacent to the exons. The primary endpoint was the prevalence of Hereditary Breast Cancer genes (BRCA1, BRCA2, PALB2, CHEK2, ATM, RAD51, BRIP1, PTEN, TP53, CDH1, NF1, STK11, MUTYH, and BARD1) in this population and the specific variants of the genes involved. Results: From 1055 patients analyzed, 141 (13.4%) individuals harbored a P/LP variant with 92 (8.7%) families harboring genes associated with an increased risk of HBOC syndrome. The most frequently mutated gene was BRCA1 (34.8%), followed by BRCA2 (15.2%), CHEK2 (14.1%), PALB2

(11.9%) ATM (8.7%), MUTYH (6.5%), RAD51 (4.3%), TP53 (3.3%) and NF1 (1.1%). In BRCA1, c.5074+2T>C (31.3%) was the most common variant, followed by C.5266dup (25.0%) and c.1961dup (12.5%). In BRCA2, the most common variant was c.4808del (35.7%), followed by c.9382 C>T (21.4%), c.2167delA (14.2%), and c.6024dup (14.2%). Within CHEK2, the most common variant was c.846+1G>C (69.2%), followed by c.349A>G (23.1%), and c.593-1 G>T (7.7%). Deletion (Exons 1-10) (27.3%) was the most common variation in PALB2, followed by c.1042C>, c.1240C>T (9.1%). In ATM, was c.1236 (25.0%), c.640del (25.0%) and Deletion (Exons 27-29) (25.0%). In MUTYH cases, it was c.536A>G (50.0%) and c.1187G>A (33.3%). In RAD51D was, c.694 C>T (50.0%). A TP53 founder pathogenic variant (R337H) accounted for 33% of all TP53 P/LP. Conclusion: These findings highlight the genetic diversity in HBOC syndrome and emphasize that regionality plays a crucial role in the distribution and prevalence of specific P/LP variants. Also, this study underscores the importance of conducting germline studies in under-represented populations to understand the landscape of HBOC syndrome better and develop unique prevention strategies. However, further research is needed to explore the underlying factors driving these regional differences and to understand the clinical implications of specific variants identified in this research.

P4-02-17: Toward Universal Germline Genetic Testing for Patients with Breast Cancer, Perspectives from a Recourse-Restricted Country

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Introduction: Breast cancer continues to be the most common cancer diagnosed among women worldwide. Median age at diagnosis tends to be significantly lower in low-resource countries compared to Western societies. Almost half of breast cancer patients, in countries like ours, are diagnosed at age 50 years or younger. Pathogenic germline variants (PGVs) in cancer-predisposing genes, mostly in BRCA1 and BRCA2 have been linked to the etiology of more than 10% of breast cancers. Identification of individuals with PGVs may reduce cancer burden by informing treatment decisions in patients themselves, whereas at-risk relatives may benefit from intensive screening and risk-reducing strategies.
Universal germline genetic testing (GGT) via multigene panels (MGP) is currently recommended for patients with ovarian, pancreatic, and metastatic prostate cancers. However, many international professional societies have established guidelines recommending GGT for high-risk patients with breast cancer. Such guidelines are somewhat complicated for most health care worker to follow; a fact that may contribute to the lower-than-expected referral rates for GGT in such patients. Age at which GGT for patients with breast cancer is recommended keep changing; moving from 40 to 45 and 50 and lately was moved to age 65 years by the American Society of Clinical Oncology (ASCO) most recent guidelines. Complexity of current guidelines may create barriers to GGT.
Methods: Medical records consecutive patients newly diagnosed and treated for breast cancer at our institution were reviewed. Patients with indication for GGT testing as per the

latest ASCO guidelines were identified based on age , breast pathology and family history. Number and percentage of patients who are not eligible for GGT will be determined.
Results: During the study period a total of 1570 patients were diagnosed with breast cancer, median age 51 (22-96) years, majority 1352 (86.1%) were Jordanian while the rest (n= 218, 13.9%) were non-Jordanian Arabs. Based on age criteria (i.e. patients aged ≤ 65 years), a total of 1346 (85.7%) were eligible for testing. Among the others 224 (14.3%), medical records were reviewed for indications, other than age at diagnosis, for GGT. Another 134 (8.5%) were found eligible for testing because of personal or family history of breast and other cancers (n=121, 7.7%), having triple-negative disease (n=9, 0.57%) and male gender (n=4, 0.25%). In total, 1480 (94.3%) patients are eligible for GGT as per the most recent ASCO guidelines, leaving only 90 (5.7%) who are not candidates for testing as per current guidelines. Among the 1570 patients, 1134 (72.2%) were referred for the genetic counselling clinic and were tested.
Conclusions: Applying a universal germline genetic testing for newly diagnosed breast cancer, regardless of their age, disease characteristics, family or personal history of breast or other cancers, would increase the pool of eligible testing by few percentage points; the burden of which can be justified given its impact on improving referral rate.

P4-02-18: Change in MRI Background Contrast Enhancement (BPE), Fibroglandular Volume, and Hormones with Bazedoxifene and Conjugated Estrogens

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Background: The proportion of MRI fibroglandular tissue exhibiting contrast is termed background parenchymal enhancement (BPE). American College of Radiology (ACR) BPE criteria are minimal, <25%; mild, 25-50%; moderate, 50-75%; and marked, >75%. Postmenopausal women have a low proportion of MRIs with moderate and marked BPE, but those with BPE of ≥ 50% have an elevated cancer risk. In cross-sectional studies, women taking combined estrogen + progestin hormone replacement are more likely to have a high BPE; while women taking tamoxifen are less likely. We explored use of abbreviated MRI BPE as a response biomarker in a feasibility study of 6 months of the SERM bazedoxifene plus conjugated estrogens (BZA+CE) vs waitlist control in high-risk postmenopausal women with vasomotor symptoms.
Methods: Inclusion criteria were age 45-64, no periods for ≥2 months, vasomotor symptoms, and ≥ 2x population risk for breast cancer. Following consent, women underwent phlebotomy, 3D mammography, and abbreviated breast MRI and were randomized to BZA +CE or waitlist with repeat procedures at 6 months. Women randomized to waitlist were offered the option of BZA+CE at 6 months. 11 women were enrolled in STUDY00145121 of BZA 20mg + CE 0.45 mg as Duavee™ vs waitlist between 5-2020 and 8-2020 which closed with the temporary withdrawal of Duavee™. 16 women

were enrolled on its replacement, STUDY00146761, between 2-2021 and 9-2023 which used imported BZA and commercial CE in the same doses as Duavee™. Following study completion all baseline and 6-month MRIs were re-assessed by a single radiologist blinded to treatment assignment. This research assessment altered ACR criteria reads by changing minimal to 5-25% BPE and adding a 5th category of almost none (<5%). Mammographic fibroglandular volume (FGV) was assessed by Volpara™ fully automated software. Baseline and 6-month serum samples were assayed together for estrogen and progesterone. Results: 27 women were randomized, 15 to BZA+CE and 12 to waitlist. Five randomized to waitlist did not have 6- month reassessment due to dissatisfaction with randomized arm (1) or unavailability of Duavee™ at 6 months in STUDY00145121 (4). Two withdrew from BZA+CE due to muscle cramps and colon cancer. Twenty are evaluable for change in FGV and MRI, and 19 for change in hormones. Using the ACR 4 category assessment with clinical interpretations, 16/20 (75%) were in the lowest BPE category (<25%) at baseline. However, with our 5-category research assessment and a single reader, only 4/20 (20%) were in the lowest (<5% BPE) category. For the 13 evaluable women randomized to BZA+CE, the median relative change in FGV was -3%. Change in MRI category was 6 decreased, 2 increased, and 5 no change despite a median relative increase in estradiol of 80% and little change in progesterone. For the 7 evaluable women randomized to waitlist, the median relative change in FGV was +3%. Change in MRI category was 2 decreased, 1 increased, and 4 no change. Baseline or directional change in MRI category was not associated with baseline or directional change in FGV or estradiol. Hot Flash Score in the BZA+CE arm was reduced from a median of 16 to 0 but unchanged in waitlist. Conclusions: A no-treatment control arm in women with vasomotor symptoms is feasible if BZA+CE can be supplied after the 6-month randomized period. Use of categorical MRI BPE as a response biomarker for prevention is limited by low level of enhancement at baseline. A fully quantitative measure should be explored. MRI BPE change after BZA+ CE appears independent of change in serum estradiol or mammographic FGV in this pilot assessment.

P4-02-19: Development and Validation of a Genetic Risk Prediction Model for Breast Cancer: The Prognostic Role of SNAI1 and CDH1 in Treatment Response and Patient Survival

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Background: Breast cancer is a highly heterogeneous disease characterized by varied molecular profiles, leading to diverse clinical treatment responses. Effective and specific classification and diagnosis are critical for optimizing breast cancer treatment. This study aims to establish a genetic risk prediction model for breast cancer patients, focusing on malignant manifestations and treatment response.

Methods: This study extracted and transformed mRNA data from 27 target genes from 486 breast cancer patients into a data matrix. A hierarchical clustering-based risk prediction algorithm was developed to identify the most influential risk molecules and protective factors. The top 10 important genes identified were SNAI1, HDAC1, DNMT3A, WHSC1, PRDM15, PRMT2, CDH1, PRDM7, HDAC3, and PRMT8. Validation was further conducted using immunohistochemistry and scoring on samples from 74 patients.

Results: The genetic risk prediction model identified SNAI1 as the most significant risk molecule and CDH1 as the primary protective factor. Pearson's analysis indicated a negative correlation between SNAI1 and CDH1. Kaplan-Meier analysis showed that SNAI1 is a poor prognostic indicator for overall survival in lymphatic invasion (LN+), chemotherapy alone (CT), chemotherapy plus radiotherapy (CT plus RT), and breast-conserving surgery (BCS). Univariate analysis highlighted SNAI1 as a prognostic indicator, while multivariable analysis confirmed that SNAI1 and CDH1 are significant independent prognostic indicators for overall survival in breast cancer after adjusting for clinical parameters. SNAI1 demonstrated excellent discrimination in CT, CT plus RT, and BCS subgroups and acceptable discrimination in overall and radical treatment groups.

Conclusion: This study proposes a genetic risk prediction platform to identify potential risk factors. Clinical specimen validation showed that SNAI1 has a significant diagnostic effect on lymphatic invasion, patient survival, and chemotherapy prognosis in breast cancer patients. These findings provide a valuable biomarker prediction model for effective diagnosis, monitoring, and treatment. The ultimate goal is to prolong patient survival while avoiding over-treatment and unnecessary medical expenses.

P4-02-20: Investigating Age-Dependent Breast Cancer Dynamics in a Mouse Model

Yun Zhang, Jyotsna Godavarthi, Abie Williams-Villalobo, Bin Liu

Breast cancer incidence increases with age, but the relationship between age and breast cancer survival remains controversial. Additionally, while younger and older patients exhibit distinct molecular profiles, the intrinsic age-related molecular events in breast cancer are yet to be fully understood. To address this gap, we investigated the age-dependence of breast cancer in a controlled setting, aiming to uncover key signaling pathways. In this study, we utilized a breast cancer mouse model we previously published, which is highly relevant to human breast cancer. This model features the conditional p53wm-R245W allele that allows Cre recombinase-mediated conversion of wild-type p53 into the p53R245W mutant (corresponding to human p53R248W, one of the most common mutations in breast cancer). Female p53wm-R245w/+ mice received mammary intraductal injections of adenovirus expressing Cre (Ad-Cre) at 2 months or 10 months of age (referred to as the 2-month and 10-month groups, respectively) and were monitored for tumor

development. Twenty-six percent of the 2-month group and 34% of the 10-month group developed mammary tumors, most of which were adenocarcinomas. The average latency for tumor onset was 15 months post-injection (p.i.) in the 2-month group and 18 months p.i. in the 10-month group. Interestingly, tumors from the 2-month group grew significantly faster but had a lower rate of lung metastasis (43% vs 82%) compared to those in the 10-month group. In addition, molecular analysis revealed significant differences between the two groups. The mRNA levels of estrogen and progesterone receptors and the erythroblastic oncogene B indicated distinct breast cancer molecular subtype distributions: luminal A/normal-like subtypes dominated in the 2-month group, while triple-negative subtypes were more prevalent in the 10-month group. Notably, tumors from the 2-month group exhibited a higher frequency of p53 loss of heterozygosity (LOH) compared to the 10-month group (37.5% vs 9.09%). To further explore the molecular mechanisms driving age-related differences in breast cancer, we performed RNA sequencing on tumors and mammary gland controls. We identified 6,799 differentially expressed genes in the 2-month group and 6,502 in the 10-month group. Ingenuity Pathway Analysis revealed that the two groups were associated with distinct signaling pathways, such as EIF2 signalling in the 2-month group and Notch signaling in the 10-month group. Additionally, Interferon gamma signaling and Th1 responses were strongly correlated with reduced tumor growth rates, while pro-metastatic pathways, such as IL-4 and FAK signaling and Phagosome Formation, may contribute to the increased metastatic potential of slow-growing tumors in our model. Further investigation is needed to elucidate the role of these pathways in the age-dependence of breast cancer. Our findings may ultimately lead to improved diagnosis and personalized therapeutic targets to extend the lives of breast cancer patients.

P4-02-21: Disparities among patients evaluated in dedicated high-risk breast cancer clinic

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Background: Annual screening mammogram is recommended for all women aged 40 years and older, as early detection of breast cancer leads to increased overall survival and better outcomes. During mammography, various risk factors such as age, BMI, and family history influence the risk of breast cancer. These data points, along with breast density classification, are used to calculate a Tyrer-Cuzick (TC) score, which estimates both the ten year and lifetime risk of breast cancer as a percentage. If the TC lifetime risk score percentage is ≥20%, the patient is considered to be at high risk for breast cancer and qualifies to be seen in high-risk breast cancer clinic to discuss additional recommendations beyond the annual screening mammogram. These recommendations include annual breast MRI, consideration of risk reduction strategies and genetic counseling. While previous studies have established that breast density and BMI are risk factors in developing breast cancer, few have explored the characteristics and trends among high-risk breast cancer patients. We hypothesize that patterns may exist among race, breast density, breast imaging

findings, genetic testing results in this population of high-risk patients. Methods: We created a 6-month database (07/01/2022-12/31/2022) of women at high risk for breast cancer across a single health system with a diverse patient population. Data collected included self-identified race, TC score, mammography and MRI imaging findings, and genetic testing. Stratification analysis was performed using SAS Software 9.4. Results: A total of 496 women were included, with mean age of 47 years. The mean TC score was 23%. Compared to Black women, White women had higher rate of heterogeneously dense, fibroglandular density or fatty imaging on mammogram (p=0.0113). Among all women who had a breast MRI, 30% had abnormal findings. More White women had abnormal findings on MRI than Black women (33.0% vs 23.5%, p=0.1066). Regarding genetic consults, more White women than Black women attended appointments with genetics (45.4% vs 34.5%, p=0.0293). Additionally, 47.1% of White women pursued genetic testing compared with 35.5% of Black women (p=0.0193, RR 1.15, 95% Cl 1.03-1.30). Among those who had genetic testing, 53.1% of Black women were found to have genetic mutations (both VUS and pathogenic) compared with 40.8% of White women (p=0.1209). Patients with a TC score of 40% or higher were 83% more likely to have abnormal MRI findings than those with TC scores of less than 40% (RR 1.83, 95% Cl 1.10-3.03). Conclusions: These data demonstrate significant disparities in genetic testing and imaging findings between White and Black women in high-risk breast cancer clinic. White women were more likely to have genetics appointments and undergo genetic testing than Black women. Despite this, Black women had a higher proportion of genetic mutations among those tested. Additionally, patients with higher TC score (>40%) were more likely to have abnormal MRI findings, emphasizing the importance of comprehensive screening in high-risk individuals. These results underscore the importance of high-risk breast cancer clinics in educating patients about breast cancer and opportunities for risk reduction. They also demonstrate the critical need for equitable access to genetic counseling and testing services to ensure that all high-risk patients receive appropriate and timely care. Further research with a larger dataset and over a longer time frame is needed to better understand and address these disparities in high-risk breast cancer patients. Research sponsor: None.

P4-02-22: Temporal Changes in Breast Cancer Reproductive Risk Factors Among Women in Southwest Nigeria: Data from The Nigerian Breast Cancer Survey

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Background: The incidence of breast cancer in Sub-Saharan Africa (SSA) has surged by 247% from 1990 to 2019, with a notable prevalence among women under 50 years of age. The understanding of evolving risk factors has however remained heavily reliant on data and guidelines extrapolated from High Income Countries (HIC), which may not accurately reflect the unique epidemiological profiles in Nigeria and other Low to Middle Income Countries (LMIC) in SSA. Given the stark mortality rates amidst rising incidence, our study

aims to refine the understanding of the trend of breast cancer risk factors in low-income countries using the Nigerian Breast Cancer Study (NBCS) data.

Methods: This is a retrospective study using the NBCS database consisting of 2314 healthy Nigerian women aged 18 and older enrolled between 1998 to 2017. Participants were grouped into six birth cohorts: <1940, 1940-49, 1950-59, 1960-69, 1970-79, and ≥1980. The primary dependent variables were age at menarche, thelarche, number of pregnancies, parity, and duration of breastfeeding. Descriptive statistics was employed to assess reproductive characteristics, while univariate regression models explored the relationship between reproductive risk factors and birth decade. Trend analyses were conducted to identify significant changes across the cohorts.

Results: The average age at menarche decreased from 16.1 years in women born before 1940 to 15.1 years in those born after 1980 ($p < 0.001$). The mean age at thelarche was centered around 13 years across cohorts. However, regression analysis indicated a slightly increasing trend in thelarche age over time (coefficient = 0.090, $p = 0.001$), with decade of birth explaining a very small portion of the variability (R-squared = 0.0048). This slightly increasing trend in thelarche age is not consistent with the sharp decreasing trend in menarche age. This discrepancy is difficult to explain physiologically and warrants further investigation. The number of pregnancies per woman similarly declined, from an average of 7 in the pre-1940 cohort to 4 in the post-1980 cohort ($p < 0.001$). Parity trends mirrored this decline, with a significant reduction over time ($p < 0.001$). The mean age at first live birth remained consistent at approximately 23 years across cohorts. The mean duration of breastfeeding per child also decreased from 18.3 months for those born before 1940 to 14.8 months for those born after 1980 ($p < 0.001$).

Conclusion: Our study provides important insights into the temporal changes in reproductive health risk factors among Nigerian women across various birth cohorts. Although some of our findings indicate shifts that might parallel trends observed in HIC, it is important to highlight notable differences such as events like thelarche and menarche which occur approximately 2 to 3 years later in the Nigerian population compared to their counterparts in HIC. There are also discernible differences in breastfeeding duration and parity trends. Future studies will evaluate the role of genomic and environmental risk factors in the etiology of breast cancer in Nigerian women. This study underscores the need for population-specific risk assessment tools to accelerate progress in breast cancer prevention in LMICs.

P4-02-23: RISK-REDUCING MASTECTOMIES FOR BREAST CANCER IN BRAZILIAN PATIENTS UNDERGOING GERMINATIVE MULTIGENE PANEL TO BREAST CANCER: impact of results on the decision of conduct

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Introduction: Hereditary breast cancer accounts for 20% of breast cancer cases in Brazil. The cumulative risk of breast cancer in patients with germline genetic mutations in high-risk genes can reach 70-80%. Identifying high-risk patients allows for adequate screening, as well as offering risk-reducing surgeries when indicated. Risk-reducing the risk of breast cancer by more than 90% and significantly impacts a woman's quality of life. In Brazil, few institutions provide services aimed at the population at high risk for breast cancer. Objective: To evaluate the behavioral changes of high-risk patients regarding risk-reducing mastectomy (RRM) before and after receiving genetic testing results, and to identify the main influencing factors on decision-making. Methods: A prospective cohort study conducted between 11/2021 and 10/2022 included women under surveillance at the high-risk outpatient clinic of the State University of Campinas. These women were asymptomatic but had a family history of breast and/or ovarian cancer diagnosed at <45 years, met NCCN high-risk criteria, or had a personal history of breast cancer and at least one relative with breast, ovarian, and/or prostate cancer. Patients completed a questionnaire and underwent genetic testing. After receiving the results, they received appropriate guidance based on the examination result. Results: A total of 373 women were included, with 188 (50.4%) having no personal history of breast and ovarian cancer, and 185 (49.6%) having such a history. In the pre-genetic testing analysis, 173 (54.1%) patients were considering risk-reducing mastectomy, while 147 (45.9%) were either not interested or unable to respond at the time. After the test, 42.2% of patients wanted the surgical procedure, with a change in behavior observed in 26.2%, mainly from "yes" to "no/I don't know" (35.3%) ($p<0.001$). Genetic testing yielded positive results in 29.7% of patients, more frequently in those with a personal history of cancer (36.9% vs. 22.7%) ($p=0.012$). Among the 90 patients with positive results, 62 (68.9%) agreed to undergo RRM, while 22 (24.4%) remained unwilling to accept RRM, regardless of the positive test result. Genetic testing influenced the decision in 11 out of the 90 patients (11.2%) who changed their opinion from "no" to "yes" after a positive test, while 14.9% changed to "no" after a negative test, and 40.4% remained inclined towards surgery regardless of the result being normal ($p<0.001$). Significant influencing factors for pre- and post-genetic testing behavioral change (towards undergoing surgery) in multivariate analysis were positive genetic testing results (OR 2.94, $p<0.001$), personal history of cancer (OR 2.7, $p=0.008$), and ages between 40-49 (OR 2.07, $p=0.008$) and ≥ 50 (OR 3.47, $p<0.001$). Conclusion: The Brazilian high-risk population utilizing the public health system shows a greater inclination towards desiring risk-reducing mastectomies. However, when genetic testing and counseling are conducted, behavioral changes are observed, especially when the result is positive. This underscores the influence of genetic testing as a prevention strategy and optimization of healthcare system resources.

P4-02-24: Frequency of HER2-low status among patients with breast cancer and germline BRCA1/2 pathogenic variants

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Background: The antibody-drug conjugate trastuzumab deruxtecan (T-DXd) has significantly improved survival outcomes among breast cancer (BC) patients with low expression of HER2 (HER2-low) as determined by immunohistochemistry. Since then, understanding the frequency of this condition in different scenarios became relevant to guide therapeutic strategies. In sporadic BC, around 60% of hormone receptor (HR) positive and 30% of HR negative tumors are classified as HER2-low. BRCA1/2-mutated breast cancers have distinct biology, with an enrichment of triple-negative breast cancer in patients with germline BRCA1 pathogenic variants (PVs), while luminal B-like tumors are common in those with germline BRCA2. Given these particularities, the objective of the study was to evaluate the frequency of HER2-low status among BC patients carrying germline BRCA1/2 (gBRCA1/2) PVs.

Methods: An observational study was conducted to evaluate patients with a germline BRCA1/2 PVs diagnosed with breast cancer. Patients were treated between 1995 and 2024 in three oncologic centers. Electronic records and pathology reports were reviewed to collect data on demographic, clinic and pathologic features. HER2-low status was defined as immunohistochemistry +1 or +2 with a negative in-situ hybridization.

Results: Ninety-three patients with breast cancer and a germline BRCA1/2 pathogenic variant were identified, with 59 having a BRCA1 PV (63.4%) and 34 a BRCA2 PV (36.5%). Triple-negative breast cancer occurred in 47% of the cases, while 44% were hormone receptor-positive HER2-negative. Only two patients had HER2-positive breast cancer.
The median age at diagnosis was 40 years (18.5 - 69.2 years), and the majority of the patients had early-stage tumors (10.7% stage I, 47.3% stage II, 20.4% stage III), with only three patients with stage IV disease. The most frequent histological type was ductal carcinoma (82%), followed by lobular carcinoma (9%); histological grade 3 was observed in 61% of the cases.
Among patients with triple-negative breast cancer, 17.8% of the tumors were HER2-low. Among those with hormone receptor-positive HER2-negative breast cancer, the proportion of HER2-low was 29.0%. Overall, HER2-low status was present in 19.4% and 26.9% of the BC from germline BRCA1 and BRCA2 PVs carriers, respectively.

Conclusion: The HER2-low status occurred in a lower proportion in BRCA1/2-mutated BC patients in this cohort, compared to what was previously described in the literature for sporadic BC patient, both in HR negative and HR positive tumors. Additional studies are required to understand particularities of hereditary tumors in terms of the frequency of HER2-low status when samples of multiple tumor sites are evaluated as well as the frequency of HER2-ultralow since these conditions can also guide the use of targeted therapy with T-DXd.

P4-02-25: Dietary-Restriction Genes as Modulators of Breast Cancer Risk Through Metabolic Pathways

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Background: Breast cancer is a prevalent malignancy among women, exhibiting significant heterogeneity due to complex genetic and environmental interactions. Despite advances in treatment, challenges such as recurrence and drug resistance persist. Dietary Restriction (DR) has emerged as a lifestyle intervention with potential anti-cancer effects, primarily through modulation of metabolic pathways and immune responses. However, the impact of DR-related genes on breast cancer risk remains underexplored.
Methods: This study utilized differential expression analysis from the TCGA breast cancer database to identify significant genes intersecting with the DR gene set ($p < 0.05$). We conducted KEGG and GO enrichment analyses to elucidate the roles of these genes in breast cancer pathogenesis. Employing Mendelian randomization, we assessed the causal relationships between DR-related genes and metabolic indicators (LDL, TG, HDL, BMI, and waist circumference), identifying key genes involved. Additionally, protein-protein interaction networks were analyzed to explore molecular interactions, followed by drug prediction and molecular docking studies to evaluate therapeutic potential.
Results: Our differential expression analysis identified 86 DR-related genes significantly associated with breast cancer. Co-localization analysis revealed that seven of these genes shared the same SNP loci with metabolic indicators. Previous studies have established that metabolic indicators (LDL, TG, HDL, BMI, and waist circumference) can influence breast cancer incidence; our co-localization study clarified that specific DR-related genes can affect breast cancer risk through shared SNP loci with these metabolic indicators. KEGG and GO enrichment analyses indicated that these specific DR-related genes influence breast cancer development via regulation of longevity-associated pathways and lipid metabolism. Furthermore, protein-protein interaction network analysis uncovered several core regulatory nodes. Through drug prediction and molecular docking analysis, we identified perfluoroundecanoic acid, perfluorohexane sulfonic acid, perfluorononanoic acid, perfluorodecanoic acid, and rifampicin as potential therapeutic agents with high binding affinity to these genes.
Conclusions: This study elucidates the causal relationship between DR-related genes and breast cancer risk, highlighting potential therapeutic targets and candidate drugs through their effects on metabolic markers. These findings provide new directions and scientific evidence for precision treatment in breast cancer, with the potential to advance personalized treatment strategies and enhance lifestyle-based clinical management.
Keywords: Breast cancer; Mendelian randomization; Dietary restriction (DR)-related genes; Metabolic markers.

P4-02-26: CHEK2-Associated Cancer Risk Beyond 1100delC: Insights from a Northeast Brazilian Cohort

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Introduction: CHEK2 is known to be a moderate-penetrance breast cancer gene, with a lifetime risk of 20–40%. Recent studies demonstrated that pathogenic and likely pathogenic (P/LP) variants in this gene are associated with the risk of other cancers, like colorectal, kidney, and thyroid cancer. However, most of what we know about CHEK2 is from the 1100delC pathogenic variant, a founder European variant. We describe the distinct phenotype of 13 families from the state of Ceará, in the northeast of Brazil, that harbors rare P/LP variants in CHEK2, with unique tumor types, rarely described as associated with germline CHEK2 variants. Materials and methods: This cross-sectional study examines patients from a private oncology clinic in Ceará – a northeast state of Brazil - who met clinical criteria for Hereditary Predisposition to Breast and Ovarian Cancer (HBOC). The molecular analyses were from commercial multi-gene cancer panel genes from accredited laboratories between 2018 and 2023. Sequencing was conducted using next-generation sequencing (NGS) capture panels, which included 27 to 84 genes depending on clinical suspicion. The primary endpoint was to describe the prevalence of CHEK2 P/LP variants as well as sex, age at diagnosis, family phenotype, and geographic distribution. Results: From 1055 patients, 141 (13.4%) harbored a germline P/LP variant in HBOC genes, and of these, 13 (9.2%) had a P/LP variant in CHEK2. Subsequently, family members of these 13 individuals were analyzed. In total, 57 individuals were included all descending from lineages that exhibited segregation of these CHEK2 variants. Only three distinct P/LP variants were identified on CHEK2: c.349A>G, c.846+1G>C, and c.593-1G>T. The mean age of this cohort was 52 years (range 20-85). The majority was female (64.9%), with a history of one neoplasm (77.2%), and from the northwest of Ceará (57.9%). Across the 13 families analyzed, 9 (69.2%) had the c.846+1G>C mutation, while 3 had the c.349A>G mutation (23.1%), and only one had the c.593-1G>T mutation (7.7%). Interestingly, all families from the northwest were carriers of c.846+1G>C, while most families with c.349A>G were from the south of Ceará. The most frequent type of cancer was breast (40.9%), followed by

papillary thyroid cancer (9.1%), colon (7.6%), melanoma (6.1%), kidney (6.1%), prostate (6.1%), hematological (4.5%), testicular (3.0%), gastric (1.5%), lung (1.5%), hepatocarcinoma (1.5%), head and neck (1.5%) and sarcoma (1.5%). The variant c.593-1G>T accounted for one breast cancer, while the c.846+1G>C variant was enriched by papillary thyroid cancer, with 26.7% of the cases. Likewise, c.846+1G>C presented a distinct phenotype, with enrichment of melanoma (8%), prostate (8%), and testicular cancer (4%). Conclusion: This study highlights the importance of investigating CHEK2 variant impacts across diverse populations, as genetic influences on cancer phenotypes may vary significantly by genetic background and geographical location.

P4-02-27: Do High-Risk Breast Cancer Patients Belonging to Ethnic Minority Groups Undergo Adequate Genetic Testing for Early Detection and Treatment

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Abstract: Background: Approximately 15% of women diagnosed with breast cancer have a familial history of the disease. Women with a first-degree relative are twice as likely to develop breast cancer. Early detection can confer a 99% chance of survival at five years. Numerous studies underline the Importance of genetic testing, however, there is limited literature on the use of genetic testing in ethnically and socioeconomically diverse populations. Therefore, our study aimed to analyze whether high-risk patients in our population with a documented family history of cancer received referrals to medical genetics before their breast cancer diagnosis.

Methods: A retrospective chart review was conducted on patients diagnosed with breast cancer between 2018 to 2023 from a single institution. All demographic data, family history, pathology, and genetics were collected.

Results: A total of 636 breast cancer patients with a familial cancer history were identified. Of the patients diagnosed with breast cancer, 414 (65.0%) cases were detected through routine mammography screening, and 207 (32.5%) patients discovered their cancer through palpation of a tumor. Out of the 636 patients, 226 (35.5%) of them had a first-degree relative with breast cancer. Of those with a first-degree relative, 49 (21.9%) were diagnosed with DCIS, 93 (42.0%) with stage I, 27 (12.0%) with stage II, 10 (4%) with stage III, and 2 (0.88%) with stage IV breast cancer. Overall, 43.3% of patients with familial cancer history received referrals to medical genetics, with 4.1% referred before their breast cancer diagnosis and 39.4% postdiagnosis.

Conclusion: Only 4.1% of patients with a family history of cancer received genetic consults before diagnosis. Early detection is crucial and identifying pathogenic variants can alter screening protocols. We propose a dual approach involving education and systemic modifications to increase genetic testing in high-risk patients. Automated electronic medical record prompts and primary care physicians' recognition of family history can aid in identifying high-risk patients.

P4-02-28: Decisional challenges regarding risk-reducing surgery for BRCA1/2 and PALB2 women

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Women with hereditary breast and ovarian cancer syndrome (HBOC) have an increased risk of breast (BC) and ovarian cancer (OC) (absolute risk > 60% and 39–58% for BRCA1-2 respectively). Options for risk management include surveillance and risk reducing surgery (RRS). Previous data reported up to 90-95% BC risk reduction after RR bilateral breast surgery (RRBBS). RR salpingo-oophorectomy (RRSO), besides a risk reduction effect on OC, impacts on overall survival for BRCA1/2 women. At present, most early BC patients with a known BRCA1/2 or PALB2 pathogenic variant discuss breast RRS during their primary surgical plan.
In this study we reviewed the Multidisciplinary Group (MD) decisions regarding cancer survivors (excluding BC women during primary treatment) and individuals with no previous cancer diagnosis, highly motivated for RRS. Between August 2020 - December 2023, 216 (215 F; 1 M) pts (median age 49 yrs) were discussed in the MD group. Genetic testing (GT): BRCA1/2 (69%), PALB2 (5%), other genes (19%) and GT not positive (7%). Cases: 114-healthy women, 97-CA survivors (6 with advanced CA) and 5 considered ineligible for RRS (1-NF1; 4-not positive GT).
Healthy women: the MD group agreed on RRBBS, RRSO or both in 37, 42 and 35 cases, respectively. After further individual discussion 5 (14%RRBBS), 2 (5%RRSO) and 6 (17% both) women refused RRS. Four RRBBS women (3 BRCA2; 1 PALB2) were diagnosed with unilateral BC in the preoperative MRI, 1 RRSO women had OC diagnosis previous to surgery. Surgical specimens of interest (5): 1- lobular invasive BC (pT1bN0), 2- DCIS, 2- STIC (1 bilateral).
Survivors were mostly (84/87%) BC survivors. While considering RRBBS, RRSO or both in 17, 49 and 25 cases, respectively, 2 (12%RRBBS), 2 (4%RRSO) and 3 (10% both) pts refused RRS. Regarding the 6 pts with advanced CA, 4 had sustained CR and RRS was agreed. Four pts (3 early BC) relapsed before RRS. Surgical specimens of interest (7): 1- multifocal BC (pT1cN1mi(sn)); 2- high grade serous fallopian tube CA (1 stage III); 4- STIC.
The decision of RRS is a challenge that should measure the competing risks, either regarding age of primary prevention for healthy women, or regarding risk of relapse versus another cancer. This decision should be as tailored as possible to each patient, respecting their individuality and preferences.

P4-02-29: Epigenetic aging as a tumor marker for predicting breast cancer susceptibility

Su Yon Jung

DNA methylation (DNAm)–based marker of aging, referred to as 'epigenetic age' or 'DNAm age' is a highly accurate multi-tissue biomarker for aging, associated with age-related disease risk, including cancer. Breast cancer (BC), an age-associated disease, is associated with older DNAm age and epigenetic age acceleration (age accel) at tissue levels. But this raises a question on the predictability of DNAm age/age accel in BC development, emphasizing the importance of studying DNAm age in pre-diagnostic peripheral blood (PB) in BC etiology and prevention. We included postmenopausal women from the largest study cohort and prospectively investigated BC development with their pre-diagnostic DNAm in PB leukocytes (PBLs). We estimated Horvath's pan-tissue DNAm age and investigated whether DNAm age/age accel highly correlates with risk for developing subtype-specific BC and to what degree the risk is modified by hormones and lifestyle factors. DNAm age in PBLs was tightly correlated with age in this age range, and older DNAm age and epigenetic age accel were significantly associated with risk for developing overall BC and luminal subtypes. Of note, in women with bilateral oophorectomy before natural menopause experiencing shorter lifetime estrogen exposure than those with natural menopause, epigenetic age accel substantially influenced BC development, independent of obesity status and exogeneous estrogen use. Our findings contribute to better understanding of biologic aging processes that mediates BC carcinogenesis, detecting a non-invasive epigenetic aging marker that better reflects BC development, and ultimately identifying the elderly with high risk who can benefit from epigenetically targeted preventive interventions.

P4-02-30: Germline Mismatch Repair Gene Mutation (MLH1) in Breast and Endometrial Cancer: A Case Report

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Background: Lynch syndrome is an autosomal dominant, inheritable genetic disorder that predisposes affected individuals to the development of malignancies such as endometrial and colorectal cancer. This condition is primarily attributed to pathogenic germline variants, particularly in mismatch repair genes. While certain literature suggests a potential link between Lynch syndrome and breast cancer, the association remains ambiguous and requires further investigation.
Case Presentation: This report presents a case of a 52-year-old female who has been diagnosed with a double primary malignancy. The patient has a strong family history of colon cancer among her first-degree relatives.
Eleven years prior (in 2012), she was diagnosed with early-stage breast cancer, Stage I, estrogen receptor (ER) and progesterone receptor (PR) positive, HER2/neu positive. For this condition, she underwent definitive surgery and completed adjuvant systemic therapy with trastuzumab, docetaxel, and carboplatin. Trastuzumab was administered for one year,

and endocrine therapy with tamoxifen was given for five years.
In 2023, the patient began experiencing back pain accompanied by vaginal spotting. A PET CT scan revealed multiple hypermetabolic, axial, and appendicular skeletal metastases, along with multilevel lytic changes in the vertebrae, ribs, and pelvic bones, including a soft tissue component in the right pubic ramus. A biopsy of the pubic ramus indicated findings consistent with a primary breast origin, ER progesterone receptor PR negative, and HER2/neu positive. A transvaginal ultrasound further revealed a thickened endometrium. Transcervical resection of the endometrium led to a histopathological diagnosis of endometrial adenocarcinoma, endometrioid type, FIGO grade 2.
Given the patient's oncologic profile, genetic counseling and testing were conducted. A pathogenic mutation in the MLH1 gene was detected.
The association of breast cancer with Lynch syndrome remains a subject of ongoing debate. Previous literature has reported no conclusive evidence of an increased risk of breast cancer among individuals with Lynch syndrome compared to the general population. Nevertheless, recent studies suggest a potential increased risk of breast cancer in those with germline mismatch repair mutations. However, the rarity of this condition presents a significant challenge in confirming the correlation between breast cancer and Lynch syndrome on a larger scale. Given these limitations, an individualized approach is recommended.
Conclusion: This paper emphasizes on the importance of genetic counseling and genetic testing in the understanding the behavior of malignancies. This approach may aid oncologists to a comprehensive risk assessment that will serve as a guide in appropriate recommendations on screening methods for early detection, and its implications in employing preventive measures as deemed necessary. There is potentially life-saving information that may be derived in this clinical context. Hence, future studies may aid clinicians to a better understanding of tumor biology and the potential role of mismatch repair genes in breast cancer carcinogenesis, and ultimately, heighten awareness of this condition.

P4-03-01: Alarming levels of obesity and overweight in Brazilian women with early-stage ER+ breast cancer in adjuvant endocrine therapy

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Background: Adiposity and the presence of overweight/obesity are associated with mortality in general and also with mortality and recurrence related to breast cancer. This study aims to evaluate obesity and overweight levels in Brazilian women with early-stage ER+ breast cancer in adjuvant endocrine therapy and its relations with clinical and

demographic charactheristics.
Methodology: Women with a history of early-stage ER+ invasive carcinoma of the breast on adjuvant ET for at least 6 months were invited to participate of this study. Body Mass Index (BMI) were assessed according to the cutoff points proposed by the World Health Organization (1998). Adherence, quality of life, sexual dysfunction and return to work were also assessed. Patients were stratified according to Body Mass Index (BMI (Eutrophy: 18.5 to 24.9kg/m^2, Overweight: 25 to 29.9kg/m^2 and Obesity: >=30kg/m^2). To investigate statistically significant differences between groups, Pearson's Chi-Square tests were used. To evaluate the relationship between variables and BMI in patients with breast cancer, multinomial logistic regression analysis was used.
Results: From June 2021 to March 2024, 557 women from 11 Brazilian institutions were recruited. Mean age was 62 y.o , mean tumor size was 2.14 cm, mean duration of ET was 3.1y. A total of 27% of patients were obese, 42% were overweight and 30.8% had eutrophy. Women with higher education had a lower prevalence of obesity (26%) compared to those with lower education (31%) ($p = 0.03$). The presence of comorbidities had a highly significant association with BMI, with a higher prevalence of comorbidities among obese women (33%) compared to those with ideal weight (24%) ($p < 0.001$). Patients treated in public hospitals had a higher prevalence of obesity (35%%) compared to those treated in private hospitals (20%) ($p < 0.001$). Patients in stage III were more likely to be obese (odds ratio = 2.88, 95% CI: 1.55-5.33, $p < 0.001$) compared to those in stage I. There was no significant association between BMI and variables such as age ($p = 0.97$), ethnicity ($p = 0.35$), marital status ($p = 0.98$), and duration on endocrine therapy ($p = 0.17$). Better QLQ C30 - Physical Functioning was associated with a lower chance of obesity (odds ratio = 0.95, CI95%: 0.93-0.97, $p < 0.001$) and overweight (odds ratio = 0.96, CI95 %: 0.94-0.98, $p < 0.001$). Arm Symptoms also showed a significant association with obesity (odds ratio = 1.01, 95% CI: 1.00-1.02, $p = 0.005$), but not with overweight (odds ratio = 1.00, 95% CI: 0.99-1.01, $p = 0.082$). In multivariate analysis, stage III disease (OR 1.72), prior lumpectomy (OR 7.2), prior mastectomy (OR 2.7), axillary lymphonode dissection (OR 2.8) and use of concomitant medication (OR 1.8) were related to obesity.
Conclusions: Only a third of the women evaluated in the study had an adequate BMI, leading to a worrying risk of morbidity such as cardiovascular disease and risk of breast cancer relapse. Some characteristics related to obesity, such as its greater presence in patients treated in the public service and in women with a lower level of education, lead to the hypothesis that economic factors may be related to this disease.

P4-03-03: Outcomes and Survivorship in Geriatric Breast Cancer Patients with Chemotherapy Dose Reduction.

Huy Nong, Ahmed Elkhanany

Background: Neoadjuvant and adjuvant chemotherapy is a cornerstone of treatment for high-risk breast cancers. Geriatric patients with frailty, poor performance status and multiple co-morbid conditions may not tolerate standard dosing regimens of chemotherapy

per clinical guidelines and may require dose reduction in order to complete therapy. However, previous studies have shown that dose reduction in chemotherapy in geriatric patients have led to poor outcomes and decreased overall survival.
Methods: This retrospective cohort reviews all geriatric patients (ages > 65 y.o.) who received chemotherapy for early-stage and locally advanced breast cancer over. RDI was calculated as the ratio of delivered to planned chemotherapy dose intensity. The primary outcome was low RDI, defined as RDI < 85%. Multivariable logistic regression was used to evaluate the association between baseline variables and low RDI. Survival probability was estimated using the Kaplan-Meier method, and the log-rank test was used to compare overall survival. Clinical data regarding patients' tumor subtype, stage, biomarkers, performance status, chemotherapy regimen, co-morbidities and clinical outcomes were recorded.
Results: In our population of geriatric breast cancer patients who received chemotherapy, 65% of patients had low RDI. Low RDI < 85% did not have any immediate clinical difference between patients with RDI > 85% at time of analysis. Patients with RDI < 85% were more likely to have multiple co-morbidities such as HTN, DM, CHF and CKD. Regimens containing taxanes and anthracyclines led to more toxicities compared to non-taxane and anthracycline based regimens
Conclusions: Geriatric breast cancer patients receiving chemotherapy are more likely to have dose reduction with low RDI due to multiple co-morbidities. Clinical outcomes were comparable between patients who had low RDI and high RDI with no significant difference in outcomes. Taxane and anthracycline based regimens led to more toxicities in geriatric breast cancer patients.

P4-03-04: Effect of a Weight Loss/Exercise Program and Hormone Therapy on Peripheral Inflammatory Markers in ER+ Breast Cancer Survivors

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Background: Obesity and chronic systemic inflammation are known factors affecting mortality and cancer recurrence in breast cancer survivors (BCS). Lifestyle interventions like diet and exercise can mitigate systemic inflammation; however, their efficacy with concomitant hormone therapy remains unclear. Peripheral blood inflammation markers, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII), hold promise as indicators of chronic inflammation but require further exploration in the breast cancer population. Hormone therapies, including tamoxifen (TAM) and aromatase inhibitors (AI), may influence inflammation differently, with TAM possibly exerting cardioprotective effects and AI showing mixed evidence regarding cardiovascular risks. This study aims to investigate the impact of a 12-week weight loss/exercise program and hormone therapy on peripheral inflammatory biomarkers among ER+ BCS.
Methods: A retrospective chart review identified 37 ER+ BCS who completed a 12-week

weight loss/exercise program consisting of supervised aerobic and resistance training exercise sessions twice weekly, and weekly balanced dietary counseling. Baseline and post-intervention assessments included anthropometric measures, body fat percentage, and laboratory results obtained within 1 week of initial and post-intervention visits, which included NLR, PLR, and SII measured as platelet count x neutrophil count/lymphocyte count. Statistical analyses included normality assessment, log10 transformation for non-normally distributed data, paired and independent t-tests for normally distributed data, and Wilcoxon signed-rank and Mann-Whitney U tests for non-normally distributed data. ANOVA assessed changes within and between groups.
Results: Participants included 36 females and 1 male ER+ BCS, mean age of 56 ± 9 years; 28 Hispanic, 9 non-Hispanic. BCS were categorized into 3 groups based on hormone therapy: TAM (N=11), AI (N=16), and no hormone therapy (N=10). No significant differences were observed in anthropometric measures or body fat distribution between the 3 groups. Age significantly differed between groups ($p = 0.046$), with the TAM group notably younger (51 ± 8 years) than AI group (60 ± 7 years) or no hormone therapy group (56 ± 10 years). TAM and AI BCS had a significantly lower baseline measure of SII ($p = 0.020$) and NLR ($p = 0.009$) compared to BCS on no hormone therapy. Post-weight loss/exercise program intervention: All BCS groups had a significant reduction in SII ($p = 0.022$), while NLR ($p = 0.026$) was significantly reduced in the TAM and no hormone therapy group. Additionally, all participants demonstrated significant reductions in weight (–2.5 kg ± 3.2, $p < 0.001$), body mass index ($p < 0.001$), waist circumference ($p < 0.001$), and % body fat ($p = 0.003$).
Conclusion: Our findings suggest that the 12-week weight loss/exercise program significantly reduces systemic inflammation and improves body composition parameters in ER+ BCS. Surprisingly, our study revealed that patients receiving hormone therapies, including TAM and AI, exhibited significantly lower baseline levels of peripheral inflammatory biomarkers compared to those on no hormone therapy. This unexpected finding underscores the potential beneficial impact of hormone therapy on modulating chronic systemic inflammation in ER+ BCS. The reduction of systemic inflammation may provide an additional mechanism through which hormonal therapy mitigates cancer recurrence in breast cancer patients. The lower levels of inflammatory markers in the TAM and AI groups highlight the importance of considering hormone therapy status when assessing inflammatory profiles in breast cancer patients.

P4-03-05: Effect of Phesgo® on Time Toxicity in Breast Cancer Patients

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Background: Time toxicity is the time spent in medical facilities, such as outpatient, travel, waiting, infusions, laboratory tests and hospitalization for any disease. Time toxicity can be an important information for treatment selection because it affects the patient's daily life such as work, housework and childcare not only for advanced cancer patients but also for early cancer patients. However, time toxicity is generally not evaluated in oncology trials.

Phesgo® is a fixed-dose combination of pertuzumab and trastuzumab for subcutaneous injection. It was approved by the U.S. Food and Drug Administration (FDA) on June 29, 2020, and has been available in Japan since November 22, 2023, with insurance coverage. The initial dose is administered subcutaneously over 8 minutes and the maintenance dose is administered over 5 minutes, which is a shorter administration time than the intravenous infusion of pertuzumab and trastuzumab, and is expected to reduce time toxicity.
Methods: We retrospectively reviewed patient background, medical condition, time spent in the hospital, time from confirmation of implementation to start of administration, and adverse events in patients with HER2-positive breast cancer treated in our hospital who switched from intravenous pertuzumab/trastuzumab to the fixed-dose combination for subcutaneous injection. Hospitalization time was defined as the time from outpatient admission to billing. Time from confirmation of initiation to start of administration was defined as the time from the physician's decision to initiate in the electronic medical record to the start of the first drug administration at the chemotherapy center. We compared the time of the last administration of intravenous pertuzumab/trastuzumab with the time of the first administration of the fixed-dose combination for subcutaneous injection.
Results: Between December 2023 and April 2024, 37 patients with breast cancer were administered subcutaneous injection, of which 24 were cases in which was switched from intravenous administration. The median age was 61.5 years (37-71 years), 5 patients had early breast cancer and 19 patients had advanced recurrent breast cancer. The mean hospital stay was 4 hours 19 minutes for intravenous administration and 2 hours 24 minutes for subcutaneous injection ($P<0.001$). The mean time from confirmation of dosing to initiation was 1 hour 11 minutes for intravenous administration and 45 minutes for subcutaneous injection ($P<0.005$). New adverse events after switching included injection site reactions (pain, redness, swelling) in 20 patients (83%) and infusion reactions in 1 patient (4%). One patient switched back to intravenous administration due to injection site pain. There was one case of a phone call regarding an injection site reaction after subcutaneous injection, but no unscheduled visits were observed.
Discussion: The fixed-dose combination for subcutaneous injection significantly reduced the time spent in the hospital compared to intravenous administration, suggesting a reduction in time toxicity. The reduction in hospital time was greater than the reduction in administration time, which may be due to the reduction in dispensing time. Evaluation of time toxicity is considered useful information for shared decision making for patients.

P4-03-06: A Single-Center Review of the Impact of an Integrated Women's Health Clinic for Breast Cancer Patients Who Received Cancer-Directed Treatment

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Background: With increasing survival of breast cancer patients, women are subjected to the long-term side effects of their treatment and remain at risk for additional cancers. A meta-analysis showed that women with breast cancer have a 3.5-fold higher risk of developing

sexual dysfunction than women without cancer, which can lead to reduced quality of life. 1-2 A retrospective study of over 500,000 patients with breast cancer showed an increased risk of second breast cancer and other primary cancers when compared to the general population. We created GARNET (Gynecologic Survivorship and Preventative Care after Cancer Treatment) as a tool to address these issues in patients receiving care for breast cancer. Through this study we hope to describe the role of this novel clinic in a safety net hospital providing care for underserved patients in New Orleans, LA.

Methods: Data was collected via retrospective char review of women enrolled in GARNET. To be included, women had to carry a diagnosis of breast cancer from January 1st, 2021 (at the initiation of the clinic) through May 30th, 2024 at University Medical Center in New Orleans, Louisiana. Patients who received any cancer-directed treatment, including surgery, radiation therapy, chemotherapy, immunotherapy and/or endocrine therapy were included. Male patients and those who did not attend GARNET were excluded. Continuous data was summarized with means and standard deviations or medians.

Results: In total, 38 women were evaluated in GARNET during the study period. Mean age was 57.9 years; 8(21.0%) were White/Non-Hispanic, 27 (71.0%) were Black, 2 (5.2%) were Hispanic and 1 (2.6%) was Asian. Most patients used Medicare or Medicaid as insurance (24 patients, 63.2%); 12 (31.6%) patients had private insurance, and 2 (5.3%) patients were uninsured. Of the treatment types patients received, 35 patients had surgery, 23 recieved chemotherapy or immunotherapy and 31 were had radiation. Once evaluated in GARNET, 28 patients (73.7%) had an updated cervical cancer screening. Of those who did not, 4 patients exceeded the recommended age to continue screening, 3 patients cervical cancer screening was no longer indicated due to lack of cervix, 2 patients were referred for cervical cancer screening but did not yet complete it, and 1 did not have screening for unclear reasons. A review of top complaints showed that patients sought care for cervical cancer screening (52.6%) vaginal bleeding (26.3%), other vaginal complaints such as dryness, itching or discharge (13.2%), ovarian cancer screening in BRCA carriers (5.2%) and contraception (2.6%)

Discussion: Patients who received treatment for breast cancer need Chemotherapy or hormonal treatment can induce early menopause which can lead to genitourinary atrophy and psychological distress that impact a woman's sexual health. A 2016 study even showed that breast surgery has a negative impact on sexual function, independent of other treatments. Additionally, it is the consensus opinion that premenopausal women receiving treatment for breast cancer are at increased risk of endometrial cancer due to estrogenic effects of tamoxifen on endometrial tissue. Women with breast cancer are also at increased risk for additional cancers for not entirely understood reasons.

Treatment in a medical oncology office is focused on cancer treatment and surveillance. Our patients social and financial barriers often prevent them from seeking care, which led to the development of GARNET. This preliminary study showed that our patients who utilized GARNET received care for cervical cancer screening, as well as vaginal bleeding or discharge, ovarian cancer screening and contraception, among others. We hope to continue to expand referrals to GARNET to evaluate the benefit to our patients and to further inspire integrated care for women's health for those underdoing cancer care.

P4-03-07: Implementing Quality of Life assessment in breast cancer patients in India

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Introduction: Breast cancer is on the rise in India and is the leading cause of cancer incidence in women. Indian Council of Medical Research - National Centre for Disease Informatics and Research report accounted for 13.5% of new cancer cases and 10% of cancer-related deaths in 2020 with higher burden in more developed states of India. The impact is seen on Quality of Life (QoL) attributed to physical, psychological and social factors . Breast cancer is a distinctive entity as it hampers physical appearance of women besides living with the fear of outcome . Treatments include chemotherapy, radiotherapy, breast surgeries all significantly influencing QoL in survivors . Depression, fertility-issues, economic burden and social issues negatively affect quality of life. European Organization for Research and Treatment of Cancer (EORTC) QoL Questionnaires-Core-30 and Breast-23 (QLQ-C30 and QLQ-BR23) are valid and reliable tools to assess QoL in breast cancer survivors (12,13,14,15). Due to high patient load in India and insufficient manpower, routine assessment of QoL in breast cancer patients is a challenge with scarce data from this part of the world. Objective: To administer QLQ-C30 and BR23 questionnaires to breast cancer patients receiving treatment at the tertiary hospital Cancer Unit to differentiate QoL among patients in varying stages of the disease and treatment.
Methodology: 175 female breast cancer patients registered in the cancer unit in the last one year with a diagnosis established at least 6 months ago were included and other gynecological cancers were excluded. After obtaining written consent, QLQ-C30 a 30-item cancer specific questionnaire designed to measure QOL and QLQ-BR23 a 23-item breast cancer specific questionnaire were administered together. The assessment comprised of nine domains (physical, role, cognitive, emotional, social, fatigue, pain, nausea and vomiting) in QLQ-C30 and five domains (body image, sexuality, arm symptoms, breast symptoms, and systemic therapy side effects) in BR23. The impact on QoL was based on the Global Health Status (GHS), Physical functioning (PF), Emotional functioning (EF), Fatigue (FA) and Pain (PA) scores.
Results: Mean age of the patients was 49.65 (±9.94) years with a mean duration of breast cancer of 2.17 (±1.61) years. 11.54% of patients did not receive counselling for various reasons. Correlation was established between GHS and PF [r=.69, p=.00001]; EF [r=.32, p=.02]; social functioning [r=.38, p=.005]; body image [r=.36, p=.01]; future perspective [r=.3, p=.03]. Analysis of PF, EF, Pain and Fatigue scores were performed based on whether treatment included only chemotherapy or was combined with radiation and surgery. Those who received only chemotherapy had better functional status compared to those with combined therapy. The symptom scale variables were negatively correlated with global health status. The study shows a higher decrease in physical functioning levels compared to other functional parameters due to factors like pain, chronic fatigue, balance impairment and lymphedema associated with breast cancer treatment. In alignment with PF scores were fatigue, pain, insomnia and appetite scores which were relatively higher.

Conclusion: With an ever increasing incidence of breast cancer in India, QoL assessment can be a valuable tool for incorporating effective management strategies. The overall quality of life was reasonably good indicating presence of positive health ecosystem. Efforts were fruitful in recruiting participants implying high feasibility of such assessments. The findings form the basis for evaluation of predictive variables that impact quality of life in future while also boosting the confidence of patients, their families and stakeholders involved in patient services. QoL assessments in routine breast cancer care is recommended for better management and good clinical outcomes.

P4-03-08: Pre- and post-diagnostic healthy lifestyle and cardiovascular disease among breast cancer survivors

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Background Cardiovascular disease (CVD) is an important cause of death among breast cancer survivors. The relationship between pre- and post-diagnostic healthy lifestyle and CVD risk among breast cancer survivors is unknown.
Objectives To examine the associations of pre- and post-diagnostic healthy lifestyle score (HLS), defined by diet, alcohol consumption, smoking status, physical activity and body mass index, with the risk of CVD incidence and mortality among breast cancer survivors.
Methods We prospectively followed for CVD incidence and mortality among 11,448 participants with confirmed diagnosis of invasive breast cancer enrolled in the Nurses' Health Study (NHS) (1984-2020) and NHSII (1991-2019) who were free of CVD before breast cancer diagnosis. Diet and lifestyle factors before and after breast cancer diagnosis were repeatedly assessed nearly every 2 to 4 years.
Results Over 124,687 person-years of follow-up, we documented 872 new-onset CVD events and 3675 overall deaths, of which 488 (13.3%) were specifically due to CVD and 1,310 (35.6%) were due to breast cancer. After multivariable adjustment, both higher pre- and post-diagnostic HLS were associated with a lower risk of CVD-specific incidence and mortality. Compared with women with the lowest cumulative average post-diagnostic HLS (0-2), the multivariable-adjusted hazard ratios (HRs) for participants with the highest score of 5 were 0.56 (95% CI: 0.32-1.00) for CVD incidence, and 0.66 (95% CI: 0.45-0.97) for CVD mortality (all p trend<0.0001). Participants who had an unhealthy pre-diagnostic lifestyle but improved to an HLS (3-5) after breast cancer diagnosis had a lower risk of both CVD incidence (HR: 0.69, 95% CI: 0.52-0.92) and CVD mortality (HR: 0.75, 95% CI: 0.53-1.07) compared with those continuing an unhealthy lifestyle during both periods. Participants maintaining a healthy lifestyle during both pre- and post-diagnostic periods showed the strongest inverse association, with HR of 0.57 (95% CI: 0.46-0.69) for CVD incidence and 0.50 (95% CI: 0.38-0.65) for CVD mortality. Each point increment of post-diagnostic HLS was associated with a 14% lower risk of CVD incidence (p=0.007), and a 14% lower risk of CVD mortality (p=0.01).

Conclusions and relevance Independent of pre-diagnostic lifestyle, a post-diagnostic healthy lifestyle was associated with a substantial, graded lower risk of CVD-specific incidence and mortality among breast cancer survivors. These findings underscore the clinical importance for health care practitioners managing breast cancer survivors to consistently promote adherence to healthy lifestyle behaviors, highlighting the opportunity to leverage the changeable moment even for those with an unhealthy lifestyle before cancer diagnosis.

P4-03-09: Breast Cancer Long Survivorship Program Experience in Mexico City

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Background: Breast cancer survivors may experience a significant burden of side effects from cancer therapies. Timely management of such effects, including strategies to promote health and wellness among breast cancer survivors, is an unmet need in low- and middle-income countries, where cancer care is centered on diagnosis and active treatments. Our aim is to describe clinical characteristics and outcomes of women with a personal history of breast cancer included in a long-term breast cancer survivorship program at a third level hospital in Mexico City.
Methods: Women with a personal history of breast cancer who completed cancer treatment and have a disease free survival period more than 5 years are referred to the Long-term Breast Cancer Survivorship Program at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City. The program offers monitoring and management of long side effects, including lymphedema, cardiovascular diseases, bone health, and cancer screening, vaccination recommendations, psychosocial and nutrition support, health and wellness education, and geriatric assessment screening (G8 tool). Disease -free survival is defined from the time after completion of treatment (excluding endocrine therapy) up to the last visit.
Results: From October 2023 to June 2024, 32 women with a personal history of breast cancer were included. Median age was 69.5 years (range 48-88). The mean disease-free survival time was 11 years (range 5-21). Breast cancer stage distribution at diagnosis was Stage I in 18.75% (n=6) , Stage II in 28.1% (n=9) and Stage III in 50% (n=16) of cases. The most common histological subtype was Invasive ductal carcinoma in 68.75% (n=22), followed by an unspecified subtype in 12.5% (n=4), ductal carcinoma in situ 6.25% (n=2) and others 12.48% (n=4). Breast cancer treatment included: unilateral mastectomy in 53.1% (n=17), breast-conserving surgery in 43.75% (n=14), bilateral mastectomy in 6.25% (n=2), radiotherapy in 62.5% (n=20), chemotherapy in 78.5% (n=25) (69.56% taxanes and 78.26% anthracyclines), anti-Her2 therapy in 6.25% (n=2) and endocrine therapy in 84.37% (n=27). At the time of evaluation, the following comorbidities were recorded: hypertension in 53.12% (n=17), dyslipidemia in 46.87% (n=15), overweight and obesity in 59.37% (n=19), hypothyroidism in 34.37% (n=11), diabetes mellitus in 28.12% (n=9), prediabetes in 15.62% (n=5), depression or anxiety in 34.37% (n=11), and obstructive

sleep apnea in 6.25% (n=2). Lymphedema screening was positive in 15.6% (n=5) (defined as ≥2cm difference between arms), 18.75% (n=6) reported pain and 9.37% (n=3) had abnormal G8 scores. Bone health screening was completed in 79.31% (n=23) with, 43.47% (n=10) diagnosed with osteopenia and 39.13% (n=9) with osteoporosis. Adherence to cancer screening for cervical cancer was 92% (n=25), for colorectal cancer 78.12% (n=25) and for breast cancer 90% (n=30) of cases, with no abnormal result reported. About 18.75% of women (n=6) reported consuming dietary supplements and herbs.
Conclusion: The breast cancer long survivorship program in Mexico City provides comprehensive care for breast cancer survivors, addressing their medical and psychosocial needs, and monitoring potential long-term side effects. Our findings reveal a notable prevalence of overweight, obesity and other comorbidities among survivors, underscoring the necessity for lifestyle interventions. Through its multidisciplinary approach, the program strives to improve survivors' quality of life and overall well-being, emphasizing the significance of continual support and monitoring in survivorship care.

P4-03-10: Body image and sexuality concerns among young women with breast cancer in a Mexican Population

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Background: The diagnosis and treatment of breast cancer involves changes in personal, family, social, professional and sexual relationships. Younger breast cancer survivors confront a distinct set of treatment-related challenges that have a significant influence on their quality of life and psychological well-being. Although there is often a profound and distressing impact of treatment on self-image andsexual function, women rarely receive any attention for these issues
Purpose: The goal of this study was to investigate the prevalence of body image and sexual issues in the first months after breast cancer diagnosis and treatment in women aged 50 and younger.
Methods: We used the EORTC QLQ-B23 questionnaire in 50 patients diagnosed with early breast cancer at our centre. Sexual concern outcomes including poor body image, poor sexual functioning, poor sexual enjoyment, and sexual inactivity were investigated. Data were obtained prospectively over the perior of 2016 to 2021. Multivariate generalized estimating equation models were used to examine associations with sexual concerns after diagnosis, adjusting for age, sociodemographic, tumor, treatment, and clinical features.
Results: A sample of 50 patients was selected, aged 24-48 years old, with a median age of 40.8 years, who had undergone breast cancer surgery, 22% by conservative surgery. 84% were premenopausal, 16% were perimenopausal. All patients were treated with chemotherapy for neo or adjuvancy. Body image and sexual problems were experienced by

a substantial proportion of women in the early months after diagnosis. More than half of the women experienced two or more body image problems some of the time (56%), or at least one problem much of the time (26%). 52% of patients were quite or very sad and depressed since starting treatment. 62% of patients believe that the treatment has worsened their body image. Among sexually active women,51% reported that during treatment their sexual relations were not satisfactory. 40% believe that their sexual desire has decreased since diagnosis.
Conclusions: Difficulties related to sexuality and sexual functioning were common and occurred soon after diagnosis of breast cancer; they appear to be frequent and insuffiently handled. Addressing these issues is essential to improve the quality life for young women with breast cancer.

P4-03-11: Patient and health care provider perspectives on oral versus intramuscular endocrine therapy for locally advanced or metastatic breast cancer

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Background: Next generation oral selective ER degraders (SERDs) have been designed to improve efficacy and tolerability and may serve as an alternative to intramuscular (IM) fulvestrant for the treatment of ER+, HER2- locally advanced or metastatic breast cancer (MBC). Thus, it is important to understand perspectives on the benefit and burden of oral and IM endocrine therapy (ET) from both patients and health care providers (HCPs).
Methods: This US-based qualitative interview study recruited female patients with MBC treated with both oral ET and fulvestrant within the past 3 years and HCPs (medical oncologists and oncology nurses). One-on-one semi-structured interviews focused on four concepts: preference, benefits, burdens, and adherence to oral and IM ET. Patients spoke directly about their own personal experience with both types of administration, while HCPs provided their perspectives based on their clinical practice experience. Interviews were conducted by a third-party vendor and occurred September 2023 through May 2024. Interview transcripts were analyzed in ATLAS.ti through an iterative process and followed a qualitative analysis plan.
Results: An asterisk is used to denote responses that are not mutually exclusive. Of the 22 patients interviewed, 64% preferred oral ET, 27% preferred IM ET, and 9% had no preference. Of the fourteen patients that preferred oral ET, they did so for convenience (71%)*, non-painful administration (36%)*, and fear of needles (21%)*. Six patients preferred IM ET due to ease of monthly administration. The benefit of oral ET reported by the greatest proportion of patients was convenience (86%)*, followed by belief that the treatment is working (32%)*. The benefit reported by the greatest proportion of patients for IM ET was belief that the treatment is working (64%)*, followed by not having to

remember to take it (36%)*. Patient reported burdens to oral ET included side effects (59%)*, remembering to take medication (32%)*, insurance (23%)*, and reminder of diagnosis (18%)*. Patient-experienced burdens to IM ET were injection site pain (91%)*, side effects (82%)*, and having to travel for monthly appointments (45%)*. Nearly all patients (91%)* reported it was easy to take their oral ET, whereas 32%* reported it was easy to receive the IM ET. Of the 20 HCPs interviewed (medical oncologists=10, oncology nurses=10), nearly all (90%) perceived patients to prefer oral ET. All HCPs stated patient convenience is a benefit of oral ET, and patients not having to remember to take medication is a benefit of IM ET. HCPs also reported inadequate insurance coverage (55%)*, side effects (40%)*, and compliance (30%)* to be perceived burdens of oral ET, and that pain (85%)* and travel (70%)* were burdens of IM ET. HCPs' perception of ease of administration was equivalent for both oral and IM ET, 80% reporting they were both easy.
Conclusion: Among patients with MBC that have been treated with both oral and IM ET, the majority prefer oral ET and report that convenience of an orally administered medication was considered a key benefit and the leading reason for preference of oral ET. Injection site pain and the need to travel for monthly appointments for IM ET were reported burdens from patients and perceived as burdens from HCPs. Notably, although most HCPs reported ease of administration for both oral and IM ET, only a third of patients interviewed reported that it was easy to receive IM ET.

P4-03-12: How do Arab World Breast Cancer Survivors feel about De-escalation of Treatment?

Rita A. Sakr, SAWA breast cancer survivors

With the improved selection of patients during the past decades, we are seeing a real trend towards de-escalation of treatment in special settings of breast cancer because of less or no benefit on survival of those previously recommended surgeries or treatments. In the Arab world, patients are tending to be more influenced by familial factors in addition to cultural factors and certain concepts such as "more is better and safer" when it comes to treatment. The aim of our study is to investigate how breast cancer survivors would feel about de-escalation of breast cancer treatment thus try to help with the educational awareness and change of unhelpful concepts.
Members of SAWA breast cancer survivors group living in United Arab Emirates were invited to share their opinion regarding the de-escalation of breast cancer treatment. They were informed about the latest studies and then were separately invited to share their opinion regarding the de-escalation of breast surgery, axillary surgery, as well as protocols of radiotherapy, chemotherapy and endocrine therapy. So far, twenty-six patients aged between 38 and 59 years attended the interviews and separately replied to the questions that were about feeling safe about de-escalation of breast cancer treatment, or unsafe about de-escalation, or wishing to follow the recommendations of doctors without sharing opinion. Three patients felt a little bit confused and preferred to reply later. Regarding breast surgery, one third of the patients felt unsafe about de-escalating lumpectomy and

16% of patients felt unsafe about de-escalating mastectomy. Almost 42% of the patients said they would just follow the recommendations of doctors. Regarding axillary surgery, 20% of patients felt unsafe about de-escalating sentinel lymph node biopsy whereas 60% of patients would simply follow the recommendations. All of them were very happy with de-escalating complete axillary clearance. As for the adjuvant treatment, the majority of the patients (96%) were very happy with de-escalation of radiotherapy protocols. The majority of patients (67%) were also very happy with de-escalation of chemotherapy protocols and more interestingly, they expressed their happiness with de-escalation of endocrine therapy too.
Thanks to the development of biology towards an improved selection of patients and to the availability of more medical treatments, the reduction of surgery and radiotherapy is continuing in all settings of breast cancer treatment. In addition, the de-escalation was shown not to impact locoregional recurrence rate and overall outcome. Implementing those new recommendations will surely need to start by standardization of the information given to patients by the doctors. It should also involve patient in making the treatment decision especially when omission of a treatment is suggested. In the Arab World region, the concept of "more is better" in addition to the familial and cultural influence can drive patients sometimes against the recommendations and guidelines. By consequence, the opinion of breast cancer survivors who have been through most of the treatment plans can be of great guidance and value. In the first part of our study, most of the patients were very happy with de-escalating the treatments where side effects can really affect their daily life and womanhood: axillary surgery, radiotherapy, endocrine therapy. However, a small but non negligeable number of patients still felt unsafe about de-escalating surgery including mastectomy and sentinel lymph node biopsy.
Awareness campaigns involving beast cancer survivors can be of great value for changing the misguiding concepts, with a special focus on educating healthcare society, patients and families about the benefits of de-escalating treatments in a specific tailored way. Less can be better.

P4-03-13: Machine learning-based sentiment analysis related to Trastuzumab Deruxtecan in online forums

Nawale Hajjaji, Elnaz Sherafat, Djamel Zitouni, Benjamin Guinhouya

Background: New antibody drug conjugates (ADCs) have significantly improved the outcomes of breast cancer patients. However, this class of therapy has side effects including interstitial lung disease, digestive disorders, alopecia, fatigue, which can impact patients' quality of life. Although patients' tolerance collected within clinical trials and real-world studies reported acceptable and manageable tolerance, the feelings expressed by patients and their relatives outside the medical context is not known. People are increasingly searching for support, advice or answers online. An analysis of the posts about Trastuzumab Deruxtecan (T-DXD) could provide additional insight to better understand patients' experience with this ADC. In this study, we performed a sentiment analysis on

large amounts of information shared online.
Methods: The online narratives related to T-DXD posted on US-based platforms, one dedicated to a breast cancer community (Breast cancer.org) and the other a wide social platform (Reddit), were extracted to conduct a sentiment analysis. Breast cancer.org is a large non-profit supportive community of breast cancer patients with more than 83000 users. Reddit gathers thousands of online communities and more than 16 billion posts/comments. Machine learning techniques and models were used to identify and extract sentiment-bearing words based on a lexicon. These words were then assigned polarity values and frequencies within the corpus. Additionally, advanced natural language processing (NLP) methods through computational linguistic software was used to perform detailed analysis of nuanced sentiment categories within the forum discussions, including the identification and analysis of side effects mentioned by users.
Results: Although T-DXD cancer indications are expanding, as of 5th July 2024, only 271 comments related to T-DXD were found on the breast cancer forum out of a total of thousands posts and 1477 posts on Reddit out of a total of million posts. Most posts about T-DXD identified on the breast cancer forum were associated with positive sentiments (56%) while most posts on Reddit were negative (59%). To draw a comparison, the number of posts related to chemotherapy (n=240) was similar to the number of posts related to T-DXD on the breast cancer forum with a trend toward positive sentiments (55%) for chemotherapy. Among the 61248 posts related to chemotherapy on Reddit, 47% were positive and 43% negative. Comments about immunotherapy were also mostly negative (62%) on Reddit. The main side effects related to T-DXD and frequently reported on Reddit were nausea, vomiting, and constipation, while those on the breast cancer forum were nausea and tiredness.
Conclusion: Experiences and opinions about T-DXD are shared on social networks. The sentiment analysis content differed according to the platform and the users' profiles with more positive post in a dedicated breast cancer online community.

P4-03-14: A review of the evidence for survival benefit of six lifestyle medicine interventions in breast cancer patients

Laura Wright, Preeti K. Sudheendra

There are currently four million breast cancer survivors in the United States and the number is expected to substantially increase in the decades to come. Oncologists are regularly managing issues of survivorship during periods of surveillance and the topic of prevention of cancer recurrence is a central theme of many outpatient oncology visits. Patients often seek counsel on tangible ways to reduce their risk of breast cancer recurrence and are met with generic suggestions to live healthfully. Overwhelmingly, clinical trials point toward substantial benefit of the adopting the six pillars of Lifestyle Medicine in a comprehensive cancer survivorship program to improve disease-free survival and all-cause mortality. These six pillars include 1) social connection, 2) physical activity, 3) whole food and plant-based nutrition, 4) stress management, 5) restorative sleep, and 6)

avoidance of risky behaviors or toxins. In a review of the breast cancer literature, we present evidence for survival and quality of life benefit in breast cancer patients with the implementation of these six pillars of Lifestyle Medicine. Socially integrated patients and those who undergo cognitive behavioral therapy for management of stress were found to live longer and have better perceived quality of life than patients who are isolated or experiencing heightened anxiety. Collectively the literature is also clear that ongoing smoking and consumption of >6 grams of alcohol per day as a breast cancer survivor significantly increases risk of death from breast cancer. Finally, prospective and retrospective studies have shown that the maintenance of a healthy body weight with adequate nutrition through dietary interventions, aerobic and resistance exercise, and balanced circadian sleep, particularly in postmenopausal women, improve breast cancer mortality. These data suggest that breast cancer patients would benefit from a comprehensive Lifestyle Medicine approach to survivorship. Consensus on survivorship programming is currently lacking. We submit that formal implementation of programs that include each of these six pillars of Lifestyle Medicine could significantly impact cancer mortality and morbidity.

P4-03-15: Clinical characterization of taxanes chemotherapeutic regiments-induced peripheral neurotoxicity and establishment of a risk model

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Objective: To describe the clinical characteristics of chemotherapy-induced peripheral and neuropathy (CIPN) occurring in patients receiving paclitaxel-based chemotherapeutic agents, and to explore the feasibility and risk modeling of age, gender, body mass index (BMI), and clinical characteristics such as hemoglobin, vitamin D, Chinese medicine identification of body mass, anxiety state, and depression state at baseline prior to chemotherapy as predictive factors for the risk of CIPN in cancer patients.
Methods: 1. This study was a prospective, single-centre, observational cohort study, which included patients who were diagnosed with malignant tumors by pathology and received chemotherapy containing paclitaxel-based single-agent or combination regimens for the first time from March 1, 2023 to November 30, 2023 in the Department of Breast, Head and Neck Oncology of the First Affiliated Hospital of Jinzhou Medical University. Strictly following the inclusion screening criteria, 90 patients were finally included in this study. Firstly, the risk factors with strong correlation were screened by reading related literature and Meta-analysis: age, cumulative dose, number of cycles, BMI, vitamin D, and paclitaxel-based chemotherapy regimen. Patients' basic information (e.g. gender, menstrual status, previous history of psychoneurological drugs, marital status, education, residence, type of work, etc.), information related to clinical medication and treatment (e.g. chemotherapy regimen and drug dosage, etc.), laboratory test indexes (white blood cell count, haemoglobin, vitamin D, etc.), and assessment of quality of life (anxiety mood state, depressive mood state, and whether or not they suffer from insomnia, etc.) were collected.

In addition, in order to optimise the model, two innovative indicators were added to the study: Chinese medicine identification of body mass and absolute count of lymphocyte subpopulations.

2. The investigators recorded the details of peripheral neurotoxicity during chemotherapy and follow-up into the electronic medical record system, and based on the complaints, symptoms, and signs related to CIPN after the application of paclitaxel-based chemotherapy and to determine whether the patients developed CIPN, the patients were classified as 0-IV using the grading criteria of CIPN grades specified in the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0 (The National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (NCI-CTCAE V5.0) was used to classify CIPN into five grades, ranging from 0-IV, and two scales were used: the European Organization for Research in Cancer Therapy (EORTC) Peripheral Neurotoxicity Quality of Life Questionnaire (EORTC Quality of Life Chemotherapy Induced Peripheral Neuropathy20, EORTC QLQ-CIPN20) and Functional Assessment of Cancer/Gynecologic Oncology Neurotoxicity Scale (Functional Assessment of Cancer Therapy/Gynecologic Oncology20). Therapy/Gynecologic Oncology Group-Neurotoxicity questionnaire (FACT/GOG-NTX) to assist in assessing the severity of CIPN and its impact on quality of life, and the Insomnia Severity Index (ISI) to assess patients' sleepiness. Index , ISI) was utilized for the assessment of patients' sleep status, and the Hospital Anxiety and Depression Scale (HADS) was utilized to assess patients' anxiety status and depression status. In order to verify whether patients' age, gender, BMI, and hemoglobin, vitamin D, Chinese medicine identification of body constitution, anxiety mood state, and depression mood state before chemotherapy were risk factors for the occurrence of CIPN.

3. In this study, data were analyzed using the presence or absence of CIPN as the study outcome indicator. SAS_JMP11.0 and R4.3.1 software were used to statistically analyze the acquired data, and the Jordon index of the receiver operating characteristic curve (ROC) curve was used to select the optimal cut-off value of the laboratory test indicators, and the differences were first analyzed by the Pearson's chi-square test or Fisher's exact The Pearson chi-square test or Fisher's exact test was used to analyze the differences, and then binary logistic one-way regression analysis was used (continuous variable: binary variable because the outcome variable was dichotomous and had a nonlinear relationship with the independent variable; dichotomous variable: chi-square test or Fisher's exact test) to filter out the risk factors associated with the occurrence of CIPN between the groups, and then binary logistic multifactor regression analysis was used to derive the risk factors associated with the occurrence of CIPN. factor regression analysis to derive independent risk factors for the occurrence of CIPN.

4. The column-line graph (nomogram) model for predicting the risk of developing CIPN was plotted using R4.3.1 software, the discriminatory power of the model was evaluated by the area under the ROC curve, the goodness of fit of the model was examined using the Hosmer-Lemeshow test and the GiViTI Calibration Belt, and the Bootstrap re-sampling method (1,000 times) was used Internal validation was performed, and Decision Curve Analysis (DCA) was used to evaluate the clinical applicability of the model.

5. The assessment of the degree of sensory-motor impairment before and after the

occurrence of CIPN was statistically analyzed using the chi-square test and paired-samples t-test, and the assessment of the efficacy of herbal fumigation treatment was statistically analyzed using the chi-square test or Fisher's exact test, with $P < 0.05$ considered statistically significant.

Results: 1. The overall incidence of CIPN in this study was about 48.9%, the main chemotherapy regimen was paclitaxel alone or in combination, the age of patients who did not develop CIPN was 51.47 ± 11.84 years, and the mean ± standard deviation of the age of patients who developed CIPN was 54.16 ± 9.98 years, and in a subgroup of patients who developed CIPN, the tumor lymph node metastasis stage (Tumor Node Metatastasis , TNM) stage II in 18 (41.86%), the majority of patients 43 (97.73%) received at least 4 cycles of chemotherapy, and among the female patients who developed CIPN, 28 (68.29%) were in postmenopausal status. Of the types of paclitaxel, CIPN occurred with albumin-bound paclitaxel in 21 (47.73%), Eastern Cooperative Oncology Group (ECOG) physical status score of 1 in 27 (61.36%), and BMI ≥25 kg/m2 in 17 (38.64%); 9 (20.45%) had a previous history of psychoneurotics; 38 (86.36%) had a partner in their marital status; 32 (72.73%) lived in towns and cities; 24 (54.55%) had an education level of junior high school or below; 20 (45.45%) were retired in their job type; 23 (52.27%) did not experience anxiety states; 23 (52.27%) did not experience anxiety states; and 20 (52.27%) did not experience anxiety states. (52.27%); 31 people (70.45%) showed depressive mood states; 25 people (56.82%) were assessed as not suffering from insomnia; Chinese medicine identification of constitution: 14 people (31.82%) with blood stasis, 12 people (27.27%) with qi deficiency, 7 people (15.91%) with qi depression, and 11 people (25%) with other constitutions; and the vitamin D level deficiency was 28 people (71.79%).

2. In 44 patients (48.9%) who developed CIPN, univariate logistic regression analysis showed that: history of previous psychoneurotics, menstrual status, place of residence, anxiety mood state, depression mood state, presence of insomnia, leukocyte count, lymphocyte count, hemoglobin, CD3+ (total T-cells), CD16+CD56 (NK-cells), CD3+AC (absolute total T-cell count) were all significantly correlated with the development of CIPN in patients after chemotherapy. Multifactorial logistic regression analysis showed that: postmenopausal patients, P=0.0067; chemotherapy baseline depression score ≥8, P=0.0018; low baseline leukocyte count (≤6.64×109/L), P=0.0399; low hemoglobin (≤134g/L), P=0.01; and high CD3+ (total T cells) (>70%), P= 0.023 were independent risk factors for developing CIPN.

3. Based on the results of binary logistic single-factor and multifactor regression analyses of the screened risk factors plotted on a column-line graph, the area under the curve of the model was 0.908, indicating that the model was well differentiated ($P < 0.0001$); the Hosmer-Lemeshow test showed that the model was well fitted (P-value of 0.339, $P > 0.05$). Bootstrap resampling method 1000 times internal validation and GiViTI Calibration Belt showed that it did not fall within the 95% confidence interval both showed that the model had a good fit, and the DCA curve was used to evaluate the clinical applicability of the model, and the clinical decision curve was higher than the two extreme curves, thus indicating that the model predicted the net clinical benefit better, and that the model had a greater application in practice The value of the model in practice is also greater.

4. The clinical characteristics of the occurrence of CIPN showed that: acute CIPN occurred in 6 people (13.64%), chronic CIPN occurred in 38 people (86.36%), according to the NCI-CTCAE5.0 classification: 28 people (63.64%) had Grade I, 16 people (36.36%) had Grade II, and Grade III and above did not occur; the site of CIPN only symptoms in both hands were 24 (54.55%), symptoms in both feet only were 12 (27.27%), and symptoms in both hands and feet were 8 (18.18%). To assess the degree of damage to sensory and motor nerves in the event of CIPN: the results of the two-point discrimination test indicated that before the patient developed CIPN, 19 patients (43.18%) passed 7/10 times and 25 (56.82%) did not pass 7/10 times, and after the development of CIPN, 7 patients (15.91%) passed 7/10 times and 37 (84.85%) did not pass 7/10 times. of 37 patients (84.09%), P=0.0028; Purdue nailboard test results indicated that the left-handed nailboarding time before the occurrence of CIPN was 56.2±6.26s, and the left-handed nailboarding time after the occurrence of CIPN was 57.5±9.23s, P=0.422; and the right-handed nailboarding time before the occurrence of CIPN was 55.26±5.05s, and after the occurrence of CIPN the right-handed Nail plate time was 56.78±4.62s , P=0.0015.In the assessment of the occurrence of CIPN scale, the occurrence of CIPN in patients was dominated by sensory nerve disorders, and motor nerve disorder symptoms and autonomic disorders were less frequent, and the scores of the severity of the occurrence tended to increase with the increase of chemotherapy cycles.The change of the FACT/GOG-NTX scores in patients with the occurrence of CIPN and the EORTC QLQ-CIPN20 scale score trends were more consistent. At subsequent follow-up of CIPN in patients who used herbal medicine after CIPN occurred versus those who did not, 16 (36%) of the overall population who developed CIPN were not in complete remission by the date of the last follow-up.

5. In this study, the efficacy of herbal fumigation was initially explored. 15 (34.09%) of the patients who developed CIPN underwent herbal fumigation, and only 1 (6.67%) remained unrelieved of CIPN symptoms as of the follow-up date. Of the patients who did not use herbal fumigation, 14 (48.28%) had remission of CIPN symptoms and 15 (51.72%) remained symptom free. The rate of CIPN remission was higher in patients who received herbal fumigation compared to those who were not treated with herbal fumigation (93.33% vs 48.28%), P=0.0034.

Conclusions: 1. The high incidence of CIPN caused by paclitaxel-based chemotherapeutic agents (48.9%) and its impact on the adequate amount and duration of chemotherapy, as well as the efficacy and quality of life of the patients, need to be of great clinical importance.

2. Menopausal status, white blood cell count, haemoglobin, CD3+ (total T-cells), and depressed mood state are independent risk factors for the development of CIPN after chemotherapy with paclitaxel analogues.

3. The validation of the logistic multifactorial risk prediction model constructed in this study was established; the model evaluation indexes, including differentiation, calibration, decision curves, and internal validation results, met the requirements; and the application of the column-line diagram of the CIPN risk prediction model was easy, which provided a reference for the clinical prediction of the risk of CIPN occurring in zymosan-based chemotherapeutic drugs and the clinical intervention.

4. Clinical assessment, observation and follow-up of the clinical features of the occurrence of

CIPN may help clinicians to inform their knowledge prior to administering paclitaxel-based chemotherapeutic agents to their patients in order to detect, assess and intervene in a timely manner in the occurrence of this side effect.
5. Through the preliminary exploration, it was observed that herbal fumigation showed a clear preliminary efficacy in patients with CIPN, and it is necessary to continue to expand the sample size in the next study to explore the efficacy of herbal fumigation in graded prophylactic treatment.

P4-03-16: Comparison of analytical and prognostic performance among various Artificial Intelligence models for Tumor Infiltrating Lymphocytes scoring in Triple Negative Breast Cancer: An independent validation on a prospective cohort

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Introduction: Tumor-infiltrating lymphocytes (TILs) have become a significant biomarker during recent years, showcasing its predictive and prognostic potential for early and metastatic triple-negative breast cancer (TNBC). However, pathologist-read stromal TILs (sTILs) remain a semi-quantitative biomarker, susceptible to inter-observer variability. With the surge in Artificial Intelligence (AI) research, various automated approaches have been proposed to score TILs with the promise to overcome the limitations of manual assessment. However, there is a lack of studies comparing different AI models in both analytical and clinical validity with respect to mimicking the challenges of clinical practice.
Methods: In this study, we aimed to investigate the variability among ten AI-based TILs scoring models (seven own-developed machine learning models in QuPath –KNN, Random Forest, Neural Network– and three pre-trained deep learning models –HoverNet Graham et al. Medical Image Analysis 2019, CellViT Hörst et al. Medical Image Analysis 2024, Abousamra et al. Frontiers in Oncology 2022–) with respect to their analytical and clinical validity on internal and external validation sets. The development cohort consisted of diagnostic tissue slides of 79 women with surgically resected primary invasive TNBC tumors diagnosed between 2012 and 2016 from the Yale School of Medicine. An independent prospective set comprising of 215 TNBC patients from Sweden diagnosed between 2010 and 2015, with 4 years median follow-up, was used for assessing the models' clinical validity. The gold standard of this study regards manual sTILs scoring from two expert pathologists.
Results: Moderate correlation in analytical validity (Internal validation set: Spearman's r= 0.72-0.84, $p<0.001$; External validation set: Spearman's r=0.63-0.73, $p<0.001$) is demonstrated across AI methodologies and training strategies. Training on progressively increasing number of samples improved the correlation with sTILs in internal (10 patients:r=0.79, 20:r=0.81, 30:r=0.82, 40:r=0.84, 50:r=0.83, $p<0.001$) but not in the external validation sets (10:r=0.70, 20:r=0.68, 30:r=0.70, 40:r=0.68, 50:r=0.73, $p<0.001$). HoverNet & CellViT achieved the second highest correlation with sTILs in the internal validation set

(r=0.83, p<0.001) but second and third to worst in the external validation set (r=0.67 & r=0.64, p<0.001). Variabilities in the distribution of TILs scores were identified across models. Interestingly, eight out of ten models (KNN, RF, NN and HoverNet), even less extensively trained ones, showed statistically significant prognostic potential, with similar and overlapping hazard ratios (HR) in the external validation cohort (Cox regression based on IDFS-endpoint and dichotomized TILs scores at 10%, HRadjusted=0.38-0.50, p<0.047). For reference, manual sTILs demonstrated a HRadjusted=0.43 (p=0.003).
Conclusion: Most AI TIL methods demonstrated similar and statistically significant clinical validity, which we believe may be attributed to the intrinsic robustness of TILs as a biomarker. The analytical discrepancies between the AI models should not be overlooked; rather, we believe that there is a need for a large and diverse clinical benchmark dataset to be used for independent model validation ensuring the comparability and reliability of AI tools before integration into the clinical practice.

P4-03-17: Enhanced spatial detection of post-neoadjuvant breast cancer minimal residual disease by tissue-based Cancer Personalized Profiling by Deep Sequencing.

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Background: Circulating tumor DNA (ctDNA) is undetectable after neoadjuvant chemotherapy (NAC) in most breast cancer patients with residual disease, limiting its utility to inform adjuvant treatment. The current standard-of-care for assessing NAC response is pathologic evaluation of resection tissues. Many patients without histologically detected post-NAC disease, however, go on to recur, and many with significant gross residual disease are cured, highlighting the need to more accurately quantify low-burden post-NAC MRD, when adjuvant treatment decisions are made.
Methods: We describe spatial post-NAC MRD detection by tissue-based Cancer Personalized Profiling by Deep Sequencing (t-CAPP-Seq), profiling tissue-based MRD (tMRD) in breast and lymph node resection tissues. Using personalized oligonucleotide hybrid capture panels derived from whole exome sequencing (WES) along with a fixed panel of recurrently mutated and biologically relevant breast cancer genes, we established spatial tMRD detection across numerous spatially catalogued samples per case using barcode mediated error suppression and a Monte Carlo statistical framework. We compared tMRD to pathologic detection in matched samples and described patterns of quantitative tMRD measurement that predict recurrence risk.
Results: We followed a median of 36 (range: 1-112) single nucleotide variants (SNVs)/case derived from WES and a fixed panel of 56 common breast cancer genes spanning 225kb across 29 tumors representing a range of breast cancer subtypes. Nine (33%) and 10 (37%) tumors harbored TP53 and PIK3CA mutations, respectively. We genotyped 797 individual spatially resolved post-surgical specimens spanning these cases (median 24 post-treatment blocks/case, range: 12-46) and established strong concordance between genomic and

histologic MRD detection (considering histology as the gold standard, sensitivity=78.8%, specificity=83.3%), with excellent classification of histologic status by tMRD (AUC=0.92). tMRD positive samples undetected by pathology had lower mean allele frequencies (AFs) than those detected by both methods ($p<0.001$), suggesting a superior limit of detection by tMRD. With a cohort median progression-free survival (PFS) of 140 months, we identified 8 progression events, including two local recurrences adjacent to a tMRD-positive surgical margin that was histologically negative for invasive carcinoma. One of these recurrences was identified post-mastectomy in a pathologic complete response case. Both tMRD positive margins contained missense PIK3CA SNVs that were absent from the pretreatment tumor, representing identification of targetable (alpelisib, capivasertib) post-NAC driver lesions that were selected under the pressure of chemotherapy. In the overall cohort, higher mean tMRD AF across the resection tissues was associated with inferior PFS (HR 1.29, 95% CI 1.08-1.55, $p = 0.004$). We defined tMRD high status as a mean case AF >3%, which predicted inferior PFS ($p=0.034$) and identified four genes (CSPP1, POLE, DNAAF4, PCNT) containing SNVs in post-NAC tissues that were significantly correlated with inferior PFS across the cohort (Cox proportional hazards model, $p<0.05$).
Conclusions: Here we introduce t-CAPP-Seq, a novel tMRD detection platform that outperforms surgical pathology for MRD assessment, quantitatively and spatially profiling patterns of genomic lesions under the selection pressure of treatment that are associated with clinical progression. We anticipate tMRD-based interventional adjuvant approaches will motivate movement of molecularly targeted therapies into the adjuvant setting, leading to superior survival outcomes and challenging current treatment paradigms.

P4-03-18: Chimerizing the HER2 receptor for functional and immunologic studies with αHER2 antibody-drug conjugates

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Background: αHER2 antibody-drug conjugates (ADCs) have revolutionized the treatment of HER2+ breast cancer, prompting numerous clinical trials investigating different combinations and indications. However, a translational gap exists between preclinical data and clinical results due to a paucity of existing models. This stems from the fact that trastuzumab, the antibody backbone of the 2 FDA approved αHER2 ADCs (trastuzumab deruxtecan (T-DXd) and trastuzumab emtansine (T-DM1)), does not bind to rodent HER2. Thus, most preclinical studies employ in vitro or immune-deficient (e.g. xenograft) models, limiting investigations of ADC function, immunologic effects, toxicity, and resistance. To address this issue, we developed a treatment-sensitive, chimeric mouse HER2 tumor model (termed HER2X) that is capable of binding to trastuzumab, while avoiding growth suppression and immunologic selection in vivo, a limitation of existing models.
Methods: The HER2X receptor was generated by substituting 3 salient amino acids from the trastuzumab binding region of human HER2 into the mouse HER2 receptor. AlphaFold and NetMHCpan4.1 were utilized to predict protein structure and murine neoantigen production of the HER2X receptor, respectively. We transduced the HER2X receptor into

EMT6 murine cancer cells and determined their ability to bind trastuzumab using a labelled trastuzumab antibody. To evaluate the immunogenicity of the HER2X model, we orthotopically inoculated immunocompetent and athymic nude syngeneic mice with parental, mouse HER2, human HER2, and HER2X-expressing tumor cells. After 17-18 days tumors were analyzed via flow cytometry to assess HER2 expression and immune cell infiltrates. To evaluate the in vivo sensitivity of the model to ADC treatment, immunocompetent mice were inoculated, as above, with parental, mouse HER2, and HER2X-expressing EMT6 cells and treated intravenously with T-DXd (weekly). The combinatorial benefit of αPD-L1 with T-DXd was also assessed in these tumor models via weekly combinations of T-DXd, αPD-L1, and trastuzumab.
Results: The predicted protein structure of HER2X preserved the general structure shared by both mouse and human HER2, but the trastuzumab binding loop of HER2X resembled that of human HER2 after in silico chimerization. The HER2X sequence also eliminated all predicted neoantigens of the fully human sequence on a murine background, permitting use of this model without concern over tumor rejection or artificial immunologic activity. The HER2X receptor was further able to bind to trastuzumab while retaining the mouse HER2 backbone sequence. In vivo, the HER2X tumor model avoided the growth suppression and immunologic selection of the full length human HER2 receptor ($p< 0.0001$ human HER2+ tumors vs. mouse HER2+ controls, n= 5-15). Upon in vivo T-DXd treatment, we observed a significant and HER2-dependent increase in survival and tumor response in HER2X tumors ($p<0.0001$, n=9-10). T-DXd response also resulted in immunologic memory formation and resistance to rechallenge with HER2X tumor cells (4/5 complete responders [80%]). Finally, αPD-L1 and T-DXd combination treatment further increased survival and response compared to either monotherapy in HER2X tumors ($p< 0.05$, n=5).
Conclusions: The HER2X tumor model is a novel and rigorous system for functional and immunologic studies with αHER2 ADCs. The HER2X receptor permits binding of trastuzumab-based drugs in immune competent models without inducing background immunogenicity associated with human HER2. We credential this model by demonstrating its sensitivity to T-DXd and combinations with immunotherapy, as well as T-DXd's ability to elicit an adaptive immune response. Overall, this model is an innovative advance to study αHER2 ADC function, bridging the translational gap between preclinical and clinical studies.

P4-03-19: Plasma-based prediction of prognosis, receptor status and organ involvement among patients receiving antibody-drug conjugates for metastatic breast cancer

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Background: Trastuzumab deruxtecan (T-DXd) and sacituzumab govitecan (SG) are approved antibody-drug conjugates (ADCs) for individuals with metastatic breast cancer (MBC). Multiple treatments are available in this setting, and prognostic and predictive biomarkers are needed to help understand tumor biology and guide treatment choices. The DNADX assay, utilizing innovative machine learning techniques, analyzes plasma tumor DNA to evaluate complex phenotypic features. This includes a 5-class subtype classification and multiple genomic signatures, which provide insight into the tumor biology in MBC (Nat Commun. 2023).
Methods: We included patients with MBC who received single-agent T-DXd or SG at Dana-Farber Cancer Institute. DNADX was applied to plasma samples collected prior to initiating the ADC. The primary objective was to investigate the association between the DNADX 5-class group classification (Tumor Fraction-low [TF-low], copy-number aberration-flat [CNA-flat], Luminal-high, Proliferative, and Basal-related) and overall survival (OS) from start of the ADC. Secondary objectives included correlating DNADX, time to next treatment (TTNT) and site of metastases. Statistical methods included uni- and multi-variable Cox models, logistic regression models, and Student's t-tests. Clinical variables considered included timing of biopsy, ER and HER2 IHC status, number of prior lines of chemotherapy, and type of ADC.
Results: Among 160 patients (38.1% ER+/HER2-, 36.3% ER-/HER2-, 25.0% HER2+, 0.6% unknown), 85 (53.1%) received SG and 75 (46.9%) received T-DXd. The median number of prior lines of chemotherapy was 2 (range 0-10). A total of 160 plasma samples were analyzed, of which 115 (71.87%) collected within 4 months and 45 (28.13%) >4 months from ADC start. DNADX subtypes were as follows: TF-low 36.9%, CNA-flat 1.2%, Luminal-high 27.5%, Proliferative 19.4%, and Basal-related 15.0%, suggesting significant biological heterogeneity.
Median OS in the entire study population was 11.0 months. The DNADX 5-subtype classification was significantly associated with OS ($p<0.001$) and TTNT ($p=0.005$) after adjusting for clinicopathological variables. The median OS for Luminal-high group, the proliferative subtype and the Basal-related subtype were 20.2, 9.7 and 8.9 months, respectively. Compared with the Luminal-high group, the Basal-related subtype and the proliferative subtype were associated with worse OS (HR 2.25 95% CI 1.13-4.46, $p=0.020$, and 2.61, 95% CI 1.14-4.99, $p=0.023$, respectively) independently of type of ADC and clinical ER status. Patients with high (vs. low) expression of the DNADX progesterone receptor signature, which is associated with Luminal A biology, were more often pretreated with ≥3 lines of ET (45% vs 15%, odds ratio=6.54, $p=0.001$), independently from HER2 and ER status. High expression of the DNADX ER signature as a continuous variable was associated with ER IHC status (AUC=0.81, $p<0.001$). Additionally, specific DNADX signatures were associated with the presence of liver, bone, and brain metastases (all $p<0.05$). High (vs. low) expression of DNADX signatures associated with Basal-like biology was linked with the presence of brain metastases (55% vs 20%, odds ratio=4.89, $p=0.027$), independently from HER2 and ER status.
Conclusions: Plasma-based DNADX analysis prior to starting ADC treatment in MBC demonstrates significant biological and prognostic diversity beyond ER and HER2 status.

Furthermore, DNADX may facilitate the identification and monitoring of clinical features, including endocrine therapy sensitivity, as well as the specific organs affected by metastatic disease.

P4-03-20: Molecular characterization of estrogen receptor (ER)+/HER2- breast cancer unveils a biologically and clinically distinct entity with ERBB2 hemizygous deletion (ERBB2del)

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Background: Among patients with ER+/HER2- breast cancer (BC), IHC-defined HER2-low and HER2-0 tumors do not appear to significantly differ molecularly (Tarantino P. et al. Nat Comm 2023). However, this may be due to lack of sensitivity of IHC, which is currently used to select patients for T-DXd treatment, despite major diagnostic challenges. To better characterize the biologic correlates of HER2-low expression, we analyzed molecular profiles by quantitative ERBB2 mRNA expression and characterized the features of tumors harboring ERBB2del, for which resistance to T-DXd has been suggested.
Methods: Genomic, transcriptomic and proteomic data from ER+/HER2- BCs were analyzed from the METABRIC, TCGA (early BC) and MSK MetTrospism (metastatic BC, MBC) databases. Genomic landscapes, gene expression and HER2 protein expression were compared between subgroups based on HER2 mRNA tertiles (ERBB2-low, -intermediate, -high), by HER2 IHC score (IHC 0, 1+, 2+/not amplified) and by ERBB2del. Further validation was conducted in two DFCI cohorts, including patients with HER2- early BC with tumors tested for OncotypeDX between 1/2018 - 12/2022 and patients with MBC with tumors that had undergone next-generation sequencing (NGS) between 7/2013 - 12/2020.
Results: All ER+/HER2- BCs from the METABRIC (n=1298), TCGA (n=524) and early DFCI (n=971) cohorts exhibited some degree of ERBB2 mRNA expression. In both METABRIC and TCGA, the ERBB2 mRNA high group was enriched in pathways related to ER signaling (Hallmark estrogen response early), while the ERBB2 mRNA low group was enriched in genes involved in cell proliferation (Hallmark E2F targets, MYC targets) and immune response (Hallmark allograft rejection, TNF signaling via NF-kB, IFN-α response, IFN-γ response). At the genomic level, PIK3CA mutations were enriched in the ERBB2 mRNA-intermediate and -high groups (both $p<0.01$), whereas TP53 mutations were enriched in the ERBB2 mRNA-low subgroup ($p<0.001$). Notably, ERBB2del were common in the ERBB2 mRNA-low group of both METABRIC and TCGA (35% and 54%), but less frequent in the ERBB2 mRNA-intermediate and -high cohorts (METABRIC: 9% and 4%; TCGA: 14% and 5%). No significant difference in the distribution of PIK3CA, TP53 and ERBB2del was noted between HER2 IHC subgroups.
Among tumors with ERBB2-low mRNA, >90% of ERBB2del tumors had a concurrent TP53 heterozygous deletion (TP53del), compared with only 25-36% of non-ERBB2del tumors

(both on chromosome 17). ERBB2del tumors were found enriched in the expression of genes related to cell proliferation and immune response, while non-ERBB2del BCs were enriched in genes related to ER signaling. ERBB2del tumors were found scattered across HER2 IHC subgroups (30.7% of IHC 0, 23.9% of IHC 1+, 15.8% of IHC 2+), had lower ERBB2 mRNA and HER2 protein levels, lower ER signaling and higher expression of proliferation genes within each HER2 IHC category.
In the DFCI cohort of ER+/HER2- MBC with clinical NGS (n=749), the prevalence of ERBB2del was 14.3%, with 87.9% of ERBB2del tumors having concurrent TP53del. A significant difference in overall survival (OS) was observed between ERBB2del vs. non-ERBB2del tumors (33.8 vs 47.8 months, p=0.037), irrespective of the presence of TP53del, whereas no difference in OS was observed by HER2 IHC score (p=0.26). Similarly, in the MSK MetTrospism cohort (n=883), patients with ER+/HER2- MBC and an ERBB2del had worse OS (16.4 vs 30.8 months, p<0.0001), while the presence of TP53del was not associated with OS (32.1 vs 34.2 months; p=0.62).
Conclusions: ERBB2del are frequent events in ER+/HER2- BC that are characterized by low ERBB2 mRNA levels, and are associated with TP53del, higher proliferation and immunogenicity, lower ER signaling and HER2 protein expression, and decreased OS in the metastatic setting.

P4-03-21: Limiting Over- and Under-Treatment of DCIS: Ten Year Breast Recurrence Rates with Personalized Management Utilizing the 7-Gene Biosignature as Compared to Clinicopathologic Factors Alone

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Purpose: Clinicopathologic (CP) factors, including age, grade, and tumor size have been considered to guide the management of ductal breast carcinoma in situ (DCIS) to minimize over- or under-treatment. However, these CP factors have failed to identify a low-risk group with little to no benefit from radiation therapy (RT) or a high-risk group with a high risk of recurrence even after RT. We compare the impact of utilizing a 7-gene biosignature (DCISionRT) to risk stratify patients as compared to standard CP factors.
Methods: Women (n=926) from four published international DCIS cohorts treated with BCS with negative margins +/- RT were categorized as CP low-risk or high-risk using age (>70 or <50), grade (1-2 vs 3), size (≤ or > 2.5 cm). Women were classified as DS (DCISionRT Score) Low Risk (DS≤2.8, no Residual Risk sybtype, RRt) or High Risk (DS>2.8 +/- RRt) using the 7-gene biosignature and stratified by the CP factors. Rates of ipsilateral breast recurrence (IBR) and benefit or lack of benefit with RT were evaluated for low-risk, high-risk CP as compared to DS Low Risk and DS High Risk.
Results: On average, 61% of patients with individual low-risk CP factors (78%, n=133 of age >70, 56%, n=356 of size ≤ 1 cm, 51%, n=101 of grade 1, and 57%, n=218 of grade 2) were re-classified as DS High Risk. Although CP low risk patients showed significant RT benefit, when re-classified as DS Low Risk patients, no significant RT benefit was noted for age >70

(HR 1.19, p=0.71), size ≤1 cm (HR 1.09, p=0.90), grade 1 (HR 1.61, p=0.70), or grade 2 (HR 0.93, p=0.92), with a corresponding average 10-yr IBR rate of 4.9% (2.3%-6.8%) without RT vs. 5.9% (4%-7.1%) with RT. In contrast, when CP low risk patients were re-classified as DS High-Risk patients, a significant RT benefit was noted for age >70 (HR 0.15, p=.02), size ≤1 cm (HR 0.23, p<0.001), grade 1 (HR 0.22, p=0.04), or grade 2 (HR 0.32, p=0.01), with a corresponding average 10-yr IBR rate of 21% (18.3%-20.3%) without RT vs. 5.4% (3%-7.4%) with RT. On average, 27% of patients with individual high-risk CP factors (42% (n=88) of age <50, or 16% (n=10) of size >2.5 cm, 22% (n=75) of grade 3) were re-classified to DS Low Risk, and IBR rates did not differ significantly by RT receipt for these re-classified patients. High-risk CP patients with concordant DS High Risk classification did benefit from RT for age <50 (HR 0.35, p=0.04), size >2.5 cm (HR 0.17, p=0.02), and for grade 3 (HR 0.19, p<0.001), with a corresponding average 10-yr IBR rate of 34% (27.9%-38.5%) without RT vs. 11% (9.1%-12.4%) with RT.
Conclusions: The 7-gene predictive DCIS biosignature more reliably identified patients with low 10-year IBR rates and no significant RT benefit than traditional CP factors as well as those with elevated long term IBR rates that benefited from RT with substantial crossover for low and high risk clinicopathologic patients, respectively. The use of DCISionRT allows for more personalized and accurate risk stratification, preventing the under- and over-treatment of patients with suspected “low-risk” or “high-risk” DCIS based on current clinicopathologic factors.

P4-03-22: Inhibiting JNK signaling enhances the antitumor efficacy of macrophage-targeting agents in triple-negative breast cancer by inducing an immunoactive tumor microenvironment

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Background: Triple-negative breast cancer (TNBC) has a unique tumor microenvironment (TME) that contributes to tumor progression and affects responses to immunotherapy. c-Jun N-terminal kinase (JNK), part of the MAPK pathway, plays a crucial role in inflammation and tumor progression and has been targeted in clinical trials for treating inflammation-related diseases and solid tumors. Indeed, JNK inhibitor (JNKi) BMS-986360 is currently being tested alone and in combination with chemotherapy or nivolumab in advanced solid tumors (NCT05625412). Our recent study showed that JNK promoted TNBC tumor growth and metastasis by creating an immunosuppressive TME via macrophage-derived CCL2 in preclinical models. Therefore, in this study, we tested our hypothesis that JNKi enhances the antitumor efficacy of macrophage-targeting agents in TNBC.
Methods: The correlation of JNK signaling activity with the immune status of the TME in TNBC was analyzed using the TCGA dataset (n = 191). The antitumor efficacy of JNKi (BMS-986360, a reversible orally active pan-JNKi; or JNK-IN-8, a covalent pan-JNKi) combined with macrophage-targeting agents (pexidartinib or sotuletinib, both of which are orally active CSF-1R inhibitors) was assessed using immunocompetent syngeneic models of

E0771 (M1 macrophage-enriched TME, sensitive to immune checkpoint inhibitors [ICI]), PyMT-M (M2-macrophage-enriched TME, partially sensitive to ICI), and 4T1.2 (neutrophil-enriched TME, resistant to ICI). The treatments' effects on immune cell tumor infiltration were determined by flow cytometry.
Results: To investigate the role of JNK signaling in regulating the immune landscape of the TME in cancer, we have developed a phospho-JNK (pJNK) gene signature to infer JNK phosphorylation status by gene expression. Using this pJNK gene signature and TCGA dataset, we found that high JNK (JNKhi) signaling activity was associated with high counts of Tregs and cancer-associated fibroblasts and low counts of CD8+ T cells, M1 macrophages, and neutrophils in TNBC tumors. TNBC tumors with JNKhi signaling activity were enriched with pathways regulating angiogenic and fibrotic stroma, e.g., Wnt and TGF-β signaling, which are the major inflammatory pathways involving tissue fibrosis and tumor progression. These results demonstrate a strong correlation between JNKhi signaling activity and an immunosuppressive TME in TNBC. Since JNK promoted an immunosuppressive TME in TNBC through macrophage-derived CCL2, we next investigated whether inhibiting JNK signaling enhances the antitumor efficacy of macrophage-targeting agents in mouse models. Compared with monotherapies, BMS-986360 or JNK-IN-8 plus pexidartinib or sotuletinib significantly reduced tumor growth in immunocompetent syngeneic TNBC models (E0771, PyMT-M, and 4T1.2; $P < 0.01$). Furthermore, compared with monotherapies, JNKi combined with pexidartinib reduced counts of Tregs and M2 macrophages and increased counts of M1 macrophages and cytotoxic CD8+ T cells in both E0771 and PyMT-M tumors. These results suggest that combination of JNKi with macrophage-targeting agents reduces tumor growth by inducing an immunoactive TME.
Conclusion: JNKi can potentiate macrophage-targeting agents in TNBC by inducing an immunoactive TME. This finding highlights the need to conduct further studies of combining JNKi with immunotherapy to capitalize on JNK's immune regulation of the TME and the potency of JNKi in synergistically improving the efficacy of immunotherapy for TNBC.

P4-03-23: Preclinical investigation for the mechanisms of action of PARP inhibition in combination with trastuzumab deruxtecan (T-DXd) in triple negative breast cancer (TNBC)

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Background: TNBC represents a significant challenge due to its aggressive clinical course and the lack of targeted therapy. Chemotherapy remains the mainstay of systemic treatment option, however resistance is common in TNBC. Next generation antibodies-drug conjugates (ADCs) have demonstrated improved efficacy compared to traditional chemotherapy as a result of more effective tumor delivery of cancer therapeutics. Two ADCs, both employing a topoisomerase 1 inhibitor payload, including Sacituzumab Govitecan (SG) and T-DXd, have received FDA approval for the treatment of metastatic TNBC. However, resistance is

evitable. Recent data indicated the potential of adding PARP inhibitor to SG or T-DXd in improving tumor control. We aim to understand the underlying mechanisms of action for the combination strategy, focusing on the T-DXd and olaparib combination.

Method: We conducted preclinical studies to assess the anti-tumour activity of T-DXd and the PARP inhibitor Olaparib, either alone or in combination, compared to the vehicle treatment, in four TNBC cell lines (MDA-MB-231, WHIM 12, HCC1806 and BT549) in vitro and four HER2-low TNBC PDX patient derived xenograft (PDX) models: WHIM2, WHIM6, WHIM12 and WHIM30 (BRCA1 mutant), and one HER2-zero PDX model (WHIM21) in vivo.

Results: In both in vitro and in vivo models, the addition of olaparib to T-DXd enhanced DNA damage and apoptosis, demonstrated by increased pH2AX and cleaved PARP, respectively. The combination therapy showed synergistic anti-tumor effect in vitro and was most effective in inhibiting tumor growth in vivo. Interestingly, combination therapy potently reduced or blocked cell migration and mammosphere formation in vitro. This was accompanied by reduced expression of epithelial-mesenchymal transition (EMT) inducers including Snail, Slug and Twist1 by Western blot. In addition, T-DXd alone increased the expression of the stress response controller ATF4, which was prevented by the addition of Olaparib. Furthermore, Olaparib combined with T-DXd inhibited the expression of MTOR. Subsequent screening of additional TNBC PDX models identified two TNBC PDX models (WHIM3 and WHIM4), which were relatively resistant to T-DXd monotherapy, was resistant to the combination with Olaparib. Additional studies including proteogenomic analysis and functional HRD assessment by RAD51 foci and markers of replication stress of baseline and on-treatment samples of these PDX models will be presented to further understand the mechanisms of resistance.

Conclusion: PARP inhibition enhances the DNA damaging and apoptotic effect of T-Dxd as expected. The findings of reducing EMT and stress response may also contribute to the enhanced anti-tumor effect. However resistance to the combination exists. Ongoing proteogenomic analysis will provide further insight into the mechanisms of action for the combination therapy.

P4-03-24: Septin 9 isoform expression and response to taxane treatment in breast cancer patients

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Drugs that disrupt the microtubule cytoskeleton, such as the microtubule stabilizing taxanes, are regularly used in the treatment of breast cancer in both the neoadjuvant and adjuvant setting. However, there are currently no molecular biomarkers that are used to guide which patients will respond to taxane treatment. Septins, a class of small GTPases, interact with both microtubules and actin to functionally link these cytoskeletal

components and regulate cell biological processes shown to be critical for the invasion and migration of cancer cells. In particular, septin 9 (SEPT9) directly interacts directly with microtubules through an N-terminal domain on the longest isoform, SEPT9 isoform 1, which is sufficient to promote migration and invasion of breast cancer cells in vitro. Additionally, both SEPT9 isoform 1 and SEPT9 isoform 3 have been shown to play a role in taxane resistance in vitro, but the possible role in clinical taxane resistance had not been studied. We hypothesized that the relative expression of SEPT9 isoforms in breast tumors could play a role in their response to treatment with microtubule-targeted chemotherapy, specifically to taxanes in the neoadjuvant setting.
We performed a prospective clinical trial to correlate the expression of SEPT9 isoforms in breast tumors with pathological response to taxanes in the neoadjuvant setting. Pretreatment levels of SEPT9 isoforms, as well as other genes associated with clinically relevant drug resistance mechanisms, including the MDR1, ABCG2, and ABCG11 drug efflux transporters and the βIII-isoform of tubulin, were determined from FFPE biopsy samples using Biomark multiplexed PCR. RNA was isolated from 3 scrolls per patient biopsy sample using the Puregen automated RNA extraction platform. Expression was normalized between patients referencing actin using the ΔCt method and the average expression for each gene was set at 1. Protein levels of total SEPT9 as well as isoforms 1 and 3 were also determined by immunohistochemistry and quantified by calculating the cytoplasm and nucleus H-scores using the Aperio eSlide Manager analysis. Patients were treated in the neoadjuvant setting with either paclitaxel (dose dense or weekly), docetaxel or nab-paclitaxel. The expression of markers was reevaluated in the post-treatment surgical samples of patients who did not have a complete pathological response. We found that the expression of SEPT9 isoforms and drug resistance genes differed amongst the pre-treatment patient samples and are determining the correlation of initial SEPT9 isoform levels with pathological response to taxane treatment as well as whether SEPT9 isoform expression changes after treatment with taxanes. Preliminary results suggest that higher initial SEPT9 isoform 3 levels correspond with better tumor response to taxane treatment compared to those with lower initial SEPT9 isoform 3 levels and that total SEPT9 and SEPT9 isoform 1 levels decrease after taxane treatment while SEPT9 isoform 3 levels increase after taxane treatment. Overall, the data support the hypothesis that the relative expression of SEPT9 isoforms in breast tumors plays a role in their response to treatment with taxanes in the neoadjuvant setting.

P4-03-25: Actionable gene alterations affecting the PI3K/AKT and MAPK signaling pathways in breast cancer

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Background: The MAPK and PI3K/AKT signaling pathways are known drivers of breast cancer (BC) tumorigenesis. They are increasingly important as targets for therapeutic intervention such as capivasertib-fulvestrant for HR+HER2- BC with PIK3CA/AKT1/PTEN

alterations, or dabrafenib–trametinib for solid tumors with the BRAF V600E mutation. The goal of this study was to determine the frequency and co-occurrence of actionable gene alterations affecting these signaling pathways in BC.
Methods: The study retrospectively reviewed results of tumor-normal, whole-exome, whole-transcriptome testing using the OncoExTra assay in tumor samples from patients with BC. Thirty-one genes were considered, 13 in the PI3K/AKT pathway and 18 in the MAPK pathway. Actionable alterations were defined as those with associated FDA-approved targeted therapies in any cancer type, with evidence for matched therapies, or required for clinical trial eligibility.
Results: A total of 2387 tumor samples (66.1% HR+HER2-, 15.0% HER2+, 14.7% TNBC, 4.2% unspecified) were included. PI3K/AKT pathway gene alterations were present in 48.2% (1150/2387); MAPK pathway gene alterations were present in 15.4% (367/2387). Of these, 222 samples (9.3% of the cohort) had alterations in both pathways. The frequency of PI3K/AKT pathway alterations varied by BC subtype (p<.0001), occurring most often in HR+HER2- BC (884/1577; 56.1%) and least often in TNBC (97/352; 27.6%). Among the 1577 HR+HER2- BC samples, alterations in PI3K/AKT pathway genes PIK3CA, AKT1, and PTEN were found in 718 (45.5%), 84 (5.3%), and 128 (8.1%), respectively. Most samples with alterations in these genes (814/872; 93.3%) had only one gene involved. PIK3CA alterations were significantly more common in local/regional samples vs metastatic sites (43.4% vs 34.9%; p=.0089). The converse appeared true for PTEN (4.6% vs 5%) but was not significant (p=.0758). AKT alterations were present in 4.6% of local/regional and 4.7% of metastatic sites (p=.9671). In the MAPK pathway, MAP3K1 and MAP2K4 alterations were present in 196 (8.2%) and 63 (2.6%) of all BC samples, respectively, compared to KRAS or BRAF activating mutations, which were found in only 37 (1.6%) and included just one instance of V600E. Alterations in TSC1, TSC2, or NF1 were found in 75 samples (3.1%) with no co-occurring alterations.
Conclusions: More than half of BC tumor samples had potentially actionable genomic alterations affecting either the PI3K/AKT or MAPK pathways. In HR+HER2- BC, actionable alterations in PIK3CA, AKT1, and PTEN were generally present independently rather than co-occurring. These findings support a comprehensive testing approach that interrogates a large number of genes to maximize the number of patients identified who might benefit from targeted therapies. In addition, the presence of MAP3K1 and MAP2K4 alterations warrants further investigation, as these may represent targets for MEK inhibitors.

P4-03-26: MINDY1 role and exceptional response to CDK inhibitors in metastatic breast cancer

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Background: Patients exhibiting exceptional response to targeted treatments represent a unique opportunity towards identifying predictive biomarkers of response. The

introduction of edits into the genome using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) creates cancer models that can unravel the mechanisms driving tumorigenesis and response to drug agents. The study aimed to identify candidate molecular alterations associated with exceptional response to CDK inhibitors (CDKi) and validate their effects using genome editing.
Methods: Patients with ER-positive, HER2-negative advanced breast cancer treated with CDKi and endocrine combination therapy at Departments of Oncology-affiliated with the Hellenic Cooperative Oncology Group (HeCOG), were included. Exceptional response was defined as either complete response (CR) to the treatment regimen or partial response (PR) for 3 years, per physician's assessment. Formalin-fixed paraffin-embedded (FFPE) tumor tissue samples, obtained prior to CDKi administration from these patients were retrospectively collected. Whole-exome sequencing (WES) was performed to analyze tumor mutational landscape of exceptional responders to CDKi. Using CRISPR-mediated genome editing, a breast cancer cell model harboring a patient-specific SNP and the negative control were prepared, and subsequently evaluated for their responsiveness to palbociclib. Transcriptomic and proteomic analyses were conducted to uncover alterations in network interactions, between the two cell lines, elucidating the exceptional response to CDKi.
Results: Overall, 12 women identified as exceptional responders, initiated CDKi treatment from March 2017 to February 2020; median age at diagnosis was 46 (range, 37 to 78). All patients had received the CDKi as first-line treatment; most commonly palbociclib (10 patients, 83.3%). Based on physician assessment, 50% of patients achieved CR with the combination therapy. After a median follow up of 65 months, 10 patients are still alive, and 7 are still receiving treatment with CDKi in combination with endocrine treatment. WES results with adequate mapped reads and acceptable technical characteristics in both tumor and control blood samples were obtained from 6 of 12 patients. A set of germline and somatic single nucleotide polymorphisms (SNPs) commonly shared in this cohort was identified. The focus was on MINDY1, a member of the MINDY family of deubiquitinases (DUBs), known as a deubiquitylase of ERα in breast cancer, the common SNP among all 6 patients. Utilizing CRISPR-based genome editing, a breast cancer cell model harboring a specific MINDY1 SNP was engineered, and their response to palbociclib was subsequently evaluated. Depletion of MINDY1 transcripts through RNA interference with short-hairpin RNA, increased sensitivity to palbociclib in both cell lines, confirming that MINDY1 protein is involved in molecular pathways related to palbociclib response. The functional role of MINDY1 rs771205 was also investigated by combining transcriptomic and proteomic data. Our data demonstrated that the MINDY1 rs771205 SNP, which causes an amino acid substitution, while it does not impact the respective transcript and protein levels, alters the MINDY1 protein's interaction network.
Conclusions: The Rs771205 single mutation alters the MINDY1 protein's interaction network, sensitizing cells to palbociclib. Our study sheds new light on the underlying mechanisms driving exceptional response in patients with ER-positive, HER2-negative advanced breast cancer treated with CDKi and endocrine combination therapy , demonstrating that it is an opportune moment to invest attention and resources towards probing the underlying mechanisms of exceptional response in cancer patients.

P4-03-28: Validation of an AI-based solution for breast cancer risk stratification using routine digital histopathology images

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Introduction: Stratipath Breast is a CE-IVD marked AI-based solution for prognostic risk stratification of breast cancer patients into high- and low-risk groups, using haematoxylin and eosin (H&E)-stained histopathology whole slide images (WSIs). In this retrospective validation study, we assess the prognostic performance of Stratipath Breast in independent breast cancer cases.

Material and methods: This study included patients (N=2719) diagnosed with primary breast cancer at two healthcare locations in Sweden. The patients were stratified into low- and high-risk groups by Stratipath Breast using H&E stained WSIs from the surgically resected tumours. The prognostic performance was evaluated using time-to-event analysis by multivariable Cox Proportional Hazards analysis with progression-free survival (PFS) as the primary endpoint. Further, we evaluated the prognostic performance of the continuous slide score from the Stratipath Breast by stratifying patients into 5-level risk groups based on the 5-equally sized bins of the continuous slide score defined by quantiles.

Results: In the clinically relevant ER+/HER2-oestrogen receptor (ER)+/human epidermal growth factor receptor 2 (HER2)- patient subgroup, the estimated Hazard Ratio (HR) associated with PFS between low- and high-risk groups was 2.76 (95% CI: 1.63-4.66, p-value < 0.001) after adjusting for established risk factors. In the ER+/HER2- Nottingham histological grade (NHG) 2 (intermediate risk) subgroup, the HR was 2.20 (95% CI: 1.22-3.98, p-value = 0.009) between low- and high-risk groups. For 5-level patient risk stratification based on the continuous slide score, we observed the adjusted HR for PFS of 3.88 (95% CI: 1.43-10.52, p-value = 0.008) comparing between the lowest- and highest-risk group in ER+/HER2- patient subgroup.

Conclusion: The results indicate an independent prognostic value of Stratipath Breast in both the general breast cancer population, in the clinically relevant ER+/HER2- and subgroup and the NHG2/ER+/HER2- subgroups. Improved image-based risk stratification of intermediate-risk ER+/HER2- breast cancers provides information relevant for treatment decisions of adjuvant chemotherapy and has the potential to reduce both under and over-treatment, shorten lead times, and reduce costs compared to molecular diagnostics.

P4-03-29: Patient reported anxiety levels during ctDNA surveillance in early stage triple negative and hormone positive breast cancer

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Background: Growing evidence has demonstrated that circulating tumor DNA (ctDNA) is a sensitive and specific assay for predicting distant recurrences from breast cancer (BC). Patients with detectable ctDNA after neoadjuvant chemotherapy have a higher risk of distant recurrence compared to patients with negative ctDNA testing. Further, ctDNA positivity precedes clinical metastatic relapse by 10.5-12 months (mos) on average. SignateraTM (Natera, Inc.) is a tumor-informed and commercially available ctDNA assay that has been CMS-reimbursed for stage IIB and higher BC since 2023. It is unknown whether ctDNA testing influences BC patient anxiety or physician decision-making. In this study, we describe patient reported anxiety levels using the Patient-Reported Outcomes Measurement Information System (PROMIS) and assess physician decision-making using a provider decision assessment tool (PDAT) in the setting of an institutional registry for patients who underwent ctDNA surveillance during routine follow-up.

Methods: This is a single institution observational study of ctDNA surveillance in patients with early stage hormone receptor positive (HR+) or triple negative BC (TNBC) with residual disease after neoadjuvant treatment. ctDNA was assessed using the Signatera assay at baseline (prior to adjuvant therapy in ER+ disease or following surgery in TNBC) and every 6 mos for 2 years or until clinical evidence of recurrence. Patients with positive ctDNA were re-staged with scans as deemed appropriate by the treating physician. Patients were invited to complete an anxiety questionnaire using the PROMIS every 6 mos, online, or in clinic. Treating physicians were invited to complete a PDAT at the time of ctDNA test results. Mean scores on the PROMIS and PDAT were calculated at baseline and 6 mo intervals. Higher PROMIS scores correspond to higher anxiety levels. This planned interim analysis is at 50% of recruitment (n=48). T scores will be analyzed and reported at final analysis.

Results: 48 patients (TNBC n=29, HR+ n=19) were enrolled in the surveillance study, with mean age of 52 years (range 24-84). ctDNA results are available for 45 participants at baseline, and 25 at 6 mos. Seven patients, 6 TNBC (4 stage IIB and 2 stage IIIB disease), and 1 HR+ (stage IIB) had positive ctDNA. Five (11.1%) were positive at baseline and two (8%) turned positive at 6 mos. Three of the 7 (42.9%) ctDNA positive patients, all TNBC, had detectable metastatic disease on imaging at the time of ctDNA positivity. PROMIS results are available for 40 patients at baseline and 23 at 6 mos. PROMIS mean total scores were similar at baseline and 6 mos (44.5 vs 42.5, p=0.648) for the whole group, in ctDNA negative

patients (44.7 vs 42.1, p=0.488), and in ctDNA positive patients (42.3 vs 40.5, p=0.508). PDAT mean scores were also similar at baseline and 6 mos (41.2 vs 41.1, p=0.588). PROMIS data is available for 3/4 participants with ctDNA positive / imaging negative results and showed a small increase in anxiety levels at 6 mos vs baseline (32 vs 30, 48 vs 38, 49 vs 44).

Conclusion: To our knowledge, this is the first study to report on patient reported anxiety levels associated with ctDNA surveillance in early stage BC. At this interim analysis, no statistically significant difference in patient reported anxiety was seen at 6 mos compared to baseline. Average anxiety scores were similar to the US general population (average 50, standard deviation 10). The majority of ctDNA results were negative; among patients with positive ctDNA testing, small increases in anxiety levels were detected, but the sample size was limited. PDAT scores remained stable. This interim analysis suggests that ctDNA surveillance may not adversely affect patient anxiety. However, further follow up and future randomized trials are needed.

P4-03-30: GPR56/ADGRG1 promotes the progression of triple-negative breast cancer by enhancing PKM2/LDH-mediated glycolysis via the PI3K/AKT/mTOR/HIF-1α pathway

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Objective: Despite increasing evidence suggesting that G protein-coupled receptors (GPCRs) may play a critical role in tumor development, their exact roles and related mechanisms in breast cancer remain largely elusive. This study aimed to comprehensively investigate the prognostic value of 33 adhesion GPCRs in breast cancer, identify key GPCRs associated with prognosis, and further explore the specific biological functions and mechanisms of these key GPCRs in breast cancer.
Methods: This study utilized transcriptomic sequencing data from the TCGA and three GEO breast cancer cohorts to explore the relationship between 33 adhesion GPCRs and RFS using Cox regression analysis. GPR56 (also named as ADGRG1) was identified as a key GPCR associated with prognosis. Pathway enrichment analyses, including GO, KEGG, GSVA, and GSEA, were performed to explore the potential biological functions of GPR56. Additionally, immunohistochemistry was conducted to validate the clinical significance of GPR56. Finally, in vitro cell experiments were conducted to verify the biological functions and related mechanisms of GPR56 in triple-negative breast cancer (TNBC).
Results: Among the 33 adhesion GPCRs, only GPR56 was significantly associated with relapse-free survival (RFS) across all four cohorts and was identified as an independent adverse factor affecting both RFS and overall survival. GPR56 was overexpressed in TNBC tissues compared to normal breast epithelial tissues and was clinically associated with high invasiveness in breast cancer. GO and KEGG analyses showed that the high GPR56 expression group was significantly enriched in biological processes and pathways such as

cell division, DNA replication, and the cell cycle. GSVA and GSEA analyses indicated that the high GPR56 expression group was significantly enriched in carcinogenic pathways such as glycolysis and PI3K-AKT-mTOR. In vitro experiments demonstrated that knockdown of GPR56 inhibited the proliferation, migration, and invasion of TNBC cells. Furthermore, GPR56 promoted TNBC cell proliferation, migration, and invasion by activating glycolysis. Mechanistically, GPR56 regulated the expression of key glycolytic enzymes PKM2 and LDHA. Through literature reports, bioinformatics analysis, and experimental validation, we found that GPR56 activated the PI3K/AKT/mTOR signaling pathway. Consequently, HIF-1α, a downstream target of the PI3K/AKT/mTOR pathway, was upregulated, leading to transcriptional regulation of PKM2 and LDHA, and fostering PKM2 and LDHA-mediated glycolysis, thereby facilitating the malignant progression of TNBC.
Conclusions: GPR56 was overexpressed in TNBC and correlated with poor prognosis. GPR56 promoted the proliferation, migration, and invasion of TNBC cells. Mechanistically, GPR56 activated the PI3K/AKT/mTOR signaling pathway, promoting downstream HIF-1α expression and activating PKM2 and LDHA-mediated glycolysis. GPR56 may serve as a potential prognostic marker and therapeutic target for breast cancer, offering new insights for the precision treatment of breast cancer.

P4-04-01: Disparities in the dissemination of new breast cancer treatments in the United States

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Background: The introduction of novel treatments and treatment approaches for breast cancer has reduced breast cancer mortality and unnecessary toxicity. As care improvements develop, it is crucial to understand whom they reach. We used insurance claims data to analyze the dissemination of four major trends in breast cancer treatment into the population.
Methods: We used SEER-Medicare data to study four changes in the treatment of local and regional breast cancer in patients diagnosed between 2010 and 2018: (1) adjuvant paclitaxel-trastuzumab (APT) for patients with HER2+ local disease, (2) pertuzumab for patients with HER2+ regional disease, (3) increasing use of neoadjuvant chemotherapy for patients with triple-negative or HER2+ regional disease, and (4) decreasing use of adjuvant chemotherapy for patients with hormone receptor-positive, HER2-negative regional disease. Patients who received no systemic therapy were excluded. We used multilevel logistic regression to identify demographic (age, comorbidity, race/ethnicity, rurality, median income of residence census tract [low, middle, high]), tumor (hormone receptor status), and care (NCI vs other academic vs community hospital, breast cancer specialization of treating oncologist) factors associated with receipt of updated care. Breast cancer specialization of a physician was defined by terciles as the proportion of breast cancer patients among their patients in SEER-Medicare with colon, lung, or breast cancer. 95% confidence intervals are shown.

Results: We identified 2,385 patients with localized HER2+ breast cancer; 1,937 patients with regional HER2+ breast cancer; 3,742 patients with regional triple-negative or HER2+ breast cancer; and 14,611 patients with regional hormone receptor-positive, HER2-negative breast cancer. In these respective populations, from 2010 to 2018, use of the APT regimen increased from 31% (24-38%) to 73% (67-78%), pertuzumab from 0% to 70% (65-75%), neoadjuvant chemotherapy from 24% (19-28%) to 61% (57-66%), and adjuvant chemotherapy decreased from 37% (34-39%) to 26% (24-28%). In multivariate analyses, higher median income of residence census tract of the patient and greater specialization level of treating oncologist were significantly associated with receipt of updated care for all four trends in breast cancer treatment. Additionally, greater cancer specialization of hospital type was associated with receipt of updated care for the APT regimen, pertuzumab, and neoadjuvant chemotherapy. We develop a model that, after adjusting for patient and clinical characteristics, estimates the probability of receiving updated care, according to tumor subtype and stage, for a SEER-Medicare patient from a low-income area treated by an oncologist with low breast cancer specialization vs one from a high-income area treated by an oncologist with high specialization: for APT, 40% (37-42%) vs 76% (75-77%), for pertuzumab 28% (25-32%) vs 63% (60-66%), for neoadjuvant chemotherapy 24% (23-26%) vs 56% (54-57%), and for omission of adjuvant chemotherapy 62% (60-63%) vs 72% (71-73%). In this model, specialization alone substantially mitigates the disparity: for example, a patient from a low-income area treated by an oncologist with high specialization has an estimated 63% (59-67%) probability of receiving appropriate APT.
Conclusions: This study reveals disparities in the dissemination of major advances in breast cancer treatment across the SEER-Medicare population from 2010-2018. Patients from lower-income areas, cared for at community hospitals, and treated by oncologists with less breast cancer focus were less likely to receive updated care. These results provide important context for targeting efforts to ensure all eligible patients benefit from the latest advancements in breast cancer care.

P4-04-02: Implementing Decision Aid Use and Shared-Decision Making in Breast Cancer Screening: A Scoping Review

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Background & Objective: International guidelines vary in recommendations on the starting age for mammography screening for women at average breast cancer risk; however, all note the importance of shared-decision making (SDM) from age 40 and older. Decision Aids (DAs) are evidence-based materials demonstrated to facilitate SDM and improve decisional conflict. The integration of SDM/DA into routine clinical practice is crucial to support patient-informed choice; however, little is known related to the implementation of SDM and/or DA into the clinical setting.
Methods: This scoping review was conducted according to JBI guidelines and reported using PRISMA-ScR. [RF1] Nine bibliographic databases were searched from January 2010 to May

2023 to identify studies using any SDM and/or DA tool or intervention used to implement SDM/DA regarding breast cancer screening. The studies could target either care providers or women at average risk of developing breast cancer and must include at least one implementation outcome (acceptability, adoption, appropriateness, fidelity, feasibility, penetration, sustainability). Studies that focused primarily on the decision-making process were included, whereas those that aimed only to increase breast cancer screening were excluded. Study selection and data extraction were conducted using Covidence with two independent reviewers using the Oxford Implementation Index. We extracted information on study design, intervention details, primary outcome of the study, frameworks used to develop DAs, and all results related to implementation outcomes[MN2] . Each study was coded as efficacy, effectiveness, or implementation. Intervention strategies were coded using Expert Recommendations for Implementing Change (ERIC) and strategies used to change clinical behaviours and support implementation outcomes were coded using Behaviour Change Techniques (BCTs).
Results: The search yielded 10,383 records of which 22 studies met the inclusion criteria, 2 targeting providers, 16, targeting patients, and 4 targeting both. Studies were classified as follows (provider cohort; patient cohort): efficacy studies (0/6, 0%; 8/20, 40%), effectiveness studies (4/6, 67%; 10/20, 50%), and primary implementation studies (1/6, 17%; 1/20, 5[MN3] %). In the provider cohort, acceptability was assessed in 6/6(100%), adoption in 2/6(33%), feasibility in 2/6(33%), appropriateness in 1/6(17%), and sustainability in 1/6(17%). In the patient cohort, acceptability was assessed in 20/20(100%), feasibility in 3/20(15%), appropriateness in 3/20(15%), adoption in 1/20(5%). In both cohorts, acceptability was the most evaluated implementation outcome(100%), while penetration and fidelity were never assessed. DAs demonstrated moderate-to-excellent acceptability, requiring the use of visual aids and plain language to facilitate understanding in patients. BCTs commonly employed to increase SDM/DA included shaping knowledge, natural consequences, and comparison of outcomes. ERIC strategies frequently used included developing and distributing educational materials; there were limited strategies related to increasing uptake amongst clinical providers. Common barriers to SDM/DA use in the provider cohort included time constraints and lack of SDM training.
Conclusions: DAs are effective in assisting with SDM related to breast cancer screening and existing DA's in the literature appear acceptable; however, these tools/techniques can only help if they are used as intended. More primary implementation studies, focused on implementation fidelity, adoption/penetration, and sustainability are required, especially targeting providers, to evaluate and understand the optimal strategies for implementation of SDM and DA in these healthcare settings.

P4-04-03: Establishment of an Inflammatory Breast Cancer Program: A Quality Improvement and Research Effort

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INTRODUCTION: Inflammatory breast cancer (IBC) is an aggressive, poor-prognosis subset of breast cancer. Given its rarity and solely clinical criteria for diagnosis, patients with IBC may face delays in diagnosis and appropriate clinical care. IBC patients significantly benefit from a multidisciplinary diagnostic and treatment approach. Therefore, care delivery pathways and concentration at high-volume centers may be advantageous for timely diagnosis and initiation of treatment. Additionally, because IBC is rare, these patients are underrepresented in breast cancer research and clinical trials. We describe the creation of a multidisciplinary IBC Program aimed at expediting intake, standardizing care delivery, improving patient outcomes, and increasing research activities.
METHODS: An ad hoc IBC working group of key stakeholders including representatives from surgical oncology, medical oncology, radiation oncology, plastic surgery, radiology, oncology rehabilitation, nursing, patient experience, scheduling, administration, and patient advocates, was formed. A systematic review of high-volume IBC programs was conducted including meeting with the leadership of these IBC programs and reviewing best practices, guidelines, and research operations. An internal assessment was done through retrospective review studies, including internal scheduling processes, timelines to multimodality treatment, and outcomes.
RESULTS: Evidence-based clinical practice guidelines for IBC patients were developed and institutionally approved. These included the creation of IBC patient-specific order sets for guideline-concordant testing and procedures as well as creating clinic visit note templates. A new patient scheduling decision tree was created to facilitate expedited scheduling of patients with symptoms suspicious of IBC or an IBC diagnosis. A process improvement cycle was established to review access and timely scheduling of new IBC patients. An IBC education module was created and disseminated to providers across the health system. Our website was updated to include the IBC Program and specific details about IBC, and an IBC patient brochure was created and distributed. As part of this effort, a set of program quality metrics that have been shown to impact patient outcomes was established, and a quality assurance dashboard was built. For example, since launching the IBC Program in 2022, 30 new IBC patients have been seen (previously averaged <5 IBC patients per year) and the average time from receipt of referral to new patient appointment was 5 days. To address research gaps, an IBC research working group was established to offer IBC-specific tissue and blood collection for translational studies. Prior to the dedicated research effort (2008-2021), only 18 IBC patients had enrolled in our breast cancer translational research biorepository with one blood sample per patient. Since the implementation of the IBC Program in 2022, we have enrolled 34 IBC patients and collected 106 blood samples to date.
CONCLUSION: Through systematic methods with a multidisciplinary approach, we successfully created and implemented an IBC program at our tertiary-care academic institution. Ongoing efforts involve tracking quality measures, subsequent quality improvement cycles, and expanding IBC-specific research. The program is fulfilling an unmet need of a rare but complex patient population.

P4-04-04: Leveraging Technology to Improve Access to Clinical Trials in Underrepresented Populations within a Safety-Net Institution

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Background: Clinical trials should be accessible to all patients regardless of race, ethnicity and socio-economic status. In the US, Blacks constituted 4-6% and Hispanics 3-6% of participants in cancer therapeutic trials despite representing 15% and 13% of people with cancer. US Food and Drug Administration draft guidance from April 2022 calls for the improvement of clinical trial enrollment of participants from historically excluded racial and ethnic populations. Safety-net healthcare systems like John Peter Smith Health Network (JPS) in Tarrant County, TX, serve predominantly racial ethnic minorities. At JPS, recruitment into oncology therapeutic clinical trials remains pitifully low. Efficiently identifying patients who meet eligibility criteria in an understaffed, busy clinic in the non-academic setting has been a challenge. Referral responsibility lands on overwhelmed providers or outside companies who lack the ability to effectively screen patients. Therefore, we piloted a program leveraging the expertise of our Information Technology business intelligence (IT) team to support our clinical research team to improve recruitment efforts for an oncology trial.
Methods: Collaboration between IT and research teams was established on 5/1/24 with a goal to identify patients for this trial: EMBER-4: A Randomized, Open-Label, Phase 3 Study of Adjuvant Imlunestrant vs Standard Adjuvant Endocrine Therapy in Patients who have Previously Received 2 to 5 years of Adjuvant Endocrine Therapy for ER+, HER2- Early Breast Cancer with an Increased Risk of Recurrence. This study was obtained by JPS via the Cancer Prevention and Research Institute of Texas Clinical Trials Network Award (CPRIT-CTNA) partnership. Using the inclusion and exclusion criteria and applying the Microsoft Structured Query Language (SQL) Service Management Studio software, queries and parameters were developed and run against the Epic Clarity database of patients seen by 15 providers spanning medical oncology, radiation oncology and survivorship clinics, to extract the requested dataset of potentially eligible patients.
Results: IT team identified 123 potential eligible patients (pre-screened) for EMBER-4 research study as of 6/5/24. EMBER-4 was open for accrual at JPS on 6/18/24. A roster of pre-screened patients' next scheduled clinic appointments was also provided by the IT team. Of the 9 pre-screened patients in the first week, 4 qualified for Ember-4, and successfully enrolled two Hispanic patients. 1 of the remining 2 patients is slated to be enrolled into Ember-4 in the third week. Manual screening of all patients in providers' clinics with a high proportion of breast cancer is performed weekly in the first month to validate IT list. Validation efforts have reflected that the current list from the IT collaboration has indeed included all possible patients for EMBER-4 trial. Pre-screening has been completed on 47 of the 123 potential patients thus far. Our efforts have yielded an additional 4 eligible patients for enrollment in the next 6 weeks for a total of 8 patients meeting all eligibility criteria within the first 9 weeks.
Conclusion: These efforts have empowered the clinical research team with statistics regarding diversity among study populations and ability to better track and engage all

appropriate patients for the EMBER-4 study. Leveraging the IT team's expertise to collaborate with clinical research team is a novel method: it has resulted in precision screening capabilities with immediate possibility of increasing enrollment in therapeutic oncology research studies. This method of patient identification can be done in tandem with opening clinical research studies and will be used for future studies at JPS not only in oncology but other specialties as well. We plan to introduce this concept to other CPRIT-CTNA sites.

P4-04-05: Enhancing Clinicians' Knowledge and Competence Regarding the Use of Antibody Drug Conjugates (ADCs) in HER2+ Advanced Breast Cancer: Impact of Continuing Medical Education

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The emergence of antibody drug conjugates (ADCs) has considerably improved outcomes for patients with HER2+ advanced breast cancer. In light of recent advancements, clinical guidelines have also been updated to reflect how to sequence ADCs in patients progressing on first-line therapy, trastuzumab deruxtecan being the preferred second-line option. As timely treatment sequencing to the next line of therapy is crucial to extend survival in patients who are progressing on current line of therapy, education is needed to improve clinicians' understanding of how to optimally sequence ADCs in practice, balancing their efficacy and safety, in order to improve long-term patient outcomes. In order to bridge knowledge and competence gaps regarding the use of ≥2nd line ADCs in patients with HER2+ advanced breast cancer, we developed a continued medical education (CME) curriculum aimed at improving clinicians' understanding of: how the latest evidence on ≥2nd line ADCs may impact treatment decision-making; what factors to consider to optimize the use of ADCs in practice; and how to mitigate treatment-related side effects.
A CME-certified educational curriculum featuring three 20-minute interactive webinars titled "A HER2-Positive Breast Cancer Proficiency Series: Expert Insights on Evolving Data, Sequencing/Patient Selection, and Adverse Event Management With Antibody-Drug Conjugates", presented by two oncologists and a nurse practitioner, were launched between February 27 to March 14, 2024. Learners could participate in three different ways- 1) attending virtually, 2) watching the replay following the launch of each event, or 3) by watching the enduring (online) highlights following the completion of all three events. Changes in knowledge and competence were measured using pre- and post-test learner analysis. Intent to change practice and potential patient impact were also measured. Data collection is ongoing.
As of June 25, 2024, a total of 784 learners participated in the virtual live setting and 2,752 in the enduring setting, with the leading learner specialties being oncologists (62% in virtual live setting and 44% in enduring setting). A subset of learners (n=65 in virtual live setting and n=460 in enduring setting) completed pre- and post-test assessments. Baseline knowledge was lowest regarding awareness of guideline-recommended sequencing and

safety considerations. Post-education, learners demonstrated a positive change in knowledge/competence related to: clinical profiles of ADCs in ≥2nd line for HER2+ advanced breast cancer (37% in virtual live setting and 41% in enduring setting); optimal sequencing of ADCs (38% in virtual live setting and 32% in enduring setting); and management strategies for common treatment-related side effects (15% in virtual live setting and 42% in enduring setting). Of the learners, about 80% intended to make changes to their practice based on the activity. Based on learners' self-report of the number of patients seen per week, this program potentially impacted 25,437 patient interactions. More than half of the learners also expressed interest in receiving further education related to guideline/evidence updates on ADCs in patients with HER2+ advanced breast cancer. Our interim findings showed that i) CME was effective in improving knowledge and competence among clinicians related to the use of ADCs in ≥2nd line for HER2+ advanced breast cancer and ii) an ongoing need/interest among clinicians to learn more about this topic.

P4-04-06: Connecting Care: Telemedicine Breaks Barriers for Low-Income Brazilians Battling Breast Cancer

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Introduction: Telemedicine stands out as a potential tool to promote equity in healthcare, particularly in low-income communities. By facilitating remote access to health professionals, it might help to overcome economic and geographic hurdles, increasing disadvantaged populations' access to medical care. This approach significantly contributes to a more inclusive, fair, and accessible healthcare system. The impact of implementing a protocol for remote consultations in a socially vulnerable population with breast cancer is particularly relevant, as patients require multiple hospital visits throughout their long treatment journey.
Objective: Evaluate, through the implementation of a protocol for remote consultations, the impact on equity in healthcare access for a low-income population with breast cancer.
Method: This is a prospective cohort study designed to evaluate patients' satisfaction with telemedicine consultation, in a tertiary public cancer center in Brazil. Due to the socioeconomic characteristics and the limited access to high end technology and digital literacy of the study population, all teleconsultations were conducted over the telephone. Sociodemographic data and information regarding patients' transportation to the hospital were collected. Also, a validated Likert-based questionnaire was applied to all participants to assess the quality of care provided. The study was approved by the local ethics committee (CAAE no. 68518523.0.0000.0071), and all participants signed an informed consent form.
Results: Our population consisted of 93 women, with a mean age of 57.3 years, the majority being white (51.6%) and married (35.5%). Over a third of the population (34.3%) did not

complete elementary school and 6.5% had no formal education. Regarding family income and work life, 64.4% had earnings of up to 2 Brazilian minimum wages (620.00 USD), while 25.8% were unemployed. Among those currently employed, 35% did not miss a day of work due to the remote consultations. Almost the entire population had some access to technological resources: 93.5% had internet access, 90.3% owned a mobile phone, and 51.6% had an email address. Regarding transportation to the hospital, 55.8% of individuals used public transportation. On average, approximately US$4 was spent per hospital visit, with expenses potentially exceeding US$20 (3.2%). This amount represents more than 3% of the average family income (2 minimum wages). The approximate travel time to the hospital was 60 to 90 minutes for 19.4% of patients, potentially exceeding 2 hours for 8.6%. As observed, the average distance to the hospital was 12.8 km, with a maximum of 39 km. We had 94 responses from the Likert-based questionnaire from 117 teleconsultations (some patients had more than one consultation, and they could answer a new questionnaire at every teleconsultation), with an average score of 4.65 (maximum of 5). The average Net Promoter Score (NPS) for telephone consultations was 79.8, within the range of excellence (75 to 100).
Conclusion: The implemented remote care protocol appears to be a useful tool, yielding a high level of patient satisfaction. This study also addresses the financial burden of vulnerable patients during its journey to treat breast cancer in Brazil. Therefore, adopting an appropriate remote care protocol is particularly valuable during the challenging journey faced by low-income patients with limited technology literacy who have been diagnosed with breast cancer.

P4-04-07: Harnessing Cognitive Engineering to Understand Breast Cancer Patient Decision Making

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Introduction: As the number of available treatment options for breast cancer increases, decision-making for patients has become complex. Patients often struggle to make decisions as treatment options can vary in terms of short- and long-term side effects, risks of recurrence, and impact on daily life.1 Numerous decision aids have been developed to support patient decision-making.2 However, sustained implementation and use of these tools remains limited. We propose that cognitive engineering approaches, such as naturalistic decision making (NDM), can provide a deeper understanding of how patients make treatment decisions, which can improve the design of decision support tools. Naturalistic decision making (NDM) is a theoretical perspective and methodological approach used to understand how people make decisions in the real world. Originally developed to understand decision-making of expert firefighters during crises, NDM approaches have been used to understand complex decision-making across domains including the military, offshore oil rigs, and healthcare.3,4 In this study, we used an NDM approach, the critical decision method (CDM), to gain an in-depth understanding of how

breast cancer patients make treatment decisions following diagnosis.

Methods: We conducted CDM interviews,5 with breast cancer patients diagnosis in the last 12 years. CDM interviews aim to understand critical or difficult events by unpacking the event using structured probes. One researcher conducted each interview over Zoom. We started each interview by asking the patient to reflect back on the beginning of their cancer journey and what they remember about their diagnosis. We then drew a timeline and asked the patient to relay the different treatments they considered or underwent for breast cancer. We then asked "Can you think of a time during your breast cancer journey when you had to make a difficult decision?" and probed patients about that decision. We continued asking patients about their treatment decision-making as time allowed. Each interview was audio-recorded and transcribed. A researcher and a patient advocate coded each interview and created a decision requirements table,6 which detailed the decisions made by the patient, what made that decision challenging, what strategies and information they used, and what their goals were at the time. We then met to discuss and come to consensus. Once a decision requirements table was created for each transcript, we developed aggregate tables and identified key themes.

Results: We conducted 20 interviews, averaging 57 minutes each; patient age ranged from 42 to 81 years. Patients described an average of 8 decisions that they made following breast cancer diagnosis. Despite many patients facing the same decisions (e.g., mastectomy vs. lumpectomy), we found variability in which decisions were most difficult for patients. We identified 11 categories of difficult decisions for patients including whether to receive chemotherapy, getting genetic testing, stopping a medication due to side effects, and deciding where to receive treatment. Patients reported feeling time pressure and urgency to make treatment decisions and a fear of regretting their decisions. We found that patients' firsthand experiences from friends who had cancer influenced their treatment decision-making. Given the heterogeneous nature of breast cancer treatment, this often presented a barrier to decision-making as patients expected to have the same experience and treatment options as their friends. Patients expressed variable goals when making treatment decisions, which often changed throughout their treatment journey.

Conclusion: In this study, we explored how breast cancer patients made treatment decisions using NDM methods. This cognitive engineering approach revealed intricacies in the decision-making process of patients that will be valueable for improving the design of decision support tools. Next steps include collaborative design with patients to develop a tool that supports the broad spectrum of treatment decisions made across the patient journey.

P4-04-08: Delays from Self-Detection to Treatment of Breast Cancer in the South Fluminense Region: A Cross-Sectional Analysis

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Background: Long waiting times (WTs) to begin treatment after breast cancer (BC) diagnosis have been reported in the Brazilian public health system. However, there is a paucity of data regarding strategies that encompass women that look for primary care units due to self- perceived breast abnormalities up to the point of diagnosis. We have conducted a cross-sectional study to describe WTs from self-perception breast abnormalities to diagnosis and from diagnosis to the initiation of cancer treatment in the South-Fluminense region.
Methods: A retrospective study was conducted by assessing medical reports of all BC patients registered at a High Complexity Oncology Assistance Unit (Unidade de Alta Complexidade em Oncologia, UNACON) from October 2021 to September 2023. Sociodemographic, clinicopathological, and WTs data were collected. WTs were subdivided into categories: 1) Waiting time (WT) from self-perception of breast abnormalities to the first imaging test (WT1); 2) WT from imaging test to core biopsy (WT2); 3) WT from biopsy collection to report release (WT3); 4) WT from biopsy report release to the first medical visit at UNACON (WT4); 5) WT from the first medical visit to the first treatment (WT5); 6) WT from the first imaging test to the first treatment (WT6); and 7) WT from breast biopsy report release to the first breast cancer treatment (WT7). The inclusion criteria required that WT data be recorded. All analyses were conducted using Python version 3.9 and p-values were adopted at 5% level of significance in all tests. The study was approved by Institutional Review Board, Coeps of Fundação Oswaldo Aranha CAAE 79726824.1.0000.5237.
Results: A total of 322 cases were registered at UNACON, with 300 meeting the inclusion criteria. The median age was 59.0 years (95% CI 57.49- 60.51). Among patients, 143 (47.66%) were married, 163 (54.34%) were non-white and 117 (39.00%) were employed. In terms of educational level, 106 patients (35.33%) had an elementary school education, 100 (33.33%) had a high school education, and 28 (9.33%) had a university degree.
The clinical stage at the diagnosis were 20 patients (6.67%) at stage 0 (in situ), 63 (21%) were at stage I, 117 (39%) were at stage II, 75 (25%) were at stage III, and 13 (4.33%) were at stage IV.
The following WTs (median) have been found: WT1 was 59.0 days, (CI 95% 42.0 to 65.0); WT2 was 59.0 days (CI 95% 50.0 - 68.0); WT3 was 13.0 days (CI 95% 12.0 - 14.0); WT4 was 29.0 days (CI 95% 27.0 - 30.0); WT5 was 65 days (CI 95% 58.0 -71.0); WT6 was 185.0 days (CI 95% 174.0 - 202.0) and WT7 was 99.0 days (CI 95% 92.0 - 108.0).
We observed a difference in WT1 between white patients 50 days versus non-white patients 60 days (p=0.04). Additionally, we observed in WT6 a difference of having a high school education 169 days compared to 225 days for those with only an elementary school education (p=0.01).

Conclusion: Timely diagnosis in BC is a critical goal to be reached for public health in Brazilian population. Brazilian law established a 60-day interval to begin treatment after diagnosis, however this interval has been frequently longer, as we demonstrated in our results. Additionally, the WT from self-perception abnormalities to diagnosis is excessively long, meaning that focusing solely on the WT after diagnosis provides an unrealistic view of the overall timeline. Variables such as race and educational level may further exacerbate disparities within the system.
By characterizing delays in diagnosis procedures and treatment beginning in the region, our data highlight weak points in the public health system. To recognize and plan a symptomatic woman directed strategy aligned with offering diagnosis procedures at an only one unit care might short WTs promoting timely diagnosis and treatment for BC. These insights can help local government managers to redirect actions to improve oncological care.

P4-04-09: Supporting the Knowledge Translation of Exercise Recommendations for People with Bone Metastases using Experience-based Co-Design

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Background: Historically, people with bone metastases have been advised to limit exercise due to the risk of adverse events, such as fractures. In 2022, the International Bone Metastases Exercise Working Group published evidence-informed recommendations indicating that regular exercise can enhance physical function and quality of life in these patients, and the risk of skeletal events should be weighed against potential health benefits. Barriers remain to the adoption of these recommendations into clinical practice, including lack of knowledge about how to exercise safely and uncertainty where to find guidance for exercise advice. This project aims to collaborate with knowledge users (KUs) through an experience-based co-design (EBCD) process to develop knowledge translation (KT) products and a dissemination plan to facilitate the uptake of exercise recommendations for people with bone metastases.
Methods: Three KU groups were engaged using EBCD: 1) patient/family partners; 2) oncology healthcare providers (physicians, nurses, allied health professionals); and 3) community-based exercise professionals (physiotherapists, exercise physiologists). Through facilitated meetings, ideas for KT products were generated, prioritized, and co-created, and a dissemination and evaluation plan was developed. A survey of exercise professionals was used to collect further information on professional development (PD) preferences.
Results: Twenty-nine KUs participated in four engagement sessions (n = 10 patient/family partners, n = 9 healthcare providers, and n = 10 exercise professionals). The prioritized KT

products for development were: 1) a patient education handout; 2) patient education videos; 3) a health information form (HIF); and 4) professional development (PD) materials for exercise professionals and healthcare providers. The HIF was designed to easily collect pertinent health and oncology-related patient information that would be necessary for an exercise professional when developing exercise prescription/recommendations. A total of 183 international exercise professionals completed the PD survey, with 80% indicating that more PD resources were needed for bone metastases, focusing on exercise safety, feasibility, and prescription considerations. Over 80% of respondents found the purpose of the HIF form was easy to understand and over 75% rated the content as excellent or very good. Our dissemination and evaluation plan included a combination of targeted social media, direct connections with key organizations, presentations, and promotion through the Canadian Cancer Society and its channels. All these products are hosted on the Bone Metastases & Exercise (BMD) Hub (bit.ly/BMEhub). In the first 30 days post-launch, the BME Hub had 5,243 views from 2,407 unique users. Full engagement results will be available at the time of conference.
Discussion & Conclusion: Collaborating with KUs through EBCD has enabled the development of diverse KT products that are more likely to be useful and acceptable to the intended audience than those designed by researchers alone. Thus far, use of the Hub has been promising; further evaluation of reach, usage, and partnership indicators of the KT products and dissemination plan will be evaluated.

P4-04-10: Improving early breast cancer care coordination - Digital care pathway: Implementation and Pivotal Results

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Introduction: Care coordination has no consensus definition. A systematic review (McDonald KM.) purposed a broad definition: "Care coordination is the deliberate organization of patient care activities between two or more participants (including the patient) involved in a patient's care to facilitate the appropriate delivery of health care services. Organizing care involves the marshalling of personnel and other resources needed to carry out all required patient care activities and is often managed by the exchange of information among participants responsible for different aspects of care." In 2007, Vanhaecht et al. defined the term ‘care pathway’ or ‘pathway’ as follows as “A care pathway is a complex intervention for the mutual decision-making and organisation of care processes for a well-defined group of patients during a well-defined period. The aim of a care pathway is to enhance the quality of care across the continuum by improving risk-adjusted patient outcomes, promoting patient safety, increasing patient satisfaction, and optimizing the use of resources.”
Methods: In 2023 a non-fixed time but phase-oriented Early Breast Cancer Care Pathway was developed in the Breast Cancer Clinic of a University Hospital. In the first phase,

interviews with breast cancer experts (medical oncologist, breast cancer surgeons, nurses, pathologist) were performed. The model was developed and the experts were requested to validate the pathway. The second phase, was testing the developed model and characterized patients (pts) included prospectively in the first 6 months, 26thOct 2023 to 30th April 2024. Time to treatment initiation (TTI) was defined as days (d) from diagnosis (using the most recent tumor board as a surrogate for diagnosis) to first treatment (surgery or systemic therapy).
Results: During 2023, using UpHill's® software it was possible to provide seamless patient journeys with automation and patient communication. Professionals can access a shared history of all actions executed for each patient as well as the expected next steps permitting a fast recognition of patient current status and forecast future needs. Go live was 26th October 2023. Here we report the first 6 months experience. The digital pathway permits real time data collection in an automatic way. 593 pts entered the pathway with a breast cancer sign/symptom warranting further investigation, of these 162 were discussed at least once in a tumor board. In 62 pts a surgery was performed and 27 pts started treatment at the Oncology Day Care Unit. In predefined key points UpHill's® software interprets the information sent by pts to automate decisions (post-surgery and post medical oncology day care session). Median TTI was 32d, 14d and 34d for medical treatment and surgery, respectively. There were 88 automatic generated interactions that resulted in 62 pts responses (70,5% response rate, rr); post-surgery 55 automatic generated interactions that resulted in 35 responses (63,6% rr); post medical oncology day care session 16 automatic generated interactions that resulted in 14 responses (87,5% rr).
Conclusion: There is the need to ensure Health systems to simplify access and reduce operational barriers to initiating treatment. Studies of interventions to simplify access, ease navigation and reduce barriers are warranted to diminish the potential harm to pts. Real time automatic data collection has the advantage to provide high quality data with minimal human effort. Automate actions eliminates repetitive tasks permitting focus on actions that truly depend on human reasoning. Clinical pathways are one of the main methodologies to organize and coordinate care processes but the methodology needs to be further improved. It is a complex intervention and our model needs to be developed and continuously followed up.

P4-04-11: Managing Wait Time Between Surgery and Treatment Initiation Plan in Patients with Breast Cancer: a Mixed-method Approach

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Oncotype DX test (ODX) is a genomic testing that is considered a standard of care requirement for a subset of breast cancer (BC) patients, meeting specific histopathological criteria, facilitating informed decisions regarding their personalized systemic treatment. However, the conventional process for ordering this test often delays the initiation of systemic therapy, as it was only ordered after the first Medical Oncology Consultation. This study evaluates a novel initiative implemented at a medium-sized cancer center providing

care for rural and underserved communities in Nova Scotia-Canada. The initiative aimed at minimizing the time interval between surgery and treatment onset, through a phone call by the Medical Oncologist (MO) directly engaging the patient, explaining the test and treatment options, securing their consent for immediate ordering. Employing a mixed-method design, this study assessed wait times at various intervals along the patient's journey from surgery to treatment initiation. Qualitatively, semi-structured phone interviews were conducted with purposefully selected 11 breast cancer patients to explore their experiences and satisfaction with the MO's communication regarding the ODX test, focusing on achieving patient-centred care and alleviating distress during the waiting period.
Results reveal that 180 breast cancer patients underwent ODX testing between July 2018 and July 2022, 55% via the standard pathway and 45% through the new intervention. Median wait time from surgery to ODX result availability decreased significantly from 60 [IQR: 49-70] to 46 [IQR: 31-91] days (p-value < 0.001). While the median wait time from MO referral to chemotherapy onset decreased from 50 [IQR: 40-82] to 43 [IQR: 40-63] days, it was not statistically significant (p-value = 0.5). However, there was a notable two-week reduction in median wait time from referral to radiation therapy initiation (p-value = 0.09). Importantly, the median wait time from the initial consultation with the MO to the chemotherapy consent date decreased from 24 to 0 days (p-value < 0.001), indicating immediate consent for chemotherapy treatment during the first consultation. Qualitative findings underscored patient satisfaction with the new intervention, particularly appreciating the clear and mindful information provided by the MO, even through remote discussion. In conclusion, this intervention effectively reduced the interval between surgery and ODX results, facilitating expedited treatment initiation and enabling informed decision-making at the time of the initial consultation. Using plain language during remote discussions further enhanced patient satisfaction and understanding of treatment options, emphasizing the significance of effective communication in patient care. Using this intervention can also enhance ODX utilization in rural communities.

P4-04-12: Covid 19 pandemic: effects on the diagnosis and treatment of breast cancer in two large teaching hospitals in the city of São Paulo - Brazil

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Objective: To evaluate the impact of the COVID 19 pandemic on the diagnosis and treatment of breast cancer in São Paulo - Brazil. Methods: Observational, analytical, cross-sectional, retrospective study, in which patients treated at the Mastology Outpatient Clinic of the Escola Paulista e Medicina - Universidade Federal de São Paulo (EPM-UNIFESP) and the Mastology Outpatient Clinic of Hospital Santa Marcelina Itaquera with diagnosis of breast carcinoma, from March to December 2019 and from March to December 2020. Results: In this study, information from 361 patients was considered. time from diagnosis to the start of radiotherapy () and endocrine therapy (p=0.019) between the two periods. In the pre-

pandemic period, a higher percentage of patients with tumors smaller than 2 cm (T1) was observed (30.2% versus 19.1%; p=0.019); on the other hand, during the Covid 19 pandemic period, there were higher percentages of patients with tumors larger than 5 cm (T3) (17.7% versus 8.7%; p=0.019). The presence of massively compromised axillary lymph nodes (N2) was greater during the pandemic period (8.0% versus 14.3%; p = 0.288). Reconstructive surgeries were less common during the pandemic. Endocrinotherapy was more indicated in the pre-pandemic period (60.2.6% versus 32.0%; p<0.001), as well as radiotherapy in the conventional form (56.0% versus 29.0%; (p <0.001) as in hypofractionated (13.0% versus 3.2%; (p<0.001). Neoadjuvant chemotherapy was more frequent during the Covid 19 period, that is 38.3% versus 25.7% (p< 0.038). The time between diagnosis and the start of treatment in the pre-pandemic period was 139.8 ± 109.2 days, with a median of 94 days (23.0 - 596.0 days) and in the pandemic, 162.7 ± 112.5 days and the median was 165.0 days (16.0 - 544.00 days); with a median of 388.0 days and during the pandemic, 249.5 days, with a median of 274.0 days (p<0.001). In relation to the start of chemotherapy, an average of 378.2 days was observed. pre-pandemic period and 327.5 days during the pandemic, with medians of 373.0 days and 311.5 days, respectively. In relation to the start of endocrine therapy, we found an average of 239.9 days and 182.0 days. median 160.3 and 113.0 days, respectively when comparing the pre-pandemic and during the pandemic periods. Conclusions: The Covid 19 pandemic had an impact on the diagnosis of breast cancer, a fact evidenced when we observed the highest rate of clinically advanced tumors and clinically positive axillary lymph nodes, causing a direct influence on the prognosis and treatment of patients. At the same time, due to the possible occupancy of hospital beds, it influenced therapeutic options, such as a reduction in surgeries using oncoplastic techniques and neoadjuvant chemotherapy, but allowed for a shorter time interval between diagnosis and the start of radiotherapy and adjuvant endocrine therapy. or neoadjuvant, the latter being a new therapeutic possibility

P4-04-13: Single-center Relative and Cause-Specific Survival in Breast Cancer

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Background: In cancer, cause-specific survival (CSS) and relative survival (RS) aim to quantify net survival but both estimates differ due to differences present in both methodologies. CSS may be miscalculated if cause of death is misinterpreted while RS requires accurate life tables for the background population. Such differences in survival outcome might be important when data are used to allocate health care decisions. We compared RS to CCS in breast cancer patients recently treated with contemporary therapies in our center.

Methods: We included consecutive patients, all stages, all tumor types, with an unilateral invasive breast cancer diagnosis, assigned to UZ Leuven on the basis of main treatment during the period 2014-2018. Local and systemic therapies were given according to institutional guidelines. Using medical files, we verified the vital status until March 1st, 2022; if a patient died, the cause of death was verified. We used unadjusted data as 'case mix' adjustment is not needed when considering single-center outcome results. The 5 year unadjusted RS was calculated as the overall observed survival for patients with a given cancer diagnosis divided by the expected survival of a similar group of patients from the general population using the Ederer II method. The cumulative incidence function was used for estimating the unadjusted 5 year BCSS. Analyses have been performed using SAS software (version 9.4 of the SAS system for Windows).
Results: For the period 2014-2018, 2326 patients (median (IQR) age 59yr (50-70)) were included in the analysis. At diagnosis, 6.4% were metastatic and 46.4% had a very early pathologic stage; 3.6% (y)p 0 and 42.8% (y)p IA-B. 89.8% were operated, 73.7% primary and 16.1 after neo-adjuvant therapy; 27% and 82.8% received adjuvant chemo- and radiotherapy respectively. Cause of death was unknown in 10 patients. On March 2022, 7.7% died of breast cancer and 87.4% were alive. The 5-yr unadjusted breast CSS was 92.7% (95% CI: [91.5, 93.7%]) and the 5-yr unadjusted RS was 95.4% (95% CI: [93.8,96.8%]). This concludes in our series a 58% lower breast cancer mortality using the 5-yr RS (4.6%) as compared to the 5-yr BCSS (7.3%) based on medical files.
Conclusion: Using different approaches to estimate the 5 year net survival in our series confirmed a difference between RS and BCSS. We confirm that cancers like breast cancer with available early screening policies and a high survival have better RS than CSS.

P4-04-14: Economic analysis of germline genetic testing to assess for hereditary breast cancer: a systematic review

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Introduction: Germline genetic testing (GGT) is included in clinical guidelines and has steadily increased in use to inform treatment decisions for breast cancer patients and those with genetic predisposition. The expanding implementation has magnified the influence of GGT on the economics of breast cancer care worldwide. The objective of this systematic review was to evaluate the economic impact of GGT among adults diagnosed with breast cancer, those at increased risk of breast cancer, and in the general population.
Methods: This analysis was part of a broader study following PRISMA methodology. PubMed-Medline and Embase were searched for manuscripts published after January 2013 that reported the clinical, economic, and humanistic outcomes of GGT in patients with breast cancer and those at risk of breast cancer. The present analysis summarizes a subset of articles specifically addressing the economic value of GGT.
Results: The initial search, inclusive of all outcomes, identified 10,198 studies. Of the 231 articles extracted for data analysis, 30 studies evaluated economic outcomes. Twenty-four

of these were cost-effectiveness analyses related to testing for hereditary breast cancer; the remaining six studies were cost comparison analyses or budget impact models. Of the 24 cost-effectiveness studies, five were multinational and evaluated the cost-effectiveness of GGT in 12 countries (US, UK, Germany, Canada, Norway, Netherlands, Spain, Israel, India, Brazil, China, and Malaysia). The US (n=10) and the UK (n=7) were studied most frequently; however, there was notable heterogeneity derived from different international healthcare systems and varying patient populations. The studies modeled the effect of testing patients diagnosed with breast cancer (n=7) or breast/ovarian cancer (n=1), testing individuals with an increased risk of breast cancer (n=6), and population-specific genetic screening (n=10). Both single-syndrome (BRCA1/2; n=17) and multigene testing strategies (n=7) were investigated. Thirteen studies employed a Markov model. Close to half of the studies (n=11/24) considered cascade testing, and almost all (n=22/24) considered risk-reducing surgery (breast and/or ovarian). All studies were analyzed from a payer perspective; six used both payer and societal perspectives. Studies evaluated cost-effectiveness against various willingness-to-pay (WTP) thresholds. Each study found at least one cost-effective strategy for all countries and perspectives. Four studies presented GGT strategies that were cost-saving.
Conclusions: This analysis presents the economic impact of GGT for hereditary breast cancer syndromes with global representation and a range of testing strategies. In all settings, the analyses found GGT to be cost-effective, supporting the positive economic impact of GGT for hereditary breast cancer. Opportunities for further exploration include the added value of cascade testing and use of multigene panels, as well as the evaluation of economic impact from the patient perspective.

P4-04-15: Oncofertility and Breast Cancer: An Analysis of Perception and Practices Among Specialists

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Introduction: Worldwide, breast cancer is considered the most common cancer among women, with advanced treatments improving survival chances. In this context, the importance of preserving fertility stands out, seen as an influencing aspect in the post-treatment quality of life for patients in reproductive age. This study aims to understand the perception of specialist doctors about oncofertility, addressing the barriers for its implementation and identifying areas for improvement. Methodology: This was an observational study, wich used an electronic questionnaire developed from literature review. The questionnaire was distributed to oncologists and breast specialists active in Brazil, through online platforms, aiming to evaluate knowledge about oncofertility, current practices, and barriers faced in preserving the fertility of breast cancer patients.
Results: The questionnaire on oncofertility was answered by 276 Brazilian doctors, with a majority of 67.4% women and 63.3% doctors under 40 years old. The majority of them (78.6%) were specialists, with 70.7% of clinical oncologists, and 33.7% of doctors working

in the oncology area for more than 10 years. Most doctors worked in the Southeast region (70.7%) and 52.9% worked in both public and private oncology centers. The patient's gestational desire was the main incentive to discuss fertility (43.8%). Regarding knowledge in oncofertility, 77.9% of doctors felt well informed about the subject, with specialists (81.6%) outnumbering residents (64.4%) in this aspect. Around 68.7% of the specialists stated that they usually consulted international guidelines related to the topic. Regarding fertility preservation practices, such as ovarian suppression with GnRHa and cryopreservation, 72.4% of specialists stated that they had knowledge on the subject, and 82.6% considered cryopreservation as the most effective method. In their practice, 77.9% of the interviewees reported addressing oncofertility, a fact that was more common among specialists (83.4%) compared to residents (57.6%). Among those who did not address it, 61 participants, the main barrier was the lack of access to fertilization techniques in public health (80.3%). The majority of specialists (86.6%) were not resistant to procedures that delay chemotherapy, and 98.9% denied personal barriers against fertility preservation. The cost of fertility preservation practices was considered high by 62.0% of the doctors, especially among residents (74.6%) versus specialists (58.5%). The structural distance from fertilization centers was considered a barrier for 24.3% of doctors. Regarding recommendations for young patients with gestational desire, 63.0% suggested ovarian suppression and/or cryopreservation as a possible modality of fertility preservation. Controlled ovarian stimulation was considered safe for all patients by 56.2% of the experts. Regarding the use of ovarian suppression with GnRH during chemotherapy, 70% of specialists described its use in practice. The majority of professionals (69.2%) did not consider pregnancy after cancer as a factor that increases the risk of recurrence, while 68.5% judged as safe the temporary interruption of endocrine treatment. Furthermore, 84.4% considered assisted reproduction techniques as safe options as well. Significant differences about the perception of pregnancy after cancer could be seen between residents and specialists. Among the experts, breast specialists and oncologists diverged in the safety of ovarian stimulation and in the indication for ovarian suppression. Opinions varied minimally by region of activity, highlighting the uniformity in the general view about treating oncofertility. Discussion: The results indicate a growing awareness about the importance of oncofertility, although there are significant challenges related to access and professional education. The majority of the interviewees showed knowledge about oncofertility, but there was a significant difference in knowledge between specialists and residents, indicating the need to integrate this topic into medical training curricula. Cryopreservation was considered the most effective method of fertility preservation, but challenges in its applicability include the structural distance from specialized fertilization centers and the lack of knowledge about current available techniques, especially in less developed regions. Although most professionals were willing to discuss fertility preservation strategies in their clinical practices, there were significant barriers, such as cost and the lack of adequate infrastructure. The study also highlights the importance of patients' gestational desire as a motivating factor for addressing fertility during oncological treatment. The research reveals the need to improve communication about fertility risks and available preservation options, in addition to addressing challenges related to cost and

access to knowledge in oncofertility, suggesting that targeted educational strategies are necessary to promote informed and uniform practices. Conclusion This study highlights the importance of oncofertility in clinical practice for young patients with breast cancer, identifying significant barriers that need to be overcome in an underdeveloped country. Greater emphasis on physician education and improved access to fertility preservation techniques is recommended.

P4-04-17: TFX06 shows remarkable safety and extracranial and intracranial efficacy in patients with ER+ /HER2- breast cancer in a phase 1 study

Charles Ding, Jian Zhang, Wenxing Qin, Yang Chen, Zhiye Zhang, Yuping Sun, Hongqiang Guo, Yehui Shi, Zhaojian Fu, Jia Song, Douglas D. Fang, Wei Sha

Background: TFX06, a third-generation oral CERAN/SERD, demonstrated favorable safety and PK profile, as well as encouraging antitumor activities both extracranially and intracranially in patients with ER+/HER2- breast cancer (Fu Z, et al., 2023 SABCS annual meeting). A multicenter Phase 1a/b study (CTR20230789) is currently recruiting in China to further evaluate PK, safety and efficacy in previously treated patients with ER+/HER2- locally advanced and/or metastatic breast cancer.
Patients and Methods:
TFX06 tablets were orally administered once daily (QD) at 60/100/150/200 mg in dose escalation cohorts and recommended dose for expansion (150mg, QD) was used in dose expansion study every 28 days of treatment cycle until disease progression or intolerable toxicity. As of the cutoff date, this Phase 1 study enrolled 24 patients at median age of 60 years (range 34-74) with a ECOG score of 0-1. Eleven out of 24 patients (45.8%) carried estrogen receptor 1 (ESR1) mutations (ctDNA). 77% of patients enrolled had visceral metastasis diseases in liver, lung or both, and 11% of patients had brain metastasis. Median number of prior endocrine therapies were 2 (1-4) including fulvestrant (33.3%), CDK4/6i (54.1%) and combination of both (29.1%) and a median number of chemotherapies was 2 (0-3). Tumor assessments by investigator according to RECIST v1.1 were performed every eight weeks.
Results: All 24 patients in this study completed dose-limiting toxicity (DLT) observation period, and no DLT was observed. TFX06 was well tolerated. Treatment-related AEs experienced in >10% of patients were hypertriglyceridemia (25.6%), anemia (20.1%), AST elevation (20.1%), blood lactate dehydrogenase increase (16.3%), and ALT increase (14.0%), all of which were Grade 1/2. Photopsia and gastrointestinal toxicities seen in other SERD studies were not observed in this study across all dose cohorts. Out of 24 response evaluable patients, 4 confirmed partial responses and ORR was 16.7%, CBR was 42%. Of three patients with brain metastases showed reduction of brain metastases greater than 70% and one of the three had disease progression with PFS of 9 months. The other two are still in treatment with their PFS at least 8 months. Exposure data (Cmax and AUC) of TFX06

in patients showed a linear relationship with doses.
Conclusions: Based on the preliminary results, 150 mg QD dose expansions are ongoing. TFX06 demonstrated rather favorable safety profile and compelling antitumor activity for those extensively treated patients. Durable intracranial antitumor activities were also observed in all 3 patients enrolled with brain metastasis.

P4-04-18: AI based Thermalytix for management of Breast Pain in Primary Care

Siva Teja Kakileti, Geetha Manjunath

Introduction: Breast pain is the most common breast complaint presented to general practitioners (GPs) and the leading cause of referrals to breast units. Although the correlation between breast pain and breast cancer is low, the high volume of these referrals often results in over-investigation, potentially delaying the diagnosis and treatment of women with actual breast cancer. Thermalytix is an artificial intelligence-based breast imaging tool that uses advanced machine learning over high resolution thermal scans to generate a breast cancer risk score (B-Score). In this study, we assess whether Thermalytix can be utilized to effectively manage patients presenting with breast pain, thereby reducing subjectivity in referrals to upstream diagnostic pathways.
Methodology: This is a post-hoc analysis of data from prior clinical studies of Thermalytix, involving a total of 1187 women. The reference standard was the final diagnosis obtained from the available reports of mammography, ultrasound, and biopsy. Exclusion criteria included women below 18 years of age, women with a history of breast cancer, and those pregnant or lactating. For this analysis, women presenting with breast pain alone as a complaint was used as inclusion criterion. The performance of Thermalytix was evaluated within this subset of the population using B-Score for triaging. Further, we estimated the optimal criterion for vascular score (an output generated by Thermalytix to characterize the vascular asymmetry in the breasts) for improving B-score computation of Thermalytix using a receiver operating characteristic curve.
Results: Of the 1187 women, 157 (13.2%) women had reported pain alone as a complaint. Of these 157 women, 13 women were diagnosed with breast cancer. Thermalytix resulted in a sensitivity, specificity, NPV and PPV of 76.9%, 85.4%, 97.6%, and 32.3% respectively, using B-Score. When the vascularity criterion is set to the optimal point at Youden's Index, the sensitivity, specificity, NPV and PPV were 92.3%, 80.6%, 99.1% and 30%, respectively.
Conclusion: Currently, management of women presenting with breast pain complaints in primary care requires clinical judgement towards a 2 week wait time. In this paper, we present a non-invasive imaging tool called Thermalytix that can be used for triaging women with breast pain in an objective manner.
Acknowledgements: We would like to thank Dr. Deepak Kumar and Dr. Sanjiv Ahluwalia for their valuable discussions and feedback on this study.

P4-04-19: Characterizing Nanomechanical Properties of Skin and Soft Tissue in Postmastectomy Implant Reconstruction

Papa Diogop Ndiaye, Mark Victor Schaverien, Victor Joseph Hassid, Paul L. Shay, John William Shuck, Mark Warren Clemens, Jessie Zexi Yu, Austin Y Ha, Gregory Reece, Margaret Jane Roubaud, Alexander Francis Mericli, Mark Villa, Ashleigh M. Francis, David Matthew Adelman, Donald Baumann, Joani Marie Christensen, Ryan Dickey, Philip Hanwright, Sahil Kuldip Kapur, Jonathon Bryce Edward Olenczak, Aysegul A Sahin, Edna Paredes, Ahmed Jizawi, Gitika Srivastava, Tia Darwich, Soufian Lasli, Jacqueline Tilley, Tobias Appenzeller, Sara Nizzero, Marko Loparic, Marija Plodinec, Smith,Benjamin

Background: Implant-based postmastectomy breast reconstruction carries a significant risk of complications, including an aproximately 10% rate of implant loss following postmastectomy radiation therapy. While prior radiation, diabetes, and obesity are recognized risk factors, the impact of the quality of skin and soft tissue on these outcomes is not well understood. Currently, no biomarkers are available to guide decisions between implant-based and autologous tissue reconstruction. Mechanical alterations of skin tissue have been shown to be a hallmark of inflammation and thus we hypothesize that detection of this in standard of care samples may provide an early assessment for failure risk.
ObjectiveThis study aims to characterize the nanomechanical properties of skin and soft tissue in patients undergoing postmastectomy implant reconstruction, and evaluate correlations between these properties and clinical outcomes, including implant loss and patient-reported quality of life. The overall goal is to detect the nanomechanical changes that are predictive of implant failure in breast cancer mastectomy reconstruction surgery patients.
Methods: This data comes from 36 evaluable study subjects from an on-going single center prospective observational study currently taking place in MDACC (NCT06584396), enrolling female patients post- prevention or curative mastectomy, who elected postmastectomy breast reconstruction. Part of the fibrous capsule resection (often containing scar) removed during the breast implantation replacement surgery is analyzed using the ART-1 Nanomechanical Phenotype System, which provides a spatial nanomechanical characterization of clinical samples. For each sample, an average of 15 maps is measured, with 400 indentation points per map, totaling 6000 data points per sample. Clinical and patient reported outcomes are being collected through chart review and a validated BREAST-Q assessment for quality of life. Tissues are further annotated by pathologists and the nanomechanical signature of each sample is correlated to the presence and pathological features of scars.
Results and Conclusions: Overall the nanomechanical signature of these samples presents distinguishing features when compared to other studies such as breast cancer and normal breast tissue. While the nanomechanical signature of breast tumor is typically multi-peak and heterogeneous, the stiffness of the skin/scar tissues presents a single tail corresponding to a continuously heterogeneous stroma with stiffer profiles compared to normal breast and breast cancer tissues. The adhesion energy channel also presents a single asymmetric population with a much narrower distribution when compared to both normal

breast tissue and breast cancer tissue. The dissipation profile is focused at very low values, whereas both normal breast and breast cancer tissue present much higher dissipation values.
As we continue to enroll for this on-going study, we are compiling interim outcome evaluation of surgical outcome. Overall, this provides a baseline characterization of reconstruction tissues which supports the further development of Nanomechanics-based biomarkers for reconstruction failure/success prediction in postmastectomy settings.

P4-04-20: Contrast-enhanced mammography-guided biopsy enhances early detection of breast cancer through precise target selection in extensive suspicious microcalcification diagnosed in screening mammography

Wai-Shan Chung, Yun-Chung Cheung, Chi-Chang Yu, Shin-Cheh Chen, Tai-Ming Ko

Background: Contrast-enhanced mammography (CEM) is a modern technique that can reveal a relevant malignant lesion through contract enhancement in the mammography image. In a background of extensive microcalcifications of mammography, contrast-enhanced mammography-guided biopsy (CEM-Bx) can help locate the suspicious microcalcification for targeted sampling for an early diagnosis of breast cancer. The diagnostic outcome of CEM-Bx has not yet been reported in previous literature.
Purpose:
Our study aimed to discover the clinical utility of CEM-Bx for the diagnosis of breast cancer in female patients with extensive suspicious microcalcifications reported in screening mammography.
Materials and Methods: This is a prospective study that included female candidates with extensive suspicious microcalcifications shown in screening-base mammography. Among all the included candidates, no mass lesions were noted in physical examination, sonography, or mammography. Then, they received a CEM image after screening mammography recalled. Candidates without enhancement in CEM underwent conventional mammography-guided (MG-Bx) biopsy for suspicious microcalcification as the standard-of-care group. On the other hand, candidates with enhancement in CEM underwent CEM-Bx as the study group. Wilcoxon rank-sum test was employed to analyze the outcome of CEM-Bx to MG-Bx for cancer diagnostic rate (CDR). This project was funded by the National Science and Technology Council of R.O.C [MOST 111-2314-B-182A-047] and approved by the institutional review board of Chang Gung Memorial Hospital (202102180A3).

Results: A total of 61 female candidates with extensive suspicious microcalcification noted in their screening mammography were included in this study from November 2021 to November 2023. Twenty-six candidates received CEM-Bx, while thirty-five candidates received MG-Bx. There was no significant difference in the presentation of microcalcification morphology or distribution in the CEM-Bx and MG-Bx groups. CEM-Bx

with associated enhancements were diagnosed as cancer in 73.08% (n=19/26) of the study group, and no cancers were diagnosed in the standard-of-care group without associated enhancement. The overall CDR of CEM-Bx and MG-Bx was 31.4% and 20%, respectively. There was a significant difference in CDRs between CEM-bx and MG-bx. The CDR of CEM-bx was 81.8% in regional microcalcifications, while 66.7% in segmental or diffuse distributions was 66.7%.

Conclusion: The cancer diagnostic rate is significantly higher by CEM-Bx with associated enhancement than MG-Bx without enhancement. Contrast-enhanced mammography-guided biopsy technique should be considered as a solution for the target selection in patients with extensive suspicious microcalcifications to further enhance the early detection of breast cancer in population-based screening mammography.

P4-04-21: Frozen gloves can prevent chemotherapy-induced nail pigmentation of the hand in early-stage breast cancer patients: a prospective self-matched case-control study

Fei Xu, Kuikui Jiang, Jianwen Chen, Junping Zhang, Moke Deng, Simei Shi, Qiulian Lin, Shusen Wang, Hanmu Chen

Background: Nail pigmentation occurs in approximately 90% of patients treated with anthracyclines, cyclophosphamide, and taxanes, but approaches to prevent the melanonychia were absent. We aimed to evaluate the efficacy of frozen glove (FG) treatment for the prevention of the nail pigmentation in early-stage breast cancer patients. Methods: This was a prospective, phase II, single-center, self-matched case-control study in early-stage breast cancer patients who planned to receive anthracyclines, cyclophosphamide or taxanes. The primary end point was the incidence of severe nail pigmentation and the other end points included the degree of nail pigmentation, the incidence of nail pigmentation, the onset time of nail pigmentation, other cutaneous toxicity, neurotoxicity, and patient comfort. Due to the lack of NCI-CTCAE standard for melanonychia, we evaluated it by a scoring system based on the area (0 for no change, 1 for less than 1/3 , 2 for 1/3 to 2/3, and 3 for more than 2/3 of the nail plate) and the color (0 for no change, 1 for light, 2 for gray, and 3 for black) of nail pigmentation. Severe nail pigmentation was defined as area score plus color score ≥5. Two investigators independently examined the nails of participants and scored the most severe nail. The patient wore a FG on one hand from 30 minutes before chemotherapy until 30 minutes after chemotherapy, and the other hand remained unprotected as the control.

Results: Between July 2022 and June 2023, 42 early-stage breast cancer patients were enrolled. The median follow-up was 13.7 months (IQR: 11.7-16.7). The median number of chemotherapy cycles was 6 (IQR: 4-8). Treatment with a single chemotherapy course was associated with higher risk for nail pigmentation and severe nail pigmentation, with odds

ratios of 43.21 (95% CI: 14.50 to 128.76, p<0.01) and 2.82 (95% CI: 2.16 to 3.68, p<0.01), respectively. Thought there was no significant difference in the incidence of pigmentation between FG-protected hands and control hands, the area score and the total score of the FG-protected hands were significantly lower than those of the control hand ($p < 0.005$) from the third FG treatment. Additionally, the color score of the FG-protected hands was significantly decreased from the fourth FG treatment ($p < 0.005$). The rate of severe nail pigmentation was significantly lower in FG-protected group compared with that of control group at the eighth FG treatment (42.11% vs 73.68%, p=0.049). The odds ratio for severe nail pigmentation in the FG group compared to the control group is 0.34 (95% CI: 0.16 to 0.71, p<0.01). The median onset time of nail pigmentation of FG-protected group and control group was 39 days and 30 days after the initiation of chemotherapy, respectively, with no significant difference. There was no significant difference in other skin toxicity and neurotoxicity between two groups. Only one participant was dissatisfied with the FG treatment, and no patient withdrew from this study due to cold intolerance.

Conclusion: FG significantly reduces the nail pigmentation induced by anthracyclines, cyclophosphamide, and taxanes in early-stage breast cancer. Our study provides an effective and well-tolerated method to prevent melanonychia and may improve patients' quality of life.

P4-04-22: Impact of screening mammography on breast cancer outcomes in women aged 80 and over

Siu-Yuan Huang, Makaelah Murray, Angelique Rubio, Nneoma Okoro, Mina Sedrak, Nicholas Jackson, Mediget Teshome, Susan A. McCloskey, Nimmi S. Kapoor

Introduction: While the risk of breast cancer increases with age, screening mammography guidelines are lacking for women of advanced age and often factor in life expectancy estimates. We sought to identify differences in outcomes among newly diagnosed breast cancer patients aged 80 or older who were undergoing routine screening mammography versus those who were not screened.
Methods: 249 patients who were diagnosed with breast cancer at age 80 or older at a single institution from 2013-2020 were identified retrospectively. Patients with a previous diagnosis of breast cancer or those with incomplete records were excluded. Data was collected on patient demographics, comorbidities, timing of mammography, symptoms, tumor characteristics, treatment, and outcomes. Characteristics of the screened group were compared to the unscreened group with Chi-square and t tests. Kaplan-Meier survival analysis and log-rank testing was done to compare overall survival and disease-free survival.
Results: Among 177 patients with breast cancer included, 77 had mammographic screening within 2 years of diagnosis, and 100 were un-screened. Mean age at breast cancer diagnosis was 83 (80-98), and the two groups did not significantly differ in race/ethnicity, Area

Deprivation Index, tumor receptor status, nodal sampling, or receipt of endocrine therapy, adjuvant chemotherapy, or radiation. Compared to the screened group, the un-screened group was more likely to have invasive disease ($p = 0.009$), larger tumors (2.9 cm vs 1.6 cm, $p < 0.001$), higher grade tumors ($p < 0.001$), and more advanced stage ($p < 0.001$). More patients in the screened group underwent breast conserving surgery, while more patients in the un-screened group either had mastectomy or did not undergo surgery (both $p = 0.001$), and more un-screened patients received neoadjuvant chemotherapy ($p = 0.003$). The screened group had a significant benefit in disease-free survival (DFS) (HR 0.553, 95% CI, 0.380-0.802, $p = 0.002$) and overall survival (HR 0.427, 95% CI, 0.206-0.887, $p = 0.018$). Conclusions: Patients aged 80 or older with breast cancer who were undergoing screening mammography had significantly improved disease-free survival and overall survival compared to those with breast cancer who were not screened. This suggests that the benefit of screening mammography persists in advanced aged women and updated breast cancer screening guidelines are needed.

P4-04-23: Reverse-sequence Endoscopic Versus Conventional Open Nipple-Sparing Mastectomy with Implant-based Breast Reconstruction: A Single-center Prospective Cohort Study (RECO study)

Tianyuan Li, Qing Zhang, Yanyan Xie, Huanzuo Yang, Kawun Chung, Hui Dai, Xiaoman Cao, Zhenggui Du

Background: Minimal-access nipple-sparing mastectomy (NSM), including endoscopic- or robotic-assisted NSM, is increasingly used in the treatment of patients with breast cancer. However, traditional minimal-access NSM is time-consuming, costly, and has a steep learning curve, hindering its widespread adoption. Our team pioneered the “reverse-sequence” endoscopic nipple-sparing mastectomy(R-E-NSM), which addresses the challenges identified in previous surgeries. This study aimed to compare the clinical outcomes of R-E-NSM with implant-based breast reconstruction (IBR) and conventional open nipple-sparing mastectomy(C-O-NSM) with IBR.
Methods: In this prospective, single-center, non-randomized study, we consecutively recruited women aged 18 years or older who underwent NSM with IBR for breast cancer or risk reduction at the West China Hospital of Sichuan University from March 2021 to March 2024. Data about patients’ demographics and operative, oncological, complication details, and cosmetic outcomes were collected before and after surgery. We conducted a prespecified risk-factor analysis using mixed-effect regression to investigate the relationship between surgery type and incidence of complication. The study is registered on the China Clinical Trials Network, and the trial registration number is ChiCTR2100047862.
Results: A total of 447 patients were enrolled in this study, including 336 (75.2%) patients who received R-E-NSM with IBR and 111 patients (24.8%) who underwent C-O-NSM with IBR. The median follow-up time was 24.33 (7.27-42.07) months in the R-E-NSM with IBR group and 27.00 (7.70-42.53) months in the C-O-NSM with IBR group, respectively. The unilateral operation time of patients in the R-E-NSM with IBR group was not longer than

that in the C-O-NSM with IBR group (153.95±50.52min vs. 144.87±40.06min, P=0.09). Compared to the C-O-NSM with IBR group, the R-E-NSM with IBR group showed significantly lower rates of any complications (22.02% vs. 37.84%, P=0.001) and major complications (3.57% vs. 11.71%, P = 0.002). The Breast-Q, Ueda scale, and Harris scale in the R-E-NSM with IBR group were significantly superior to those in the C-O-NSM with IBR group (P＜0.05). The local-regional recurrence (P = 1.00) and distant metastasis (P = 0.37) were not different between the two groups. The R-E-NSM with IBR group showed significantly lower unilateral expenses (7043.84±771.16 USD vs. 7373.45±558.09 USD, P＜0.001) and hospital stay(3.24±2.86 days vs. 6.29±2.67 days, P＜0.001), respectively.
Conclusion: Compared to C-O-NSM with IBR, R-E-NSM with IBR can bring the same surgery efficiency and a sufficient cost-effectiveness ratio, lower incidence of postoperative complications, better aesthetic outcomes, and the same oncologic safety, indicating a broader application potential.
Keywords: Breast cancer, Reverse-sequence endoscopic nipple-sparing mastectomy, Implant-based Breast Reconstruction

P4-04-24: Cytokeratin 19 mRNA copy number by one-step nucleic acid amplification (OSNA) in the sentinel lymph node biopsy (SLNB) as prognostic factor in Luminal early Breast Cancer (LeBC).

Maria Yeray Rodriguez Garces, Stephanie Saide Cobelas Cartagena, Beatriz Buendía Cruz, David Morales Pancorbo, Yeray Rodríguez Garcés, Juan L Bayo Calero

Background: Unlike the number of infiltrated lymph nodes, the tumor burden in the sentinel lymph nodes is not well defined as a prognostic factor.
We studied the relationship between axillary infiltration using OSNA and the results of the genomic platform in LeBC with positive SLNB.

Methods: Observational, retrospective study in LeBC with positive SLNB defined as > 250 CK 19 mARN copies by OSNA in addition to genomic risk platform (OncoType or Mammaprint) from 2018 to 2023 in our area (Huelva, Spain).
We carried out a univariate (xi-square) and multivariate analysis (binary logistic regression) that included other prognostic factors. The SPSS Statistics 22 was used for data assessment.

Results: 212 LeBC with genomic assays were analyzed, 95 patients presented positive SLNB. Median age was 56 years, 98.9% female and 67.3% were postmenopausal. According to phenotype, 73.7% were Luminal A and 26.3% Luminal B1. Regarding prognostic factors: 40% with T > 2cm, Ki67> 20% in 31.6% and grade 3 in 5.3% of cases.
In the bivariable analysis of the overall population, both Ki-67 > 20% and > 10,000 -15,000 copies of CK19 mRNA were associated with high genomic risk (p = 0.000 and p = 0.037). These results were consistent in subgroup analysis among postmenopausal patients. In the case of the premenopausal subgroup, a significant result was also obtained for patients with

a histological grade ≥ 3 (p=0.038).
In the multivariate analysis, Ki-67 > 20% is an independent prognostic factor in relation to high genomic risk OR 7.79 [95% CI (2.03-29.9), p=0.003]. OSNA and grade 3 is also related with high genomic risk but no significance achieved.

Conclusions: Tumor genomic assays may not be necessary in LeBC with positive SLNB with elevated ki 67 and OSNA values and could even be sufficient to decide on adjuvant therapy in this patients.
It would be interesting to expand the sample size to reaffirm our hypothesis and better adjust the appropriate CK 19 mRNA copy number.

P4-04-25: HER2 Assay for the Detection of Low Expressing HER2 (IHC Score 1+/0) in Circulating Tumor Cells Enriched from Peripheral Blood using the CELLSEARCH® System

Damodara Gullipalli, Thai Bui, Margaret LaCava, Zoltan Simandi, Christina Dorris, Richard G. Del Mastro

Estrogen receptor positive/Human epidermal growth factor receptor negative (ER+/HER2-) represents 70% of metastatic breast cancer (mBC) patients. ER+/HER2- mBC is a challenging disease with significant unmet clinical needs. Approximately 60% to 65% of women with mBC, who are considered HER2-negative based on immunohistochemistry (IHC) staining of a tissue biopsy, are HER2-low and an additional 20% to 25% may represent a new category of HER2-ultra-low. This is problematic, as these patients are often ineligible for effective HER2-targeted therapies. Furthermore, the disease can evolve, and the receptor status of the tumor may change, necessitating periodic reassessment. However, the current NCCN guidelines strongly discourage repeated tissue biopsies after the second line of treatment, as they are invasive, costly, and can be exacting to perform. To address this issue, advanced liquid biopsy approaches that can assess HER2 status without tissue biopsy are needed.
Using the current CLIA certified CELLSEARCH® Circulating Tumor Cell-HER2 test, Circulating Tumor Cells (CTCs) that are EpCam+/Cytokeratin+/DAPI+/HER2+, can be detected in 7.5mls of peripheral blood only to a HER2 expression level equivalent to an IHC score 3+/2+. The next generation assay, CELLSEARCH® CTC-HER2 Low, has been designed to improve sensitivity and detect HER2 expression in CTCs to a level equivalent to IHC score of 1+/0 in 7.5mL of blood. The assay has been developed using a brighter fluorophore (Phycoerythrin), conjugated to the HER2 antibody, and was tested against 6 breast cancer cell lines (SKBR3, JIMT-1, BT-483, BT-20, MCF-7, DU4475) ranging in IHC scores 3+ to 0 and the MCF-7 HER2 knockout cell line. Analytical sensitivity and specificity were found to be >85% and >90% respectively. Linearity testing, where the cell lines were spiked in 7.5mL blood, showed high levels of reproducibility across the clinical range (1000 cells to 0) with an R2 = 0.99. The CELLSEARCH® CTC-HER2 Low test meets a critical clinical need that breast cancer patients, positive or classified as negative from a tissue biopsy, in need of a

current evaluation of their metastatic breast cancer HER2 status, through any line of treatment, can do so from one blood draw using a test that can detect HER2 expression to very low levels.

P4-04-26: A New Option for post-CDK4/6is Resistance Era: Multicenter Real-world Study of Anlotinib-based Combination Therapy in Hormone Receptor-positive Metastatic Breast Cancer Resistant to CDK4/6 Inhibitors

Quchang Ouyang, Binliang Liu, Zhanhong Chen, Xiaohong Wu, Ying Zhang, Tao Sun, Fangyuan Dong, Tao Wu, Bin Shao, Yifei Chen, Lu Gan, Hong Zong

Background: Cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors (CDK4/6is) combined with hormonal therapy are the current standard frontline treatment for patients with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER-2)-negative metastatic breast cancer (MBC). However, the optimal treatment after progression on CDK4/6is remains unknown. Anlotinib is an oral multi-target tyrosine kinase inhibitor (TKI) that strongly inhibits VEGFR, PDGFR, FGFR, and c-kit. This study aimed to evaluate the safety and efficacy of anlotinib-based combination therapy in patients with HR+ MBC previously treated with CDK4/6is.
Methods: 53 patients from 8 medical centers with pathologically confirmed HR-positive, HER-2-negative MBC were retrospectively reviewed. All included patients had received at least one line of CDK4/6is therapy before disease progression prior to anlotinib-based combination therapy. Anlotinib (12 mg daily, Day 1-14 of each cycle) was administered orally to fasting patients, with dose reductions to 10 mg or 8 mg in cases of intolerable toxicity. Combination agents included eribulin, nab-paclitaxel, etoposide, capecitabine, pembrolizumab, sintilimab, or fulvestrant, among others. Hospital medical records and imaging systems were used to assess clinical characteristics. The primary endpoint was progression free survival (PFS) and secondary endpoints were objective response rate (ORR), disease control rate (DCR), and overall survival. The safety profile has also been assessed.
Results: Between January 2020 and August 2024, 53 patients were included, with a median age of 52 years (range: 32-77 years). In the 39 patients whose efficacy could be evaluated, the median follow-up was 9.2 months (95% CI, 5.03-13.37), and the median PFS was 7.1 months (95% CI, 4.90-9.30). The ORR was 30.8% (95% CI, 0.17-0.47), and the DCR was 94.9% (95% CI, 0.83-0.99), with partial response and stable disease observed in 12 and 25 patients, respectively. The most common adverse events (AEs) were alanine aminotransferase elevation (22 patients, 41.5%), anemia (17 patients, 32.1%), and leukopenia (14 patients, 26.4%). It also includes widely observed adverse reactions such as hypertension (7 patients, 13.21%), albuminuria (7 patients, 13.21%), among others. Grade 3/4 treatment-emergent adverse events occurred in 9 patients (16.7%), with the most common being aspartate aminotransferase elevation (3 patients, 5.7%). Dose reductions of anlotinib were required in 6 patients (11.3%).
Conclusion: Anlotinib-based combination therapy has demonstrated good efficacy and

acceptable safety in HR+/HER2- MBC patients previously treated with CDK4/6is, making it a viable treatment option following resistance to CDK4/6is.

P4-04-27: Next generation antibody drug conjugates targeting HER2 and TROP2: Multi-Payload Conjugates™ targeting orthogonal mechanisms of cell killing

Marco Lobba, Richard Kendall, Devin Trinter, Maxwell Nguyen, Daniel Gutierrez, Samantha Brady, Chanez Symister, Andrew Lau, Derek Garcia-Almedina, Saurabh Johri, Matthew Francis

Introduction: Antibody-Drug Conjugates (ADCs) have had tremendous impact on patient outcomes in breast cancer and are now second-line therapy for stage IV HER2 high expressing metastatic breast cancer as well as HER2 low expressing tumors. However, many patients fail to respond or relapse after treatment with ADC therapies due to tumor heterogeneity and eventual resistance to the ADC payload. We are developing next generation Multi-Payload Conjugates™ (MPCs™) that deliver targeted combination chemotherapies within a single molecule.
Method: CatenaBio has developed novel, highly stable single-molecule targeted combination therapies, MPCs™, with tunable drug-antibody ratio (DAR). Our selective conjugation platform allows the attachment of distinct payloads targeting different mechanisms of action at three unique sites on the antibody scaffold.
Results: We screened combinations of different payloads targeting several different mechanisms of cell division attached to trastuzumab as well as sacituzumab at different DARs to optimize tumor cell killing. These targeted combination ADCs demonstrated robust killing in multiple cell lines containing high and low expression of HER2 and TROP2, as well as an Enhertu resistant cell line. Additionally, these novel MPCs show potent inhibition and excellent tolerability in mouse models of tumor growth in HER2 high and HER2 low/TROP2 expressing xenograft models including models of TNBC.
Conclusion: While advances have been made in the design of ADCs to expand to previously unaddressed populations, high patient relapse and the failure of recent mono-payload ADCs in late-stage trials indicate a need for novel conjugate modalities. Multi-payload Conjugates™ offer the next step in ADC design and allow for the combination of multiple mechanisms of action in a single MPC™ that are highly effective across multiple breast cancer cell lines and target expression levels. Successfully constructed with both HER2 and TROP2 targeting antibodies, these molecules offer the potential to circumvent tumor resistance pathways and deliver deeper and more durable patient responses.

P4-04-28: PF-07248144, a first-in-class KAT6 inhibitor, in patients with HR+ HER2– metastatic breast cancer: Updated results from phase 1 dose expansion study

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Background: Histone lysine acetyltransferase KAT6A, and its paralog, KAT6B regulate lineage specific gene transcription via H3K23 acetylation. PF-07248144 is a novel selective catalytic inhibitor of KAT6 (6A & 6B). We report updated results (with 7 months additional follow-up) of clinical safety, efficacy, and additional subgroup analyses from the phase 1 study (NCT04606446) of PF-07248144 as monotherapy and with fulvestrant (fulv) in heavily pretreated ER+HER2− metastatic breast cancer (mBC).
Methods: Patients (pts) had metastatic ER+ HER2− mBC and had progressed after a CDK4/6 inhibitor (CDK4/6i) and endocrine therapy (ET). Pts received PF-07248144 at the recommended expansion dose (RDE) of 5 mg QD as monotherapy or with fulv. Primary objective: safety and tolerability per CTCAE 5.0. Secondary objectives: antitumor activity per RECIST 1.1 and pharmacokinetics. Exploratory objectives: Pharmacodynamics and predictive biomarkers. Circulating tumor DNA (ctDNA) and gene mutations were evaluated by Guardant 360 assay.
Results: 35 pts received PF-07248144 monotherapy and 43 pts with fulv. All 78 pts had received prior CDK4/6i and ET in the metastatic setting. 59% (46/78) had detectable ESR1 mutations at baseline.
PF-07248144 demonstrated tolerable safety profile at RDE and encouraging antitumor activity as monotherapy and with fulv. In all 78 pts, the most frequent treatment-related adverse event (TRAE) was grade (G) 1/2 dysgeusia (n=66, 84.6%), mainly G1 (n=50, 64.1%); no dose reduction and treatment discontinuation due to dysgeusia. The most frequent G≥3 TRAE was neutropenia (G3: n=29, 37.2%; G4: n=5, 6.4%), reversible and manageable with dose modification. No febrile neutropenia or G5 TRAEs.
As of 30 Apr 2024, we observed increased antitumor activity with longer follow-up (median 16.4 mos) for fulv combination (n=43). The objective response rate (ORR) was 37.2% (95% CI 23.0, 53.3), median duration of response was not reached (95% CI 7.2 mos, not evaluable), clinical benefit rate was 55.8% (95% CI 39.9, 70.9), and the median progression-free survival (mPFS) was 10.7 mos (95% CI 5.3, 13.8). Antitumor activity was observed irrespective of ESR1 (wild-type [WT] n=18, mutant [MT] n=24; ORR 33.3 vs 41.7%; mPFS 10.9 vs 10.7 mos) and PIK3CA/AKT1/PTEN (WT n=23, MT n=19; ORR 43.5 vs 31.6%; mPFS 13.7 vs 7.3 mos) mutation status.
Additional subgroup analyses revealed broad antitumor activity of PF-07248144 fulv combination in pts treated as second-line (2L) therapy (following 1L CDK4/6i + ET) or as third-line or beyond (3L+) therapy (ET, targeted therapy or chemotherapy in between; ORR 30.4% [7/23] vs 45.0% [9/20]; mPFS 13.8 vs 10.7 mos); and irrespective of primary or

secondary endocrine resistance (ORR 57.1% [4/7] vs 33.3% [12/36]; mPFS 9.0 vs 10.8 mos), presence or absence of prior treatment with fulv (ORR 60.0% [3/5] vs 34.2% [13/38]; mPFS not reported [n≤5] vs 7.5 mos), and rapid progression on prior CDK4/6i (duration of treatment <6 mos) or prior CDK4/6i treatment duration >12 mos (ORR 50.0% [2/4] vs 36.4% [12/33]; mPFS not reported [n≤5] vs 10.9 mos).
After 8-wk treatment, the median reduction in total ctDNA and ESR1 mutant allele frequency was 95.0% and 100.0%. Pts with non-detectable ctDNA after the initial 8-wk treatment had longer PFS compared with those with detectable ctDNA.
Conclusions: PF-07248144 in combination with fulv demonstrated an acceptable safety profile and promising efficacy in pts with ER+ HER2− mBC post CDK4/6i and ET. Antitumor activity was observed irrespective of ESR1 and PIK3CA/AKT1/PTEN mutation status, endocrine sensitivity or resistance, duration of prior CDK4/6i treatment (<6 mos or >12 mos), prior fulvestrant treatment, and 2L or later line therapy. These findings suggest that PF-07248144 in combination with ET may potentially overcome endocrine resistance and CDK4/6i resistance and provide a novel mechanism to address high unmet medical need in HR+ mBC after prior CDK4/6i and ET.

P4-04-29: The Impact of Race and Neighborhood on Young Breast Cancer Patients

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Background: Current United States guidelines recommend mammogram screening for breast cancer (BC) to begin at age 40 for women of average risk, however diagnosis (dx) under age 40 accounts for ~7-9% of all BC cases. Patients with BC under age 40 have more aggressive cancers, present at later stages, and have poor outcomes. Factors that can negatively affect BC outcomes, regardless of age, include African-American (AA) race, Triple Negative BC (TNBC) dx, and lower socioeconomic status. Data show that AA patients with BC have increased mortality when compared to White (W) counterparts. Louisiana (LA) has one of the largest AA populations in the country at 32.8%, compared to ~12% of overall US population. When compared across the US, LA also has an exceptionally large number of high Area of Deprivation Index (ADI) neighborhoods, a variable encompassing socioeconomic status. We set out to examine the impact of race and ADI on patients with BC dx under age 40. Methods: We performed a retrospective chart review of the Ochsner Health System for all patients with a BC dx under age 40 from 2012-2022. ADI rankings are summarized as median and interquartile range (IQR) and the race-ADI association is evaluated using the Wilcoxon rank-sum test. Other continuous measures are reported as mean and standard deviation (SD), and categorical measures as frequency and percentage. Associations with race are assessed via two-sample t-tests, and chi-squared tests or Fisher's exact tests. Crude associations between outcomes (metastasis, mortality) and potential confounders of race (dx age, BMI, subtype, stage at dx) in total sample are estimated via Cox proportional hazards (PH) regression models and reported as hazard ratio (HR) for AA vs W and 95% confidence interval (CI). Three models are considered for each outcome. Model

1 (M1) includes a fixed race effect only; Model 2 (M2) adds covariates for age and BMI to M1 and is stratified by BC subtype; Model 3 (M3) additionally stratifies M2 by cancer stage at dx. All analyses were carried out using SAS/STAT software, version 9.4 for Windows. Results: A total of 719 patients were analyzed. 689 patients had ADI data. Total sample (AA =273 vs W =446) HR metastasis M1: 1.16 (0.85, 1.59), M2: 1.21 (0.87, 1.69); mortality M1: 1.20 (0.81,1.76), M2: 1.21 (0.80,1.82), M3: 1.09(0.71, 1.65) HR for patients with stage I-III (N=672; AA = 255 vs W = 417) metastasis M1: 1.23 (0.84, 1.79), M2: 1.30 (0.87, 1.94), M3: 1.27 (0.85, 1.90); mortality M1: 1.17 (0.74, 1.81), M2: 1.11 (0.71, 1.73), M3: 1.05 (0.67, 1.65). HR for TNBC subgroup stage I-III (N=207; AA = 103 vs W = 104) metastasis M1: 0.96 (0.51, 1.80), M2: 1.01 (0.53, 1.92), M3: 1.00 (0.52, 1.91); mortality M1: 1.08 (0.55, 2.17), M2: 1.20 (0.59, 2.45), M3: 1.13 (0.56, 2.31). HR stage I-III in ADI subgroups (N=643; AA = 239 vs W = 404), metastasis M1: 1.29 (0.88, 1.88), M2: 1.35 (0.90, 2.01), M3: 1.30 (0.86, 1.94); mortality M1: 1.28 (0.81, 2.00), M2: 1.30 (0.81, 2.09), M3: 1.23 (0.76, 1.98) ADI-adjusted HR stage I-III in ADI subgroups (N=643; AA=239 vs W=404), metastasis M1: 1.11 (0.73, 1.69), M2: 1.08 (0.70, 1.67), M3: 1.07 (0.69, 1.67); mortality M1: 1.02 (0.62, 1.68), M2 0.93 (0.55, 1.58), M3: 0.91 (0.53, 1.55). Highest (most deprived) ADI quartile (ADI >75): total 26.7%, AA 47.7%, W 14.3%. Lowest (least deprived) ADI quartile (ADI<25): total 9%, AA 4.3%, W 11.8%. Discussion/Conclusion: These results demonstrate that ADI is the primary driver of outcomes when compared to race, BMI or BC subtype. When incorporating ADI, differences in outcomes noted between AA and W patients diminish, especially when evaluating mortality among young women with TNBC. While there are differences in disease biology, the impact of ADI on outcomes should not be underestimated. Improvements in BC awareness, access to care, and support programs should target areas of highest ADI to improve long-term BC outcomes, especially in young populations, regardless of race.

P4-04-31: A phase Ib/II study of PARPi Mefuparib Hydrochloride (CVL218) in combination with PD-1 inhibitor plus chemotherapy in metastatic or recurrent triple-negative breast cancer (TNBC)

Jian Zhang, Rujiao Liu

Background: Triple-negative (negative for human epidermal growth factor receptor 2 [HER2] and estrogen and progesterone receptors) breast cancer (TNBC) is the breast cancer subtype with the worst prognosis. It represents approximately 15% -20% of all breast cancer cases. BRCA 1 and /or BRAC 2 mutation accounts for about 15% of all TNBC and remains poor outcomes for such patients. TORCHLIGHT trial is a randomized, double-blinded, phase 3 study assesses the efficacy and safety of toripalimab (programmed cell death-1 inhibitor) combined with nab-paclitaxel (nab-P) as a first-line treatment for patients diagnosed with metastatic or recurrent TNBC. The (progression-free survival) PFS and (overall survival) OS analysis, showed a significant improvement in PD-L1 positive (CPS ≥ 1) tumors. Phase III OlympiAD study has established the efficacy of Poly ADP-ribose polymerase inhibitors (PARPi) olaparib in patients with germline BRCA gene mutation

(gBRCAm) and human epidermal growth factor receptor 2 (HER2) negative metastatic breast cancer. Mefuparib hydrochloride (CVL218) is a second generation of PARPi with low hematological toxicity and the ability to cross the blood-brain barrier. The major purpose of this study is to explore the RP2D, efficacy and safety of CVL218 combined with PD-1 inhibitors and paclitaxel nanoparticle albumin-bound in patients with TNBC who have failed previous first-line treatment. The second endpoint of this study is to analyze the efficacy of each subgroup according to PD-L1 expression and HRD (Homologous Recombination Deficiency) mutation status.
Methods: This open- labeled, phase Ib/II clinical study was conducted in China. In phase Ib, the "3+3" principle is used to explore the RP2D of CVL218. Two dose escalation of CVL218 were preset, which are 500mg twice daily (BID) and 700mg BID. CVL218 administered orally in combination with toripalimab 240 mg on Day 1 of every 21-day cycle and paclitaxel nanoparticle albumin-bound 125 mg/m2 on Day 1 of every 21-day cycle. The first cycle (21 days) after administration was also defined as the DLT (dose-limiting toxicity) observation period. At last, no DLT was observed in the 3 evaluable subjects in the CVL218 500 mg BID dose group, and DLT was observed in 2 evaluable subjects in the 700 mg BID dose group, including creatinine increased and aspartate aminotransferase, Alanine aminotransferase increased. Therefore, the RP2D was determined as 500 mg BID. Patients in phase II cohort A were advanced TNBC with either CPS ≥ 1 or HRD gene pathogenic variants. All these patients received CVL218 (500mg BID) + toripalimab (240 mg) + paclitaxel nanoparticle albumin-bound (125 mg/m2,).
Results: From July 2023 to 15 July 2024 data, a total of 6 subjects were enrolled in Phase Ib and 5 subjects were enrolled in Phase II. The median age of the 11 subjects was 55.2 years. In phase Ib, The RP2D of CVL218 was identified as 500 mg BID. The preliminary efficacy was perfomed every 6 weeks in the first half a year and then once every 12 weeks thereafter in both phase I and II according to RECISTv 1.1. Among 11 patients, ORR (Objective Respond Rate) was 72.7% (8 PR, 2 SD), and DCR (Disease Control Rate) was 90.9%. The most common Grade 3 or higher treatment-related AEs (TRAEs) were hepatic function injury (2.6%), adynamia (1.8%), creatinine increased (0.8%), neutrophils decreased (0.8%), white blood cell decreased (0.8%), exanthema (0.8%), and peripheral sensory nerve disorder (0.8%), No Grade 5 TRAE. A total of 9 treatment-related SAEs in 6 patients (54.5%) were observed, including 2 aspartate aminotransferase increased, 2 alanine aminotransferase increased, 1 creatinine increased, 1 liver function injury, 1 adynamia, 1 neutrophils decreased, 1 fever, and 1 ventricular premature beats. All these SAEs recovered after symptomatic treatment.
Conclusion: Mefuparib Hydrochloride(CVL218) combined with PD-1 and chemotherapy appears to be well tolerated and has preliminary efficacy in TNBC patients. In this phase II study, stratified analyses will be conducted for PD-L1 expression and HRD status. In patients with BRCA 1 and /or BRAC 2 mutation TNBC, this CVL218 combination therapy is expected to further break through the clinical treatment effect.
Keywords: Triple negative breast cancer; PARPi.

P4-04-32: Clinical study of 99mTc-Rituximab combined with dyes double tracing for axillary sentinel lymph node biopsy after neoadjuvant chemotherapy for breast cancer

Hui Zhang, Minxue Zhuang, Mengbo Lin

Background and Objective: Whether sentinel lymph node biopsy (SLNB) after neoadjuvant chemotherapy (NAC) for breast cancer is an alternative to axillary lymph node dissection (ALND) remains controversial.In this study, the results of SLNB performed on patients with 99mTc-Rituximab combined with dyes were analyzed, and the application value of the double-tracing method of 99mTc-Rituximab combined with dyes in SLNB after breast cancer NAC was evaluated, the feasibility of SLNB after NAC and the clinical application of the novel tracer 99mTc-Rituximab and its value in internal mammary sentinel lymph node was discussed.
Methods: A retrospective analysis of 106 breast cancer patients who underwent post-NAC SLNB from August 2019 to August 2023 at Fujian Provincial Hospital, where SLNB was performed using 99mTc-Rituximab combined with dye imaging or dye imaging alone. The detection rate, sensitivity, false-negative rate, accuracy and the detection rate of internal mammary lymph node biopsy were compared between the two tracing methods.
Results:70 cases were included in the dual tracing group, with a detection rate of 97.14% (68/70), an average number of detected SLNs of （6.06±5.29）， a sensitivity of 92.86% (26/28), a false negative rate of 7.14% (2/28), and an accuracy of 97.14% (68/70).36 cases were included in the single tracing group, with a detection rate of 66.67% (24/36), an average number of detected SLNs of （3.17±3.073）， a sensitivity of 54.55% (11/22), a false negative rate of 45.45% (10/22), and an accuracy of 72.22% (26/36). There were significant differences in the detection rate and the average number of detected SLNs between the two groups (detection rate: P=0.004; detection number: P=0.038), but there were no significant differences in the sensitivity, accuracy, and false negative rate (P>0.05).A total of 70 patients underwent double-tracing internal mammary lymph node biopsy, and 22 patients were detected with an imaging rate of 31.42% (22/70), and a detection rate of 72.72% (16/22).
Conclusions:SNLB using radionuclide with dye double-tracing method after neoadjuvant chemotherapy for breast cancer can improve the detection rate of sentinel lymph nodes, reduce the false negative rate, and improve the prognosis.Compared with other tracers, 99mTc-Rituximab can improve the detection rate of internal mammary sentinel lymph nodes, with the characteristics of rapid clearance of injection site, less secondary lymph node imaging, and low sensitization, which can be used as an ideal tracer for further research.

P4-04-34: Use of Intravaginal Physical Energy for Treating Genitourinary Syndrome of Menopause in Breast Cancer Survivors: A Systematic Review

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Background: Breast cancer survivors (BCSs) often experience more severe symptoms of Genitourinary Syndrome of Menopause (GSM). Since hormonal therapies, such as estrogen, are typically avoided in BCSs, physical methods may offer promising non-hormonal alternatives for these women. However, the efficacy and safety of these treatments remain uncertain.
Objectives: To evaluate the efficacy and safety of physical methods, including laser and radiofrequency, for treating GSM in BCSs.
Methods: We searched eight databases from their inception through July 2024 without imposing language or date restrictions. We included randomized controlled trials (RCTs) that assessed the efficacy and safety of any physical methods for treating GSM in BCSs. Two authors independently screened studies based on titles, abstracts, and full texts, applying predefined inclusion criteria. Data were extracted, and the risk of bias was assessed using the Cochrane risk-of-bias tool (RoB 2). Due to methodological and outcome heterogeneity, a meta-analysis was not feasible, and a narrative synthesis was conducted. The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach was used to assess the strength of the evidence.
Main Results: Three RCTs, involving 185 participants and three different physical methods (microablative fractional carbon dioxide [CO2] laser, erbium photothermal yttrium-aluminum-garnet [Er:YAG] laser, and radiofrequency), met the inclusion criteria. All three studies reported improvements in dyspareunia with physical method therapies, with no significant differences compared to control groups. Vaginal Health Index (VHI) scores and quality of life (QoL) showed short-term improvements, but again, with no significant differences from control groups. Only one study evaluated sexual function using the Female Sexual Function Index (FSFI) and Vaginal Maturation Index (VMI), showing improvement in sexually active women, although this was not significantly different from the sham group. Minimal adverse events were reported. Overall, only one study had a low risk of reporting bias, while two studies raised concerns due to critical weaknesses. Confidence in the evidence is low and critical across all studies.
Conclusions: Physical methods appear to be safe for the short-term treatment of GSM in BCSs. However, due to the limited number of blinded clinical trials, the efficacy of these treatments remains uncertain. Further research, particularly high-quality RCTs with long-term follow-up, is needed.
PROSPERO Registration Number: CRD42023387680

P4-04-35: Thioredoxin-Interacting Protein (TXNIP) as a Key Regulator of Tumor Growth and Progression

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Thioredoxin-interacting protein (TXNIP) plays a critical role in glucose metabolism and redox signaling, and has recently emerged as a potent inhibitor of cell proliferation in cancer. In our previous study, we demonstrated that TXNIP activation significantly suppressed MDA-MB-231 breast cancer cell proliferation in vitro and reduced tumor growth in vivo. In this study, we show that TXNIP knockdown accelerates tumor growth and metastasis in MDA-MB-231 cells in a mouse model. Conversely, reintroducing TXNIP into TXNIP-deficient HCC-1954 breast cancer cells reduced cell proliferation and migration, while increasing reactive oxygen species (ROS) production and decreasing mitochondrial respiration, membrane potential, and glycolysis. To further investigate TXNIP's tumor-suppressive mechanisms, co-immunoprecipitation and proteomic analyses identified calpastatin (CAST) as a novel TXNIP-interacting protein in both MDA-MB-231 and HCC-1954 cells. Overexpression of CAST, an endogenous calpain inhibitor, significantly promoted xenograft tumor growth in both cell lines, revealing a previously unrecognized role for CAST as a tumor promoter. Furthermore, we observed an inverse correlation between TXNIP and HER2 expression in multiple breast cancer cell lines, where HER2+ cells exhibit low or undetectable TXNIP levels. Notably, TXNIP expression was found to reduce HER2 levels in HER2+ breast cancer cell lines. These findings uncover novel TXNIP-dependent pathways that contribute to breast cancer pathogenesis, offering new insights into TXNIP's complex role in tumor biology and presenting potential avenues for therapeutic intervention.

P4-04-36: Mixed Reality-Assisted Breast-Conserving Surgery Using HoloLens 2: Initial Results from a Phase II Trial

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Background: Achieving clear surgical margins in breast-conserving surgery (BCS) for early-stage breast cancer is essential to minimize local recurrence rates. Traditional imaging methods may not provide sufficient intraoperative guidance, potentially leading to positive margins and the need for additional surgeries. Mixed reality (MR) technology offers real-time, three-dimensional visualization of tumor anatomy, enhancing surgical precision. By integrating MR into existing surgical methods, it may be possible to omit certain procedures and expand the eligibility for breast-conserving surgery.
Methods: We are conducting a prospective, single-arm, phase II trial to evaluate the feasibility, safety, and efficacy of MR-assisted BCS using the HoloLens 2 in patients with stage I–II breast cancer. Eligible patients are females aged 20 years or older with invasive ductal carcinoma and an Eastern Cooperative Oncology Group (ECOG)

performance status of 0–1. Preoperative imaging includes contrast-enhanced breast MRI and CT scans, processed using VINCENT software (FUJIFILM) to create three-dimensional models of the tumor and surrounding breast tissue. These models are further integrated into Holoeyes MD software and visualized through the HoloLens 2 (Microsoft) head-mounted device.
In September 2024, three patients underwent MR-assisted BCS using this protocol. During surgery, the lead surgeon donned the HoloLens 2, which projected the 3D tumor model onto the patient's breast, accurately aligning with anatomical landmarks. This MR overlay provided enhanced spatial awareness, allowing for precise tumor excision. By incorporating this technology, certain intraoperative procedures, such as extensive palpation or additional imaging guidance, could be omitted, potentially simplifying the surgical workflow and expanding the applicability of breast-conserving surgery.
Results: All three surgeries were successfully completed without intraoperative complications. The operative times were approximately 2 hours for each case, consistent with standard BCS procedures. Intraoperative blood loss was minimal and difficult to quantify due to its negligible amount, indicating that MR assistance did not increase bleeding risks. Postoperative follow-up over three days revealed no complications such as infection or hematoma. Pathological examination confirmed negative surgical margins in all cases, with no need for secondary resections. Surgeons reported that the MR system was ergonomically acceptable after a brief training period and did not interfere with the surgical workflow. The enhanced visualization provided by the MR technology facilitated more precise resections while potentially reducing the reliance on intraoperative ultrasound or other imaging modalities.
Conclusions: Preliminary results from this phase II trial suggest that MR-assisted BCS using the HoloLens 2 is feasible and safe for patients with early-stage breast cancer. The integration of MR technology into existing surgical methods may improve surgical precision without extending operative times or increasing intraoperative risks. Minimal bleeding and the potential omission of certain intraoperative procedures highlight the practicality of this approach. Additionally, MR assistance may enable breast-conserving surgery in cases previously considered challenging, thereby offering more patients the benefits of less invasive treatment options. Ongoing enrollment and longer-term follow-up are needed to validate these findings and assess their impact on local recurrence rates and overall patient outcomes.

P4-04-37: SS Induces BRCA1 Reduction and Sensitizes Breast Cancer Cells to PARP Inhibitor Therapy

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Background: Poly (ADP-ribose) polymerase inhibitors (PARPi) have been approved for the treatment of BRCA-mutant breast cancer and high-grade serous ovarian cancer, demonstrating significant efficacy. The presence of a BRCA mutation is critical for the

prescription of PARPi, as their mechanism is based on synthetic lethality. Previous studies have suggested using CRISPR/Cas9-mediated BRCA1 mutation strategies to restore PARPi sensitivity in BRCA wild-type cancers. While promising, this approach poses challenges for clinical implement. Sulindac sulfide, an active metabolite of the non-steroidal anti-inflammatory drug (NSAID) sulindac, has emerged as a potential agent that can enhance cancer cell death when combined with cisplatin and doxorubicin, potentially by impairing DNA repair mechanisms.

Methods: We investigated the mechanism of sulindac sulfide's regulation of BRCA1 using 26S proteasome inhibitors and RT-PCR. The efficiency of homologous recombination (HR) and DNA damage levels were evaluated using γH2AX-ubiquitin foci formation assays and slot blot assays, respectively. To assess the therapeutic potential of combining sulindac sulfide with PARPi, we conducted both in vitro and in vivo studies.

Principal Findings: Sulindac sulfide induced a dose-dependent reduction of BRCA1 through post-translational mediated regulation. Additionally, sulindac sulfide treatment in TNBC cells revealed DNA repair deficiency and increased sensitivity to PARPi therapy. This study provides a basis for further exploration of sulindac sulfide as a potential adjuvant therapy in BRCA1 wild-type cancers, particularly those resistant to conventional PARPi therapy.

P4-04-38: Real world study of racial disparities in toxicities of Antibody Drug Conjugates in advanced breast cancer treatment

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Background: Antibody drug conjugates have revolutionized the treatment paradigm in breast cancer. Fam-trastuzumab-deruxtecan (TD) is used in unresectable or metastatic HER 2 positive and HER 2 low scenarios. In the metastatic hormone receptor positive and triple negative breast cancer, sacituzumab govitecan (SG) has also shown significant benefit. The efficacy of these drugs is well-known but data regarding racial differences in their toxicities is lacking. We analyzed real-world data to explore and contribute to the literature on racial disparities in treatment-related toxicities aiming to reduce drug failure or early discontinuation.

Methods: We utilized TriNetX, a multi-health care organization electronic health record database, to identify African American (AA) and Caucasian (C) cohorts for outcome comparison. Inclusion criteria required a C50 ICD-10-CM diagnosis code and at least one SG or TD treatment. Age, gender, and race-controlled Cox proportional-hazard Andersen-Gill models were applied to analyze time to complete each SG cycle and the time gap between TD treatments, accounting for recurrent treatment events. Multiple logistic regression was used to evaluate the association between race and toxicity outcomes (controlled for age and gender) for cytopenias, nausea, inpatient encounters, cardiotoxicity, interstitial lung disease and diarrhea. Odds ratios (OR) and hazard ratios with 95% confidence interval (CI) are

reported to estimate effect size and statistical significance.

Results: For SG 862 C, 174 AA and for TD 831 C, 145 AA met the inclusion criteria. The mean age, in years, at initiation of treatment was 59.1 +/- 12.4 for C, 56.8 +/- 12.0 for AA among SG and 61.7 +/- 12.3 for C, 57.6 +/- 12.8 for AA among TD. In both SG and TD groups, AA patients had a lower hazard and thus less likely to complete treatment cycle on time at any given time compared to C: 15% (CI: 8-22%) and 14% (CI: 8-19%) respectively. AA experienced 1.46 (CI: 1.04 - 2.07) times higher odds of having a delay longer than 7-days between 2 doses of 1st cycle SG. For TD, AA experienced a 2.7 (CI: 1.7 - 4.2) times increased odds of having a delay longer than 21-days between 3rd and 4th doses. This is associated with an increased odds (OR=7.6, p=0.008) of cardiotoxicity in AA during this time window. No other toxicities resulted in a significant difference between AA and C cohorts.

Conclusions: This study revealed critical findings regarding treatment adherence and delays among patients receiving SG and TD, specifically linked to a particular race. Among both the groups, AA patients demonstrated a lower hazard or less likely to complete their treatment cycle on time at any given point compared to C patients. Furthermore, the odds of delay was noted to be 46% higher in AA during 1st cycle of SG. The cytopenias were analyzed as probable cause but noted to be non-significant. The median number of SG cycles were 3.5 for AA and 4 for C. Interestingly, AA have 170% higher odds of having a delay between 3rd and 4th doses of TD. This correlates with a significant 7.6 times increased odds of cardiotoxicity among AA. The median number of TD cycles were 5 for AA and 7 for C. These findings emphasize the need for future research to focus on intricacies contributing to these disparities and improve patient outcomes.

P4-04-39: Excellent and Prolonged Response to Sacituzumab-govitecan in Triple Negative mBC

Elena Giontella, Elena Fiorio

Background: Triple negative breast cancer (TNBC) is defined by the absence of estrogen and progesterone receptor expression and HER2 expression. This subtype of breast cancer has the worst prognosis and the fewest therapeutic options. In recent years, there has been the advent of both immunotherapy (first in metastatic stages and then also in early breast cancer) and ADCs. Antibody-drug conjugates are used in advanced stages of the disease. In fact the ASCENT study has been shown that sacituzumab govitecan provides a significant benefit compared to single-agent chemotherpay in progression-free survival (PFS) (5.6 months vs 1.7 months) and overall survival (OS) (12.1 months vs 6.7 months) compared to single-agent chemotherapy). Therefore SG is currently used as a second-line treatment for metastatic TNBC. Additionally, the TROPICS-02 study demonstrated a benefit PFS and objective response rate (ORR) in patients with HR+/HER2- metastatic breast cancer (mBC) who had previously been treated with at least one line of endocrine therapy and two to four lines of chemotherapy.

TROP-2 expression is elevated in HER2-negative breast tumors (HR+/HR-) and correlates with poorer survival outcomes. Sacituzumab govitecan (SG) is a pioneering TROP-2-directed antibody-drug conjugate (ADC) that combines an anti-TROP-2 antibody with SN-38, a topoisomerase inhibitor linked through a hydrolysable connector.

Clinical Case: In 2021, a 57-year-old woman presented with a palpable lesion in her left breast. Diagnostic tests, including breast ultrasound and subsequent MRI, revealed a unifocal lesion measuring 4 cm and two ipsilateral axillary lymphadenopathies. A biopsy confirmed the diagnosis of ductal infiltrating carcinoma with ER expression at 9%, PgR negative, HER2 1+, and Ki67 at 40%, indicating locally advanced disease due to axillary lymph node involvement. She underwent neoadjuvant chemotherapy with epirubicin and cyclophosphamide for 4 cycles, followed by carboplatin and weekly taxol for 12 cycles. Despite the low estrogen receptor expression, carboplatin was considered due to the early triple-negative subtype. At the end of neoadjuvant chemotherapy, the patient was underwent a left mastectomy and axillary dissection, With the finding of a pathological complete response at the site of the primary tumor, but with residual disease at the axillary lymph node level. Thus, she was treated with adjuvant capecitabine and adjuvant radiotherapy.
Unfortunately, in January 2023, CT scan presented multifocal recurrence (bone, lung, liver and spleen lesions). A biopsy of a liver lesion was performed, which showed triple-negative histotype.
Since the disease recurrence occurred within 6 months after the completion of adjuvant chemotherapy, we considered the patient for treatment with sacituzumab govitecan plus denosumab (for bone metastasis). At the first follow-up, the CT scan showed a significant response in the liver and lung disease. The patient continued treatment with SG, with total body CT scans every 3-4 months showing maintained disease response. By February 2024, there was complete radiological response of the liver and lung lesions, with stable bone disease. The complete response is still maintained today, after 20 months the beginning of SG.
As adverse events, we report: grade 3 (CTCAE) neutropenia, so it became necessary prophylaxis with G-CSF; grade 1 diarrhea and grade 1 fatigue.

Conclusion: This clinical case offers significant insights. Currently, the PFS has not yet been reached at 20 months, maintaining a complete response in visceral sites and stability in bone disease, while in the ASCENT study, the mPFS was noted at 6.7 months.
The fact that there is weak estrogen receptor positivity may lead to a better response to SG and should it be classified as HR+ or TN? Could it be useful to identify any new biomarkers that may have predictive value, like Trop-2?

P4-04-40: Sentinel Lymph Node Biopsy in the Prognosis of Women Undergoing Neoadjuvant Chemotherapy for Breast Cancer Treatment including advanced Stages: A Real-World Study in a public hospital in Brazil

Rafael Machado, Marcelo Belo, Anke Bergman, Luiz Claudio Thuler

Objectives: To evaluate the impact of Sentinel Lymph Node Biopsy (SLNB) on the prognosis of women undergoing neoadjuvant chemotherapy for breast cancer (BC) treatment, including advanced stages of BC (8th Edition, American Joint Committee on Cancer - AJCC).

Methods: A retrospective cohort observational study was conducted in women diagnosed with BC, enrolled at the Cancer Hospital III of the Brazilian National Cancer Institute José Alencar Gomes da Silva (HCIII/National Cancer Institute - INCA), from January 2013 to December 2015. Patients without a primary indication for surgery, who underwent neoadjuvant chemotherapy, were included. Eligible women were followed until May 30, 2024. Data were collected from both physical and electronic medical records using a specific instrument developed for this purpose. Sociodemographic, clinical, tumor-related, oncological treatment, and prognostic variables were collected. Disease-free survival and overall survival were evaluated using the Kaplan-Meier method and Cox regression with the Stepwise Forward method. This study was approved by the Research Ethics Committee of INCA (CAAE: 06794512.3.00005274).

Results: During the study period, 918 women were eligible, with a mean age of 51.58 years (SD 11.46). Clinical stage III was the most frequent (53.5%), and most patients were classified as luminal B (52.2%) and luminal A (17.8%) subtypes.SLNB was performed in 266 women, mostly with dual-agent labeling (88.2%). In six cases (2.3%), the sentinel lymph node could not be identified, and these patients underwent direct Axillary Lymph Node Dissection (ALND). ALND was performed after SLNB in 105 patients. A median of two lymph nodes (0 to 12) were removed during SLNB, most being negative in both intraoperative analysis (74.1%) and paraffin-embedded hematoxylin-eosin (HE) analysis (67.3%).A higher frequency of SLNB with or without ALND was observed in women with cT1 and cT2 tumors, whereas ALND was more frequently performed in patients with cT4 tumors ($p<0.001$). Regarding clinical lymph node involvement, SLNB was performed in 65.8% of women with cN0, 32.7% with cN1, and 1.5% with cN2 or cN3 ($p<0.001$).A total of 37.8% of the patients had local or distant recurrence during the follow-up period, with a median disease-free survival of 87.8 months (95% CI 84 to 91), which was longer in patients undergoing SLNB (93 months) and SLNB+ALND (90 months) compared to those undergoing ALND (82 months) ($p<0.001$). In the Cox regression analysis adjusted for clinical stage and BC subtype according to immunohistochemistry, patients who underwent ALND had a higher risk of recurrence or metastasis compared to those who underwent SLNB (HR=2.27, 95% CI 1.50–3.45; $p<0.001$), as did those who underwent SLNB+ALND

(HR=1.95, 95% CI 1.18–3.22; p=0.009).Regarding Overall Survival, there were 334 deaths, with an average survival time of 102.2 months (95% CI 99 to 105), which was longer in patients undergoing SLNB (107 months) and SLNB+ALND (103 months) compared to those undergoing ALND (97 months) (p<0.001). In the Cox regression analysis adjusted for clinical stage and BC subtype, patients who underwent ALND had a higher risk of death compared to those who underwent SLNB (HR=2.52, 95% CI 1.61–3.93; p<0.001), as did those who underwent SLNB+ALND (HR=2.40, 95% CI 1.43–4.06; p=0.001).

Conclusions: This study demonstrated the safety of performing SLNB in women undergoing neoadjuvant chemotherapy, including advanced BC stages in a real-world setting of patients treated in the Brazilian Nacional Cancer Institute.

P4-04-41: The impacts of neoadjuvant or adjuvant chemotherapy on surgical complications and cosmetic outcomes of reverse-sequence endoscopic nipple-sparing-mastectomy with immediate breast reconstruction.

Kawun Chung, Yanyan xie, Faqing Liang, Zhongjian Zhu, Qing Zhang, Hui Dai, Tianyuan Li, Xiaoman Cao, Zhenggui Du

Abstract: sBackground The reverse-sequence endoscopic nipple-sparing mastectomy (R-E-NSM) combined with immediate breast reconstruction (IBR) has gained widespread attention in China, becoming a preferred surgical option for breast cancer patients. However, there is a significant gap in understanding how different chemotherapy regimens affect the outcomes of R-E-NSM with IBR. To bridge this knowledge gap, this prospective study aims to comprehensively compare postoperative complications and cosmetic outcomes of R-E-NSM with IBR among patients who received no chemotherapy (NC), neoadjuvant chemotherapy (NAC), and adjuvant chemotherapy (AC). We hope to provide valuable insights that will inform clinical decision-making and optimize patient care.
Method Patients who underwent R-E-NSM with IBR at the Breast Center of West China Hospital, Sichuan University, between April 2020 and December 2023, were stratified into three distinct groups: NC, NAC, and AC. This classification enabled a comprehensive analysis of surgical complications and cosmetic outcomes across the different treatment cohorts, providing valuable insights into the impact of chemotherapy timing on surgical outcomes and patient satisfaction.
Result: A total of 692 patients were prospectively enrolled in this study, including 241 (34.8%) patients who received NC, 113 patients (16.3%) who underwent NAC, and 338 (48.8%) who underwent AC. Compared to the other two groups, patients in the NAC group were younger (P < 0.05) and had a shorter median follow-up (P < 0.05). The AC group, but not the NAC group, showed higher rates of any complications compared to the NC group (26.6% vs. 14.2%, 2.188 [1.415-3.383], P=0.001). Specifically, the AC group had higher rates of particular treatment complications [Clavien-Dindo classification (CDC) ≥ 2] (20.7% vs.

12.6%, 1.820 [1.144-2.895], P=0.036) and surgical site infections (SSI) (18.0% vs. 8.8%, 2.240 [1.322-3.797], P=0.003). Further analysis found that these differences mainly arose more than 30 days after the surgery (any, CDC ≥ 2, SSI, P<0.05). On multivariable analysis, there were significant differences in any complications across the entire time (OR, 1.680 [95% CI, 1.015-2.711], p=0.044) and SSI that occurred more than 30 days after surgery (OR, 2.346 [95% CI, 0.991-5.556], p=0.052) between NC and AC groups. After controlling for clinical covariates, no significant differences were observed between the three groups in terms of the BREAST-Q subscales and Ueda score.
Conclusion: When performing R-E-NSM followed by IBR, administering AC but not NAC (if the surgery is done more than 21 days after NAC) may increase the incidences of any complications compared to those who do not undergo chemotherapy. As measured by breast-Q and Ueda score results, cosmetic outcomes are not affected by NC, NAC, or AC.

P4-04-42: A novel 3D-printed scaffold for patient-specific partial breast reconstruction: A Prospective, Single-Arm Clinical Trial

Lan Hou, Changjiao Yan, Mingkun Zhang, Liu Yang, Zhe Wang, Yuan Qin, Huan Zhang, Zijie Meng, Qing Yao, Rui Ling, Jiankang He, Juliang Zhang

Background: The current scarcity of appropriate breast fillers restricts the availability of breast-conserving surgery for breast cancer patients. Although breast reconstruction can be conducted following a mastectomy, the existing methods for reconstruction are constrained. Three-dimensional (3D) printing technique enables the production of patient-specific and custom-designed scaffold, which may solve this problem.
Methods: A prospective, single-arm clinical trial was performed. This study enrolled breast cancer patients who were not suitable for traditional breast-conserving surgery, including primary tumors larger than 1.0 cm and less than 8.0 cm, or those presenting as multiple diffuse lesions limited to a single quadrant. 3D-printed scaffold was fabricated according to patient's own magnetic resonance imaging (MRI) one week before surgery. Patients were assigned to receive the patient-specific 3D-printed scaffolds transplantation surgery. The primary endpoint of this study is to determine the safety and cosmetic outcomes of partial breast reconstruction using 3D-printed patient-specific scaffolds. The secondary endpoints include degradation of 3D-printed scaffold, complications and satisfaction of patients received this partial breast reconstruction surgery.
Results: Between August of 2016 and August of 2023, 26 patients received partial breast reconstruction using 3D-printed patient-specific scaffolds. The median follow-up was 58.9 months (range, 29 to 88 months). Patients who received partial breast reconstruction with 3D-printed scaffolds have natural-looking and symmetrical breasts after surgery. One year after operation, mild depression at the implantation site was observed in four patients (15.4%). This number increased to seven (26.9%) after two years of surgery. No flap necrosis or ischemia was observed in the nipple and areola area in all patients. Hematoxylin & Eosin showed that fibrous connective tissue, fibroblast cells and blood vessels were observed inside/around the scaffold after one year of surgery. The average degradation rate

of 3D-printed scaffolds is 54.07% at 12 months, 74.48% at 24 months, 86.94% at 36 months, 87.36% at 48 months, and 92.76% at 60 months. The breast satisfaction score rating by Breast-q scale is 68.5±15.7 at 6 months, 65.4±14.2 at 12 months, and 62.8±15.9 at 24 months.
Conclusion: This is the first report of 3D-printed biodegradable scaffolds for breast reconstruction. Partial breast reconstruction using a 3D-printed scaffold presents a viable alternative for breast cancer patients who are not suitable candidates for conventional breast-conserving surgery, yielding promising outcomes.

P4-05-01: Retrospective Analysis of fam-Trastuzumab Deruxtecan Treatment in Advanced Breast Cancer Patients: Real World Experience at Mayo Clinic in Arizona (2022-2024)

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Background: Fam-trastuzumab-deruxtecan (T-DXd) was first approved for metastatic breast cancer disease with iHER2 3+ positivity by IHC and/or FISH amplification . Subsequently, it gained FDA approval for HER2 low disease. Recent clinical trials demonstrated significant benefits in a broader patient population, including those with HER2 ultra-low disease potentially expanding its use in more than 85% of patients with advanced breast cancer. Treatment-induced interstitial lung disease (ILD) associated with T-DXd remains a concern that poses unique challenges for the treating oncologists. Understanding the prevalence, characteristics, and risk factors of ILD with T-Dxd in real world clinical settings is crucial for improving patient outcomes .

Methods: In this retrospective study, we included patients with advanced breast cancer treated with T-DXd at the Mayo Clinic Comprehensive Cancer Center in Arizona from July 1, 2022 to January 31, 2024. The electronic medical record extract tool (SlicerDicer) was utilized for patient identification and data collection. Data was collected on patient demographics, breast cancer subtype, treatment course, ILD diagnosis and treatment. The objectives of this retrospective review was to identify the rate of and risk factors for developing ILD with T-DXd. Analyses were conducted using SAS version 9.04. Continuous variables were summarized using median and interquartile range (IQR). Categorical variables were described using frequencies and proportions. Kruskal-Wallis tests and Fisher's exact tests were used to assess the differences between patients with and without ILD.

Results: A total of 64 patients with breast cancer who received at least one dose of T-DXd were identified. Most patients were females (96.9%) and white (82.8%). Approximately, 10.9% of patients were Black and 6.3% were Asian. 40.4% had HER2+ disease, 59.6% had HER2 low disease. Pulmonary comorbidities included asthma (25.8%), history of pneumonitis (9.7%), COVID-19 infection (3.2%), and COPD (1.6%), with 35.9% of patients having a prior history of smoking. Of the 64 patients who received T-DXd, more than 20%

of patients required at least one dose reduction of TDXd with 14.1% requiring 1 dose reduction and 6.3% requiring 2 dose reductions because of side effects. 8 developed ILD (12.5%). The median age at ILD diagnosis was 56.6 (IQR, 55 - 66) Patients who developed symptoms presented with cough (42.9%), shortness of breath (57.1%), and had a median spO2 87% (IQR, 85-89%). The median time to ILD development was 197 days (IQR, 165 – 634). Ground-glass opacities and consolidations were commonly seen on imaging (75% and 50%, respectively). Of the 8 patients diagnosed with ILD, 3 were hospitalized during their presentation. Of these, 2 were required to be admitted to the ICU and were then released from the hospital and 1 patient transitioned to inpatient hospice. All patients with ILD were treated with high dose corticosteroids and none of them were rechallenged with T-DXd. Analysis of demographics data between patients with vs. without ILD revealed that there is no difference between the two groups with the exception of race. Race was an independent predictor of ILD, with 4 out of 53 vs. 2 out of 7 vs. 2 out of 4 patients developing ILD in the White vs. Black vs. Asian patient population with a significant p-value of 0.0178.

Conclusion: In this study, the incidence of ILD with T-DXd was 12.5%. Demographic differences were not significant, except for race. However, due to the small sample size, we should be cautious when extrapolating these findings. Larger studies are needed to confirm these results and identify additional risk factors for ILD in real-world clinical settings. Recognizing risk factors can guide monitoring and improve guidelines for managing ILD in breast cancer patients receiving T-DXd.

P4-05-02: Strategies for premedication and neutropenia prophylaxis in sacituzumab govitecan treatment of patients with triple-negative breast cancer: multicenter insights

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Background: Sacituzumab govitecan (SG) is approved for use in the second line and beyond for advanced triple-negative breast cancer (TNBC), improving survival outcomes compared to standard chemotherapy. As demonstrated by the ASCENT trial and real-world data, typical adverse events (AEs) of SG include myelosuppression, diarrhea, nausea, vomiting, or hepatic toxicity. Premedication strategies should address these AEs. In patients with specific AEs, additional prophylaxis, such as granulocyte colony-stimulating factor (G-CSF), may be required. The purpose of this study was to evaluate premedication policies, adjustments, and G-CSF application in patients with TNBC treated with SG.

Materials and Methods: In this ambispective multicenter cohort study (five oncology units in Poland), we collected data on the prophylactic application of G-CSF and initiation and modification of premedication with various agents during SG treatment in patients who

completed SG therapy for any reason by April 2024 and received at least one full SG cycle (day 1 and day 8). We evaluated the use of drugs such as acetaminophen, corticosteroids, atropine, H1/H2 blockers, 5-hydroxytryptamine 3 (5-HT3) receptor antagonists, neurokinin-1 (NK1) receptor antagonists, filgrastim, and pegfilgrastim/lipegfilgrastim. Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0.

Results: Fifty female patients met the inclusion criteria. The median age at treatment initiation was 52.6 years, and the median number of SG cycles completed was 4 (cycle 4, day 8). During the first cycle, 47 patients (94%) received 1 gram of iv acetaminophen, while 3 patients did not receive it throughout their treatment. All patients received steroids (typically 8 milligrams of iv dexamethasone) and 5-HT3 antagonists (usually 8 milligrams of iv ondansetron). One patient required the addition of NK1 antagonists (aprepitant at typical doses for 3 days) in later cycles. Thirty-six patients (72%) received H1 blockers (2 milligrams of iv clemastine), with one patient receiving it in later cycles due to a hypersensitivity reaction. Thirty-eight patients (76%) received H2 blockers (40 milligrams of oral famotidine), with none requiring its addition in later cycles.
18 patients (36%) received G-CSF as primary prophylaxis starting on day 2 or 3 of cycle 1 due to a prior history of neutropenia or febrile neutropenia. However, as many as 40 patients (80%) required G-CSF as primary or secondary prophylaxis throughout the SG treatment. Typically, filgrastim was administered for 3-4 days starting at day 2-3 and for 5 consecutive days starting at day 9-10 (or pegfilgrastim/lipegfilgrastim as a single dose on day 9-10 in 10 patients). G-CSF was usually initiated due to grade 3 neutropenia, with a median onset after cycle 2, day 1. None of the patients received subcutaneous atropine on cycle 1, day 1, but 3 required it before subsequent cycles due to grade 3 diarrhea. In 6 patients, various schedules and types of steroids were used for secondary prophylaxis of neutropenia, and in 1 patient for thrombocytopenia.

Conclusions: The majority of patients received prophylaxis with acetaminophen, H1/H2 blockers, steroids, and 5-HT3 antagonists. SG AEs are well-known and predictable, resulting in similar prophylactic approaches for majority of patients. The most significant disparities were related to G-CSF application in primary and secondary prophylaxis. Patients typically required G-CSF initiated as early as during the second cycle of SG. The use of steroids as secondary prophylaxis for neutropenia remains controversial and should be avoided. Only a few patients required atropine as secondary diarrhea prophylaxis.

P4-05-03: Acceptability and feasibility of early cancer physiatry consultation in management of aromatase inhibitor-induced musculoskeletal symptoms (AIMSS) in early-stage hormone receptor positive breast cancer

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Aromatase inhibitors (AIs) are the mainstay of adjuvant therapy for postmenopausal hormone receptor (HR)-positive breast cancer (BC). AI-induced musculoskeletal symptoms (AIMSS) are a common side effect seen in up to 50% of patients, leading to discontinuation in 20-30%. This non-adherence is an independent predictor of mortality. While guidelines for AIMSS management are lacking, exercise, physical therapy, and acupuncture offer benefit, and pharmacologic options exist. However, preventative measures remain unexplored. Physiatrists, physicians who specialize in physical medicine and rehabilitation, can employ conservative measures that may help to treat and/or prevent AIMSS. This study evaluated the feasibility and acceptability of early physiatry consultation for AIMSS.

Methods: Patients with early-stage HR-positive BC starting AI therapy were enrolled in this prospective study. Patients underwent baseline physiatry consultation before AI initiation, followed by visits at 6 weeks, 3 months, and 6 months. Primary objectives were feasibility (determined by appointment attendance) and acceptability (measured by the validated Treatment Acceptability and Preference (TAP) score at 6 months), a mean TAP score above mid-point scale of 2, deems the intervention acceptable.

Results: 25 patients enrolled in this prospective study. 76% were African American, and 24% were Caucasian. The mean age was 66.2 years. The mean BMI was 28.2. 100% of patients attended all physiatry clinic visits, demonstrating the intervention is feasible. The average TAP score of the cohort was 2.7 (SD +/- 0.9) indicating that early physiatry consultation is acceptable. During physiatry visits, diagnostic imaging was performed in 24% of patients and 40% were offered referral to physical therapy. 2 patients were offered bracing, 2 patients received injections, 3 patients were given prescription medications, and 6 patients were offered over the counter (pain) medications. More than 50% were prescribed topical NSAIDS.

Conclusion: Early cancer physiatry consultations are feasible and acceptable for BC patients on AI therapy. A larger prospective study with a control group is needed to evaluate if this multidisciplinary approach can reduce AIMSS, thus promoting adherence and potentially improving survival outcomes. Physiatry consultation can offer counseling and diverse pharmacological/non-pharmacological interventions for AIMSS management.

P4-05-04: Evaluating the ePRO-based follow-up system (Pink Ribbon Diary) on quality of life and treatment adherence in patients with breast cancer receiving CDK4/6 inhibitors (JPRO-B)

Rie Ozeki, Hideo Shimizu, Kotaro Iijima, Misato Okazaki, Ayumi Watanabe, Sachiko Taka, Kouji Yamamoto, Junichiro Watanabe, Mitsue Saito, Goro Kutomi

Introduction: Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) improve the prognosis of estrogen receptor-positive (ER+) HER2 negative (HER2-) advanced breast cancer (ABC) patients, however, our concerns are treatment-related toxicities ≥ Grade 2 during treatment, decrease of QOL and influence on adherence to medication. We have recently developed an electronic patient-reported outcome (ePRO), the Pink Ribbon Diary (PRD), that allows patients to record their medication and their physical conditions easily to detect side effects as early as possible, in order to maintain treatment adherence and QOL. We investigated the clinical efficacy of the PRD in patients with ER+HER2-ABC undergoing CDK4/6i-based therapy.

Methods: Seventy patients were enrolled in this single-center, randomized, open-label, placebo-controlled, phase II trial. Patients with ER+HER2-ABC receiving oral CDK4/6i-based therapy were assigned 1:1 to either arm A using the ePRO system or arm B receiving usual care. Participants in arm A were required to record their medication taken and physical conditions daily for 3 months, and report adverse events weekly using PRO-CTCAETM. Participants in arm B were also asked to answer to questions about adverse events using PRO-CTCAETM at monthly visits during the three-month period. All participants were asked to complete to the EORTC QLQ-C30 at their monthly visits and were administered an adherence questionnaire survey before and after the study. The primary endpoint was the change in global health status/QOL (EORTC QLQ-C30) from baseline to 3 months.

Results: Sixty-five participants were eligible for data analysis. Three months global health status/QOL score went from 64.8 to 67.4 in arm A, a change from baseline of 2.6 points (N=32), and from 65.7 to 64.1 in arm B, a change from baseline was -1.5 points (N=33) (P=0.4552). In a post questionnaire, 96.9% of patients reported that the PRD was easy to use, with the most useful feature of the PRD being the ability to record medication (81.3%). All also indicated that communication with their health care providers in the PRD helped patients feel more relief and motivated to take treatment.

Conclusions: The use of the PRD, while not significant, tended to improve QOL, and support patients in taking their medications, indicating that communication with health care providers leads to safe medication use.

P4-05-05: Investigating the Efficacy and Safety of a Dose-Dense Paclitaxel, Cyclophosphamide with Trastuzumab in Stage I-III Human Epidermal Growth Factor Receptor 2 (HER2) Positive Breast Cancer

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Background: The evolution of systemic therapies has improved outcomes for patients with human epidermal growth factor receptor 2 positive (HER2+) breast cancer. Nonetheless, the tolerability and safety profile of systemic therapies represent an area for further improvement. Here we report the results of a phase 2 trial evaluating a non-anthracycline, non-platinum adjuvant treatment regimen for patients following initial surgical resection.

Methods: We enrolled patients with stage I or II HER2+ breast cancer who underwent upfront surgery to receive adjuvant treatment with 6 cycles of dose-dense Paclitaxel, cyclophosphamide and Trastuzumab (PC-H) every 2 weeks, followed by 13 cycles of maintenance trastuzumab every 3 weeks to complete 52 weeks of treatment (comprising 19 cycles). The primary objective was to determine the safety and feasibility of adjuvant PC-H measured by the completion rate and the frequency and the grade of adverse events, using National Cancer Institute Common Terminology Criteria. The secondary objective was to estimate relapse-free survival and overall survival.

Results: Between 2010 and 2019, a total of thirty-nine patients were enrolled. Of those, 34 patients (87.18%) completed the planned treatment. Sever adverse events of grade 3 or 4 occurred in 27 patients (69.23%), including 3 patients (7.69%) with grade 3-4 decrease in ejection fraction. At median follow up of 5.6 years, all 39 patients were alive. The 5-year relapse-free survival was 94.30% (95% CI: 75.3-100).

Conclusions: PC-H regimen demonstrated overall safety and efficacy, yielding high rates of relapse-free survival among patients with early stage (HER2+) breast cancer.]

P4-05-06: The role of music therapy, guided meditation and acupuncture in attenuating chemotherapy-induced cognitive impairment in breast cancer.

Jules Cohen, Gaurav Sharma

Background: Breast cancer treatment, particularly chemotherapy, is known to cause cognitive impairment, often referred to as "chemo fog" or "chemo brain," which can persist for months to years after treatment has been completed. Currently, there are no proven options to attenuate the development of cognitive impairment in chemotherapy patients.

Some non-pharmacological interventions that may help alleviate chemotherapy-induced cognitive impairment include music therapy, guided meditation (or mindfulness), and acupuncture. Music therapy has been shown to activate cortical areas in the brain that help with emotional processing and stress while acupuncture is hypothesized to promote neuroplasticity, reduce inflammation and improve cerebral blood flow. Guided meditation has been shown to improve memory and executive function potentially by preventing neuronal loss during chemotherapy. Acupuncture, guided meditation and music therapy have all shown promise in alleviating cognitive and emotional symptoms in those with mild cognitive impairment and dementia. Their efficacy in addressing chemotherapy-induced cognitive impairment (CICI) in breast cancer patients remains underexplored.

Objective: This study aims to evaluate the effectiveness of music therapy, guided meditation and acupuncture in mitigating cognitive impairment in breast cancer patients undergoing chemotherapy. We hypothesize that these complementary interventions will blunt any cognitive impairment in patients receiving neoadjuvant and adjuvant chemotherapy.

Methods: Participants will be randomly assigned to one of four groups: (1) guided meditation and music therapy; (2) guided meditation and acupuncture; (3) music therapy and acupuncture; and (4) no intervention. Cognitive function will be assessed using a PROMIS NIH questionnaire across domains related to anxiety, depression, cognition, fatigue, sleep, companionship, and emotional support. Data will be analyzed to compare the efficacy of the intervention groups against each other as well as against the control group.
Results: We anticipate that the combined intervention of acupuncture and music therapy will result in significant improvements in cognitive function compared to other interventions and the control group. We believe that the domains most likely to be affected include anxiety, depression, cognition, and emotional support. We anticipate that the (1) guided meditation and music therapy and (2) guided meditation and acupuncture intervention groups will also show improvements in cognitive function compared to the control group.

Conclusions: The study aims to provide evidence that the combination of acupuncture and music therapy offers a novel, non-pharmacological approach to alleviating chemotherapy-induced cognitive impairment in breast cancer patients. If successful, these interventions could be incorporated into standard supportive care practices, improving the overall quality of life for patients suffering from cognitive deficits post-chemotherapy. Further research will be needed to fully understand the underlying mechanisms and optimize treatment protocols to attenuate chemotherapy-induced cognitive dysfunction.

P4-05-07: Estimating Clonal Hematopoiesis Prevalence in Women with Breast Cancer: A Meta-Analytic Approach Stratified by Age

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Introduction: Clonal hematopoiesis (CH), characterized by genetically distinct blood cell subpopulations arising from somatic mutations, becomes more prevalent with age and is linked to higher risks of hematologic malignancies and cardiovascular diseases. In breast cancer (BC), the relationship between CH and cancer-related mutations is an emerging area of study, as the genetic interplay between these mutations and cancer development is puzzling. CH-related mutations have been shown to alter solid tumor microenvironment and are speculated to significantly influence angiogenesis, which could underscore CH as influential to BC development and progression. Moreover, cytotoxic treatments' myelotoxic effects on this parameter are not fully understood. This meta-analysis synthesizes data on CH incidence in women with BC, estimating its prevalence and helping elucidate the necessity of a specific CH screening in this population.

Methods: We searched the PubMed, EMBASE, and Cochrane databases for studies reporting the prevalence of somatic mutations related to CH in women with BC. For studies providing data at both baseline and during treatment, we selected the treatment data to account for possible treatment effects. To account for age-related differences, as this highly influences CH incidence, we stratified the data into three groups: young (≤40 years), characteristic (41-69 years), and older (≥70 years) patients. We excluded studies that did not report the mean or median age of patients, had a variant allele frequency threshold for CH mutations lower than 0.02 (2%), or only made composite assessments with other tumors available. Proportions of CH incidence were pooled using a meta-analysis of proportions with logit transformation, performed in the software R.

Results: Three full articles and five abstracts were included in the meta-analyses. Of these, five studies had patients with a mean or median age in the characteristic group (41-69 years), which had an estimated CH incidence of 15% (95% CI: 13%-17%). Two studies focused on older populations (≥70 years), with an estimated CH incidence of 55% (95% CI: 42%-68%). Only one study focused on younger women (≤40 years), with an estimated CH incidence of 4% (95% CI: 3%-6%). The subgroup differences were evident in the forest plot and confirmed by moderator analyses ($p < 0.001$ for all comparisons).

Conclusion: Our meta-analyses underscore the prevalence of CH in women with BC, which closely mirrors statistics and age-related tendencies observed in the general population. Our data reaffirms age as a significant factor in CH development. This research does not show evidence supportive of CH specific screening for BC populations, although it reinforces

the standard investigation in older patients. Nonetheless, specific treatments or risk factors may make investigation in younger ages valid. Further research, spanning different age groups and treatment modalities, is crucial for advancing our understanding of CH in BC and optimizing therapeutic interventions.

P4-05-08: Impact of antiemetic prophylaxis on maintaining dose intensity of trastuzumab deruxtecan in HER2-positive advanced breast cancer

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Introduction: Maintaining dose intensity is crucial for optimizing outcomes in patients with HER2-positive advanced breast cancer (ABC) treated with trastuzumab deruxtecan (T-DXd). Nausea and vomiting are among the most frequent adverse events associated with T-DXd and often lead to dose reductions, potentially compromising efficacy. While T-DXd is classified as moderately to highly emetogenic, clinician education on appropriate antiemetic prophylaxis is crucial, especially given the extended treatment duration due to its remarkable efficacy. This analysis investigated the impact of guideline-recommended antiemetic prophylaxis in the first cycle on maintaining T-DXd dosing over time.

Methods: Data from patients with HER2-positive ABC treated with at least two cycles of T-DXd were extracted and analyzed from the clinical data warehouse of Samsung Medical Center. Patients were grouped based on the use of a guideline-recommended triple antiemetic regimen (TAR) in the first cycle of T-DXd. The TAR consisted of either the fixed combination of netupitant/palonosetron (NEPA) or the combination of an NK1 receptor antagonist (RA) and a 5-HT3 RA plus dexamethasone. The proportions of patients requiring a reduction from the 5.4 mg/kg starting dose of T-DXd to 4.4 mg/kg and 3.2 mg/kg were calculated for both TAR and non-TAR groups.

Results: Between June 29, 2022 and May 5, 2024, 59 patients with HER2-positive ABC received a median of 7 cycles (range, 2–30) of T-DXd. The median age at initiation of T-DXd was 55 (range, 28–79). Prophylactic TAR was administered to 49 (83%) patients in the first T-DXd cycle: 46 with NEPA and 3 with other NK1 RA and 5-HT3 RA combinations. Ten patients (17%) initially received either a 5-HT3 RA alone (n=9) or no prophylaxis (n=1). Patients who received TAR in cycle 1 were less likely to require T-DXd dose reduction to ≤4.4 mg/kg (24% vs 50%, p=0.133) or ≤3.2 mg/kg (0% vs 10%, p=0.169), with a significantly greater magnitude of dose reduction observed in patients who did not receive TAR in cycle 1 than those who did (P=0.011). Following an institutional policy change favoring NEPA due to the high incidence of nausea and vomiting with other regimens, 8 of 9 patients initially on a 5-HT3 RA alone and 2 of 3 patients initially on other NK1 RA and 5-HT3 RA combinations switched to NEPA. At the data cutoff, no patients discontinued T-DXd due to intractable nausea and vomiting; 44 (75%) patients continued to receive T-DXd, while 8 (14%), 4 (7%), and 2 (3%) patients discontinued due to disease progression, death,

or pneumonia/pneumonitis, respectively. One patient was lost to follow-up after cycle 2.

Conclusion: These results highlight the previously unreported finding that administering guideline-recommended TAR prophylaxis facilitates maintaining the dose intensity of T-DXd throughout treatment. Using an appropriate upfront antiemetic regimen when initiating T-DXd is critical to optimize patient outcomes.

P4-05-09: ERIBULIN'S ARCANE ODYSSEY: UNRAVELING STEVEN JOHNSON SYNDROME (SJS) /TOXIC EPIDERMAL NECROLYSIS (TEN) IN METASTATIC BREAST CANCER

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Abstract: Eribulin mesylate is a non-taxane inhibitor of microtubule dynamics belonging to the halichondrin class of antineoplastic drugs. It is a novel agent that inhibits the microtubule growth phase without affecting the shortening phase, causing tubulin sequestration into non-productive aggregates. Eribulin is used as monotherapy in heavily treated metastatic breast cancer patients resistant to taxane or anthracycline-based chemotherapy. Common Grade 3 or 4 adverse events with Eribulin include neutropenia (52%), leucopenia (23%), thrombocytopenia (19%), and peripheral neuropathy (35%). Cutaneous manifestations with Eribulin are uncommon. However, Steven Johnson Syndrome (SJS) / Toxic Epidermal Necrolysis (TEN) is a rare and severe mucocutaneous reaction characterized by extensive necrosis and detachment of the epidermis, often triggered by medications. Here we present a case of SJS/TEN caused by Eribulin in a woman with Metastatic Breast cancer.
Introduction: A 55-year-old post-menopausal hypothyroid woman was diagnosed with triple-negative breast cancer (TNBC) with metastasis to the liver and non-regional nodes. She was found to be refractory to anthracycline, docetaxel, capecitabine, and platinum agents and was thus planned for Eribulin monotherapy as a third-line agent. On day 11 of Cycle 1 of Eribulin at 1.4 mg/m^2, she presented with high-grade fever and a rash over her torso and arms, which gradually spread to approximately 80% of her body surface area. Further examination revealed Grade 2 oral mucositis as per the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. The rash initially presented as targetoid lesions involving the torso and then gradually covered up to 80% of the body surface area, sparing the scalp. Later, the rash became confluent and formed flaccid bullae, but the Nikolsky sign was negative. She had a persistent high-grade fever with temperatures reaching up to 104°F. All infective workup evaluations were negative. Her lab parameters suggested Grade 2 transaminitis, Grade 2 hypokalaemia, and hyponatremia as per CTCAE v5.0. Blood culture and swab culture from the skin lesions were negative. She was non-neutropenic throughout the course. A skin punch biopsy of the largest lesion on her back showed trans-epidermal neutrophilic infiltration, suggestive of a drug-induced hypersensitivity reaction. Ruling out

other infective etiologies and considering the temporal relationship between initiating Eribulin therapy and the development of the rash, she was clinically diagnosed with Stevens-Johnson Syndrome (SJS) / Toxic Epidermal Necrolysis (TEN). Further probing confirmed that her drug history did not indicate any alternative medications intake. Due to persistent high-grade fever, she was started on empirical antibiotics, meropenem, and teicoplanin. As there was no clinical improvement over 24 hours, she was started on dexamethasone at 24 mg/day. Over 15 days, with gradual tapering, her skin lesions healed by more than 75% from baseline. She was discharged in a hemodynamically stable condition. On her first follow-up visit after 15 days, her lesions had healed, with mild hyperpigmentation persisting over her face and arms.

Conclusion: SJS/TEN is a life-threatening mucocutaneous reaction that typically is not associated with chemotherapy. We present a case of SJS/TEN where the drug eribulin was identified as the causative agent. Despite the high mortality rate of TEN (>30%), our patient successfully recovered, likely due to the prompt recognition of the syndrome, immediate discontinuation of the causative drug, and timely treatment with steroids. This case underscores the critical importance of considering SJS/TEN in any patient presenting with an extensive, desquamative rash following eribulin chemotherapy. Quick intervention can be the difference between life and death, highlighting the need for vigilance and rapid response in managing adverse drug reactions.

P4-05-10: Outcomes of Chemotherapy-Induced Peripheral Neuropathy (CIPN): Psychological Impact and Predictive Modeling

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Introduction: CIPN from taxanes involves numbness, tingling, or burning or shooting pain in the hands and feet. In some patients, these effects are debilitating and can interfere with treatment, leading to taxane dosing changes or discontinuation. The aim of this study was to use a machine-learning approach to build a predictive model of CIPN from clinical data and patient reported outcomes (PRO) collected before taxane treatment. The study hypothesis was that predictive model of CIPN would identify patients that would develop CIPN.

Methods: In a multicenter observational study, breast cancer patients receiving taxane treatment were invited to participate over 12 months period. PRO were collected at seven visits before (visit 1), during (visits 2-4), and after (visits 5-7) taxane treatment. The outcomes included assessments of CIPN, anxiety, and depression. The EORTC QLQ-CIPN20 was used to evaluate the presence and severity of CIPN. Moderate to severe CIPN was defined as an 8-point increase from baseline. Anxiety was measured using Generalized Anxiety Disorder 7-item scale (GAD-7). The GAD-7 score was interpreted as no (0-4), mild (5-9), moderate (10-14) and severe (15-21) anxiety. Depression was measured using PHQ8.

PHQ8 depression raw scores are categorized as follows: none (0-2), mild (3-5), moderate (6-8), and severe (9-12). Wilcoxon-ranked sum tests were used to explore differences in patient reported outcomes between time points and between patients that did and did not develop CIPN. P values were adjusted using a False Discovery Rate (FDR) approach. Using machine learning, a Random Forest model, based on 500 trees and three variables per split (randomly selected features for each decision point), was built using clinical features before taxane initiation to predict CIPN. Features included GAD7, PCS (Pain Catastrophizing Scale), PHQ-8, BPI (Brief Pain Inventory), PROMIS physical and sleep scores, dietary habits, baseline CIPN scores, age, race, smoking status, chemotherapy regimen, CCI (Charlson Comorbidity Index), and BMI. Model accuracy was assessed using Out of Bag (OOB) error analysis, providing an unbiased estimate of the generalization error.

Results: From January 2021 to February 2024, 400 patients were enrolled. Clinical and PRO data from visits 1 through 7 were used in this analysis. 241 patients completed all 7 visits, out of which 182 developed CIPN while receiving taxanes. For the patients who completed all 7 visits (241), median anxiety scores dropped from 4 (IQR = 1-7) at baseline to 2.3 (IQR 1-5.3) (FDR P = 7.1 x 10-9) during treatment and 2.3 (IQR 0.3-5) (FDR P = 7 x 10-10) after treatment. While on treatment, median anxiety scores were 2.5 (IQR = 1-5.9) and 1.5 (IQR = 0.4-3.5) (FDR P=.03) for patients with and without CIPN. The PHQ8 depression scores were analyzed at baseline for all 400 patients. Median raw PHQ8 depression scores at baseline were 4 (IQR 2-7) and 3 (IQR 1-5) (FDR P = 0.03) for patients with and without CIPN. Using data from visit 1 (baseline visit before taxane therapy), the Random Forest model's ROC predictive accuracy was 0.72 in identifying patients that would develop CIPN. Of all the clinical and patient reported outcomes, BMI, age, anxiety scores, and depression scores were the most influential in predicting CIPN.

Conclusion: While on taxane treatment, although anxiety and depression scores were statistically different between patients with and without CIPN, scores in both groups suggested no clinically relevant anxiety or depression.
Model prediction of CIPN was poor suggesting that clinical and PRO data alone may be inadequate to predict CIPN. Future work is warranted exploring the use of molecular biomarkers to improve model accuracy.

P4-05-11: Physical changes caused by low-dose Tamoxifen therapy

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Background: Ten-year data of the phase III trial TAM-01 showed that low-dose Tamoxifen reduced recurrence of invasive breast cancer and ductal carcinoma in-situ.
Purpose: The first objective of the study was to learn about the gynecological and other side effects caused by low-dose Tamoxifen therapy in daily practice. A second objective was to report disease outcome.

Methods: The study group consisted of women with DCIS who received low-dose Tamoxifen and did their follow-up at Davidoff Center in Israel. Consecutive patients from July 2019 to July 2021 were enrolled. The endometrial thickness, uterine fibroid and uterine polyps were assessed by gynecological US. General potential side effects were assessed by a questionnaire.
Results: A total 33 patients were enrolled. 23 of them were included in the study. The median follow-up was 50 months (38, 60). The median age at diagnosis was 51 (40,66) years. 8.6% of the women were referred to an endometrial biopsy or polypectomy during the study period, none of them discovered malignancy. The median endometrial thickness before the treatment with Tamoxifen was 4 (2,9) mm, the median maximal endometrial thickness during the treatment with Tamoxifen was 8 (4,15) mm. New symptoms were developed during the treatment, 47% of the women reported hot flashes, 43% leg cramps and 37.5% of premenopausal women reported prolongation of the menstrual cycle. One (4.3%) of the women developed DCIS recurrence.
Conclusions: Low-dose Tamoxifen therapy is associated with increased endometrial thickness, formation of new endometrial polyps, and general side effects such as hot flashed and leg cramps.

P4-05-12: A meta-analysis of adverse events associated with anti-PD-1 therapy

Balazs Gyorffy, Janos Tibor Fekete

Anti-PD-1 therapies have revolutionized cancer treatment by significantly improving clinical outcomes. However, the safety profile of these therapies remains a critical concern. This meta-analysis aimed to evaluate the correlation between anti-PD-1 therapy and the occurrence of adverse events (AEs).
We systematically analyzed data from ten studies, encompassing a total of 4,379 patients treated with anti-PD-1 agents and 3,720 control subjects. The statistical platform available at www.metaanalyisonline.com was used for data analysis. Using a random effects model with the Mantel-Haenszel method, we observed a statistically significant increase in the risk of AEs in the anti-PD-1 cohort compared to controls (risk ratio: 2.15, 95% CI: 1.39 - 3.32, $P<0.05$). Despite the observed association, substantial heterogeneity was detected among the studies ($P<0.01$, $I^2 = 95\%$), indicating variability in the magnitude and direction of effects. Funnel plot analysis and Eggers' test revealed potential publication bias (intercept: 3.85, 95% CI: 1.39 - 6.3, P=0.015). Additionally, a Z-score plot indicated that the cumulative sample size (n=8,099) did not reach the optimal threshold for a definitive conclusion (n=24,056 at a significance = 0.05, type II error level = 0.2, and minimal clinically relevant outcome = 30%).
These findings highlight the importance of continuous monitoring and reporting of AEs in patients undergoing anti-PD-1 therapy to ensure a comprehensive understanding of its safety profile. However, currently available studies show considerable heterogeneity and insufficient number of cases, suggesting the need for extensive further research.

P4-05-13: The quality of life of women after treatment of breast cancer is lymphostasis as a life-changing complication.

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Relevance: Lymphedema (lymphostasis) is an edema of the hand characterized by the accumulation of fluid in the intercellular space, which develops as a result of dysfunction in the lymphatic system. Lymphedema develops in about 40% of patients within 8-10 years after surgical treatment in the volume of axillary lymphadenectomy. In the case of axillary lymphadenectomy in combination with radiation therapy, the risk of lymphedema in some cases reaches 60%. Also, even in the case of a biopsy of "signal" lymph nodes, the risk is 4-5%. Complete Decongestive Therapy (CDT) is a method of treating lymphedema (lymphostasis), which is currently considered the "gold standard" in the treatment of lymphedema in Europe and the United States. This method involves a special lymphatic drainage massage and banding the upper limb with the subsequent wearing of compression knitwear.

Research methods: Materials and methods: Two doctors and 2 nurses have been specially trained in our surgical department to introduce conservative techniques into practice. 30 patients who had previously received treatment in our center and who were diagnosed with stage 2-4 lymphostasis using ICG lymphography, lymphoscintiography, volumetric measurements, physical examination were examined. The main complaints of patients are the feeling of heaviness and schedule in the upper limb on the side of the operation, swelling of the upper limb, dysfunction of the upper limb on the part of the operation, recurrent erysy inflammation. The average volume difference between a healthy hand and a hand in which lymphostasis developed was 5-6 cm. At the same time, there was a positive symptom of Stemmer and Pitting test on the affected arm.

Results. Patients were given courses of complex physical anti-edema therapy. The number of courses ranged from 5 to 10 courses. After the courses, the patients subjectively noted an improvement in general well-being in the form of a decrease in gravity in the affected limb, a decrease in puffiness, restoration of limb function. The average difference in volume between a healthy hand and a hand in which lymphostasis developed before the procedures was 5-6cm, after the procedures 1-2cm. Patients were performed a control ICG lymphography, on which positive dynamics was observed.

Conclusions: Complete Decongestive Therapy (CDT) is the "gold standard" of lymphostasis treatment, which leads to an improvement in the quality of life. This method does not create an economic burden, as it requires only specially trained medical personnel and special bandages. The high effectiveness of this method and the lack of alternative conservative methods of treatment of lymphostasis indicate the need to introduce this method of

treatment into medical standards of care for patients with lymphostasis. Mastering this technique in a surgical hospital allows you to better understand the mechanisms of lymphedema development, as well as to take timely measures for prevention through special surgical interventions and/or training of patients.

P4-05-14: Prognostic factors of one versus two pregnancy associated breast cancer

Maxim Izquierdo

Aims. The five year interval pregnancy associated breast cancer has resulted in patients with two pregnancies. Study ans assess the diferences in the prognostic factors with one and two pregnancies associated with breast cancer
Methods. Study 134 pregnancies associated breast cancer treated in the same hospital, 110 with one pregnancy associated breast cancer and 24 with two pregancies associated breast cancer. Analyze prognostic factors: estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2), histological grade and Ki67, with one and two pregancies associated breast cancer
Results. 110 patients with one pregnancy associated breast cancer, estrogen receptor is positive in 89 (80'90%) patients and estrogen receptor inegative in 21 (19'09%) patients. 24 patients with two pregnancies associated breast cancer, estrogen receptor is positive in 14 (58'33%) patients and estrogen receptor is negative in 10 (41'66%) patients (p<0'05). One pregnancy associated breast cancer is progesterone receptor positive in 76 (69'09%) patients and progesterone receptor negative in 13 (54'16%) patients (p<0'05). HER. Ki67 and histological grade there were no differences with one or two pregnancies associated breast cancer (p=ns)
Conclusion. One or two pregnancies associated breast cancer over 5 years influence estrogen receptors and progesterone receptors, and do not influence HER2, Ki67 and histological grade.

P4-05-15: EVOLVE: Harnessing the Power of AI, RWD, and Precision Education to Minimize Breast Cancer Care Disruptions

Shashi Shankar, Jeff Carter, Josh Zalis

Background: HER2-targeted therapies have significantly advanced the treatment of HER2+ breast cancer. However, many of these therapies are associated with interstitial lung disease (ILD), a rare but potentially life-threatening adverse event (AE). The perceived risk of ILD often leads to premature discontinuation of effective HER2-targeted therapies, potentially compromising patient outcomes. Better understanding of ILD incidence and management using real world data is crucial to optimize care continuity and treatment efficacy in HER2+ breast cancer patients.

Methods: Project EVOLVE, a two-part quality improvement study, integrated Novellia's AI-powered personal health record platform with Prime Education's expertise in medical education. The study aimed to uncover ILD patterns and develop targeted interventions to minimize unwarranted therapy discontinuations.
Part 1: A real-time observational study analyzed over 500,000 unique health records of HER2+ breast cancer patients using Novellia's advanced AI-enabled data modeling to identify ILD-related patterns and treatment gaps.
Part 2: Development and implementation of precision educational interventions based on Part 1 findings for patients and their treating HCPs. Patient interventions included personalized modules on cancer basics, treatment adherence, and side effect management. HCP interventions focused on patient-specific care strategies and advanced ILD management techniques.

Results: Part 1 Findings:

Only 4.17% of documented respiratory AEs were identified as ILD, lower than generally perceived risk (n=24)
Primary respiratory AEs: asthma (37.5%), common cold (20.8%), COVID-19 (8.3%)
65.79% of patients lacked COVID-19 vaccination history
Secondary survey (n=187): 67% cited fatigue as the most challenging side effect; 38% lacked awareness of cancer subtypes and stages

Part 2 Findings:
Patient Improvements (n=187):

Treatment plan adherence confidence: 72% to 89%
At-home side effect management confidence: 55% to 79%

HCP Improvements (n=275):

Correct identification of ILD as a boxed warning AE for trastuzumab deruxtecan: 19% to 47%
Confidence in individualizing treatment selection: 66% to 90%
AE identification and management confidence: 69% to 92%
Appropriate recommendation of trastuzumab deruxtecan in specific scenarios: 36% to 58%

Conclusions: Project EVOLVE demonstrated the efficacy of integrating AI-driven real-world data analysis with targeted educational interventions in optimizing HER2+ breast cancer care, particularly in managing ILD. The study revealed a lower-than-perceived ILD incidence, challenging prevailing risk perceptions. Significant improvements in HCP confidence and decision-making regarding AE management and treatment recommendations were observed. Patient outcomes improved substantially, with increased confidence in treatment adherence and side effect management. Novellia’s personalized

education approach was identified as a key benefit by 53% of patients. These findings suggest that this novel methodology effectively minimizes care disruptions due to ILD concerns, potentially improving treatment continuity and patient outcomes in HER2+ breast cancer. This innovative approach presents a promising model for addressing complex adverse events in oncology and beyond, paving the way for more personalized and effective cancer care strategies.

P4-05-16: FATTY ACID PROFILE ON RED BLOOD CELL MEMBRANE PREDICT BREAST CANCER SUBTYPE

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Introduction. Metabolic reprogramming is a hallmark of cancer development and progression. Regarding breast cancer (BC) limited evidence assert an association between metabolic changes and its impact on BC biology and subtype. Yet no clear data has evaluated the link between lipid metabolism, measured through red blood-cell membrane (RBCm) composition, and BC subtype determination. Because RBC are the main circulating cells and 90% of its surface is membrane, the fatty acid (FA) measurement on the RBCm seems to be a good marker for FA body status and BC cell relationship with systemic normal cells. Our study sought to determine if RBCm FA profiles, determined on a routine blood sample, could predictBC subtypes in non-metastatic BC patients. Methods. A prospective study assessing FA levels (Saturated:SFA; monounsaturated: MUFA, and polyunsaturated: PUFA) on RBCm from baseline peripheral blood samples obtained in BC patients, prior systemic or local treatments. The primary objective was to determine the relationship between FA lipidomic profiles measured in the RBCm and the four main BC subtypes, defined by the IHC of diagnostic biopsy. Subtypes were classified based on estrogen receptor positive (ER) and epidermal receptor growth factor type 2 (HER2) status determinations:ER+/ HER2-, ER+/HER2+, ER-/HER2+ and ER-/HER-. Results. A total of 77 stage I-III patients with BC were included. Regarding CcClinico pathological variables measured include (age, body mass index, histological grade, Ki67 andor stage). An elevated total SFA, and lower MUFA showed a significant association with ER+/HER2- BC (p= 0.0028). No differences for total FA values were found for other BC subtypes. Regarding specific FA analysis, the concentration of PUFAs alpha-linolenic acid (C18:2n6c, linoleic (ALA), p= 0.0001), C18:3n3 (a-linolenic, p= 0.0000), C20:5n3 (EPA, p= 0.0009) and C22:6n3 (DHA, p= 0.0000) were differentially expressed based on IHC subtypes. Conclusions: RBCm FA profile determination showed a significant diagnostic potential as a biomarker for BC subtype in non-metastatic BC. Measuring FAs in RBCm could offer a convenient, minimally invasive strategy to identify BC subtypes. Further research is needed necessary to validate the performance of FAs and their thresholds using an independent dataset for cross-validation to ensure robustness.

P4-05-17: PER2 Expression as an Independent Predictor of Shorter Disease-Free Survival in Breast Cancer Patients Undergoing Neoadjuvant Chemotherapy

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Background: The PERIOD2 (PER2) gene is a core component of the circadian clock and is crucial for maintaining daily rhythms. PER2, regulated by melatonin, is often linked to tumor development. Studies have shown that PER2 expression is downregulated in various tumors, and in vitro studies indicate that PER2 overexpression in tumor cells, including breast cancer cells, promotes apoptosis and reduces proliferation. Since melatonin interacts with estrogen receptors, hormone receptor status may influence PER2 expression. This study aimed to evaluate PER2 expression in different breast cancer (BC) molecular subtypes using immunohistochemistry (IHC) and to examine its association with disease-free survival (DFS) and overall survival (OS).
Materials and Methods: A retrospective cohort study was conducted at the Instituto do Câncer do Estado de São Paulo from January 2012 to September 2023. The study included women with stages II-III BC, who completed neoadjuvant chemotherapy (NAC) followed by surgery. Following histological and immunohistochemical analysis, samples from 100 patients were classified into the following molecular subtypes: Triple-negative, Luminal A, Luminal B, HER2 enriched, and Luminal B – HER2+. IHC reactions using the primary antibody for PER2 (C6 -sc-377290 Santa Cruz Biotechnology, Inc.) were performed and qualitatively classified as follows: absence of expression, weak/moderate expression, and strong/intense expression of PER2. Two independent researchers analyzed the IHC results. Clinical, demographical and outcome data were extracted from electronic medical records. Associations between categorical variables were evaluated using the Chi-square test. OS and DFS were assessed using the Kaplan-Meier method and the Log-Rank test. Statistical significance was defined as p-values less than 0.05.
Results: One hundred patients equally divided among Luminal A, Luminal B, HER2 enriched, Triple-negative and Luminal B – HER2+ molecular subtypes were selected. Five patients were excluded because of inadequate PER2 IHC staining. A total of 95 women were included, with a mean age of 54.4 years (range: 29-84) and a median BMI of 30.1 kg/m^2. Additionally, 14.9% were nulliparous and 54.7% were post-menopausal. Before NAC, 28.4% of patients presented with stage II BC and 71.6% presented with stage III BC. 4.2% of the patients had absence of expression of PER2, 44.2% presented weak/moderate expression and 51.6% presented strong expression. There was no difference in PER2 expression according to molecular subtype (p=0.769). After NAC,18.1% of the patients achieved pathological complete response (pCR), with a statistically significant association between pCR and molecular subtype (p=0.001). At a median follow-up time of 90.2 months (IQR 62.6-123.9) there were 31 recurrence events (32.6%) and 29 overall deaths (30.5%). There was a statistically significant association between DFS and PER2 expression (log-rank p=

0.0288). Median DFS was 56.1 months (95%CI 28.3 – NR) for patients with no expression of PER2, 48.5 months (95%CI 24.6-81.8) for patients with moderate expression and 25 months (95%CI 15.7-37.7) for patients with strong expression of PER2. In a Cox multivariate analysis, adjusting for molecular subtype, PER2 expression remained associated with DFS with an HR of 3.02 (95% CI 0.52-17.481, p=0.22) for moderate expression and an HR of 13.13 (95% CI 1.89-91.15, p=0.009) for strong expression when compared to absence of expression. There was no statistically significant OS difference according to PER2 expression in our cohort (p=0.92).
Conclusion: Our findings suggest that strong PER2 expression is an independent predictor of shorter DFS in breast cancer patients treated with NAC, highlighting its potential role as a prognostic biomarker. Further research is warranted to understand the underlying mechanisms and to explore potential therapeutic interventions targeting PER2 pathways.

P4-05-18: Modelling MHC-I downregulation in triple negative breast cancer to rescue resistance to ICB using diacylglycerol kinase inhibitors

Jacey Marshall, Justin M. Balko

Background: Immune checkpoint blockade (ICB) has recently revolutionized the treatment of triple-negative breast cancer (TNBC) and improved patient outcomes. However, most patients are intrinsically resistant and do not benefit from ICB. Moreover, no biomarkers to predict outcome have been clinically successful.
Antigen presentation by MHC-I molecules is required for CD8 T cell mediated tumor death and response to immune checkpoint blockade, e.g. a-PD-1/a-PD-L1 therapy. TNBCs downregulate MHC-I to suppress anti-tumor immunity and patients with decreased antigen presentation have worse outcomes to ICB. Thus, loss of MHC-I expression may be a candidate biomarker for lack of ICB response, and therapeutic strategies to overcome MHC-I loss are an area of high translational relevance and interest.
Diacylglycerol kinase α/ζ inhibitors (DGKis), which increase T cell activation, are being investigated to treat patients resistant to ICB. DGKis increase concentrations of diacylglycerol (DAG) in CD8 T cells by inhibiting conversion of DAG into phosphatidic acid, lowering the threshold for activation and amplifying TCR signaling and effector T cell function. We hypothesized that DGKis will improve response to ICB in TNBC with low MHC-I expression by improving CD8 T cell response to low antigen stimulation. However, modeling low MHC-I expression (as opposed to CRISPR-mediated KO) presents a technical challenge. Thus, we created a novel model of MHC-I downregulation, leveraging miRNA binding element mutations to titrate MHC-I expression. We assess changes in the tumor microenvironment and ICB response created by stepwise-decreased MHC-I expression and evaluate the potential of DGKis to treat MHC-I low tumors in combination with ICB.
Methods: We assessed the effect of DGKi/a-PD-L1 dual therapy on an MHC-I competent, baseline model of TNBC using the murine EMT6 cell line, which is moderately responsive to a-PD-L1 therapy. To model titrated levels of MHC-I downregulation in vivo, we utilized miRNA silencing-mediated fine-tuners (misFITs). We created an MHC-I null variant of EMT6

cells through knock out of beta-2-microglobulin (B2m), a required component for MHC-I expression. To create the misFITs, we reintroduced the B2m gene into the cells with the addition of varied binding regions for the endogenously expressed miR-17. Presence of an miR-17 binding region allows for miRNA-mediated suppression of B2m and thus, MHC-I. Seven different binding regions were introduced resulting in stepwise titration of MHC-I expression. To evaluate the effects of titrated MHC-I expression on tumor growth and survival, we orthotopically inoculated mice and treated weekly with a-PD-L1.
Results: In the EMT6 TNBC model, treatment with DGKi every 3 days in combination with weekly a-PD-L1 administration resulted in decreased tumor growth (Isotype control v. DGKi/a-PD-L1, p=0.0023, n=20) and doubled complete response rates from 20% in single-agent a-PD-L1 to 40% with combination therapy. Re-injection of complete responders with the original tumor cell line to assess persistence of response resulted in no tumor growth. Simultaneous bilateral injection of MHC-I null isogenic cells also resulted in significantly reduced growth, indicative of an MHC-I-independent memory response (p=0.0012, n=5-12). MisFITs representing 60%, 30%, and 10% of MHC-I levels in EMT6 cells grew at differential rates – faster with less MHC-I expression. Additionally, preliminary data demonstrates reduced a-PD-L1 benefit in mice with lower MHC-I expression.
Conclusions: We determined that DGKi/a-PD-L1 treatment enhances response in the EMT6 TNBC model and establishes a memory immune response. We have created a model of MHC-I downregulation and begun to establish how MHC-I affects a-PD-L1 response and will utilize the misFITs to assess the mechanism of resistance to response and the efficacy of DGKi/a-PD-L1 treatment in improving response to ICB in MHC-I downregulated tumors.

P4-05-19: Magnetic Resonance Elastography: A Novel Imaging tool to predict response in patients undergoing Neo-Adjuvant Chemotherapy for Breast Cancer

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Introduction: Patients undergoing neoadjuvant chemotherapy (NACT) for breast cancer are monitored using Magnetic Resonance Imaging (MRI) with Dynamic Contrast Enhancement (DCE), the gold-standard imaging technique to assess tumour response.
DCE-MRI has a sensitivity of 80-90% but a low specificity of 37-97% when assessing complete response (CR). Although alternative methods to assess CR have been attempted, such as biopsies, no highly accurate method has been identified. Magnetic Resonance Elastography (MRE) is a non-invasive imaging technique that uses biomechanics to identify tissue alterations within the tumour during NACT. With recent discussions regarding the de-escalation of surgical treatment for breast cancer post-NACT, an accurate assessment of CR is essential.
Methods: This prospective, first-in-human study includes patients undergoing five MRI-

MRE scans at different time points during the course of NACT. Breast MRE is a 7-minute sequence added to the routine clinical breast MRI, with mechanical vibrations applied to the patient's breast via paddles attached to a gravitational driver incorporated into a Siemens biopsy coil for a 1.5T system. The tissue occupying the tumour at diagnosis on DCE imaging was defined throughout the NACT treatment. Biomechanics within these regions were quantified, primarily observing relative changes in tumour stiffness (elasticity) between pre-NACT and post-NACT (tumour stiffness ratio: TSR). Relative changes in phase angle (the phase lag between viscosity and elasticity) in the specific tumour region after the 1st cycle from pre-NACT were also observed (phase angle ratio: PAR). Post-surgical histopathology was used to determine complete and partial responders. Furthermore, a repeatability analysis was done on nine patients to assess concordance of the scans.
Results: The analysis included complete datasets from forty-one patients. After NACT, the TSR significantly decreased for complete pathological responders and increased for partial responders ($p<.001$). The PAR's post-cycle 1.1 predictive ability for a complete pathological response was also significant ($p<.001$). When TSR was combined with DCE imaging, the specificity improved considerably compared to DCE alone (43.5%→95.7%), while maintaining the high sensitivity of DCE (94.4%). The repeatability analysis demonstrated excellent agreement for elasticity (ICC=0.969, RC=8.9%) and phase angle (ICC=0.876, RC=12.6%).
Conclusion: This novel technology, which uses a combined approach including biomarkers (DCE+MRE), shows great promise as a non-invasive imaging method for assessing complete pathological response at the end of NACT. As a stand-alone method or combined with other investigative techniques to diagnose complete responses accurately and non-invasively at the end of chemotherapy, this can aid consideration in de-escalating surgical treatment. Furthermore, the information after the 1st cycle can help predict if tumours will eventually have a complete response or partial/no response; the latter may need early re-discussion in multi-disciplinary meetings to consider an alteration to their chemotherapy regimens. Further studies are required to strengthen these findings.

P4-05-20: Development and validation of a combined ultrasound-pathology model to predict axillary status after neoadjuvant systemic therapy in breast cancer

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Background: For patients with axillary lymph node metastasis before neoadjuvant systemic therapy (NST), axillary lymph node dissection is still the mainstream surgical procedure after NST, but this procedure will bring many complications to the patients. Currently, ultrasound-guided fine-needle aspiration (FNA) is commonly used in the clinic to determine whether the axillary lymph nodes have metastases, but the false-negative rate of FNA is too high. Therefore, there is a need for a more accurate method to assess whether axillary lymph nodes achieve pathological complete response (pCR) after NST to help clinicians make treatment decisions or even try to perform axillary de-escalation surgery for these

patients.
Methods: This study retrospectively collected data from breast cancer patients who underwent NST and were considered to have axillary lymph node metastasis at the Department of Breast Surgery of the First Affiliated Hospital of Nanjing Medical University from 2015 to 2022. The clinical information, ultrasound results and postoperative pathological results of patients were collected, and all patients were randomly divided into a training group and a validation group at a ratio of 7:3. Independent predictors of axillary pCR were screened in the training group using univariate and multivariate logistic regression analyses, and a nomogram model was developed and validated based on these factors, and then the accuracy of the model was compared with that of FNA.
Results: Ultimately, 657 patients were enrolled in this study, and after randomization, there were 460 patients in the training group and 197 patients in the validation group. Univariate and multivariate logistic regression analyses based on clinical and ultrasound factors were performed to screen clinical node stage (cN) (P =0.014), estrogen receptor (ER) status (P <0.001), human epidermal growth factor receptor 2 (HER2) status (P <0. 001), imaging regression of the primary breast tumor (P =0.011), the hilum structure of the axillary lymph nodes after NST (P <0.001), and the blood flow distribution of the axillary lymph nodes after NST (P =0.004) were independent predictors of axillary pCR, and an ultrasound-based model was developed, which had an AUC value of 0.821. Univariate and multivariate logistic regression analyses based on clinical, ultrasound and breast pathology information were again performed to screen cN (P =0.003), ER status (P <0.001), HER2 status (P =0. 009), the hilum structure of the axillary lymph nodes after NST (P <0.001), the blood flow distribution of the axillary lymph nodes after NST (P =0.003), longest diameter of breast tumor after operation (P =0. 001), vascular invasion of breast tumor (P =0.043) and Miller-Payne grade (P =0.006) were independent predictors of axillary pCR. A combined model incorporating ultrasound and breast pathology was developed, which had an AUC value of 0.883. The calibration curves, decision curve analyses, and clinical impact curves of the training and validation groups demonstrated that the discrimination ability, calibration ability, and clinical application value of this combined model were significantly better than those of the ultrasound-based model and FNA.
Conclusion: In this study, a combined model incorporating axillary lymph node characteristics under ultrasound and breast pathology to predict axillary pCR after NST is developed, validated and found to be highly accurate. This model will help surgeons select patients who are likely to achieve axillary pCR after NST and thus attempt to perform axillary de-escalation surgery for these patients.

P4-05-21: Dynamic ctDNA tracking stratifies individual relapse risk for early triple negative breast cancer patients receiving neoadjuvant chemotherapy

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Xing-Rui Li, Jun Zhang, Hua-Wei Yang, Yaping Yang, Mengzi Wu, Qin Li, Fang Li, Yuhua Gong, Xin Yi

Background: Early Triple negative breast cancer (eTNBC) is the breast cancer subtype with the least favorable outcome. Tools to identify their individual relapse risk are in great need. Circulating tumor DNA (ctDNA) analysis is shown to predict the prognosis in breast cancer, but its utility in eTNBC remains unclear.
Patients and methods: In this prospective study, 130 eTNBC patients receiving neoadjuvant chemotherapy (NAC) were successfully enrolled. Their blood samples were taken at the baseline, post-NAC, post-surgery and during follow-up, and were subjected to tumor-guided ctDNA analysis.
Results: ctDNA positivity at post-NAC and post-surgery, but not at baseline, was associated with significantly worse prognosis. A threshold of 1.1% maximum variant allele frequency (MVAF) at baseline better stratified eTNBC patients with different relapse risk, which was validated both internally and externally. A systemic tumor burden model integrating baseline and post-surgery ctDNA was highly prognostic and independent of clinical characteristics. Combining systemic tumor burden with pathologic response identified a highly curable subgroup and a subgroup of high-risk eTNBC patients that need more effective adjuvant treatments. ctDNA surveillance during follow-up showed that the patients with negative ctDNA had 100% distant recurrence free survival (DRFS), but the ones with positive ctDNA had high relapse rate with relatively short lead time.
Conclusions: To our knowledge, this study is the first to set a ctDNA threshold in eTNBC patients to stratify their relapse risk. This systemic ctDNA analysis from baseline to follow-up demonstrates the utility of baseline ctDNA with a threshold and a systemic tumor burden model in risk stratification of eTNBC patients, which may guide future treatment escalation or de-escalation trials.

P4-05-22: Discovery of Molecules Synergistic with (Z)-endoxifen for the Treatment of Breast Cancer

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Background: (Z)-endoxifen is a potent selective estrogen receptor modulator (SERM) with 100-fold more potency than its parent drug, tamoxifen. It is hypothesized that (Z)-endoxifen has a dual concentration dependent mechanism of action. At lower concentrations, (Z)-endoxifen is an ER antagonist while at higher concentrations (Z)-endoxifen additionally inhibits and degrades protein kinase C beta 1 (PKCβ1). Preclinical and clinical studies in bipolar disease, ER+ breast cancer and other solid tumor types have demonstrated (Z)-endoxifen to be safe and well tolerated. Specifically, in ER+/HER2- breast cancer (Z)-endoxifen has shown promising anti-tumor activity (EVANGELINE; NCT05601004). The objective of this study was to evaluate the synergistic potential of (Z)-endoxifen by identifying new combination therapeutic solutions in breast cancer.

Methods: Insilico modeling: The artificial intelligence-driven platform PandaOmics was used to collect and aggregate omics-based gene signatures of breast cancer and (Z)-endoxifen and combine these with over 3000 small molecule perturbations from the LINCS database to predict compounds that could be synergistic with (Z)-endoxifen. All possible combinations with (Z)-endoxifen were evaluated by several ranked scores which included effects on disease signature and direction of gene expression changes, applying a gene-gene synthetic lethality component. Thus, drug pairs with highest combined scores are predicted to be potentially synergistic. Chemical screen: A total of 5251 compounds from the Drug Repurposing Library (SPECS) were screened for synergy with (Z)-endoxifen. MCF-7 cells (ER+ breast cancer) were treated with 0.5μM (Z)-endoxifen, dispensed into 384-well plates and exposed to 1 μM of compounds diluted in DMSO. After 4 days, cells were fixed and stained with Hoechst to visualize nuclei. Cells were imaged via high throughput microscopy (INCell 2200 microscope) and nuclei count was analyzed using CellProfiler. Statistical and data analysis was done using KNIME Software. All values were normalized to DMSO, no (Z)-endoxifen condition. Compounds reducing the viability of cells more than (Z)-endoxifen alone (mean viability -3x StD), and showing higher viability than Doxorubicin (10μM) combined with (Z)-endoxifen (mean viability +3x StD), were considered as hits and subjected to validation screening.
Results: Insilico modeling identified compounds with inhibitory mechanism of actions against mTOR/PI3K, tyrosine kinase, topoisomerase, HDAC and CDKs in combination with (Z)-endoxifen as potentially synergistic. To validate these results, a chemical screen was conducted in MCF-7 cells. Results from this screen yielded a total of 354 compounds with synergistic potential with (Z)-endoxifen's mechanism of action. The top-ranking compounds in the screen have mechanisms of actions centering on inhibition of DNA topoisomerase, CDK, mTOR/PI3K, AKT and HDAC. Nineteen compounds showed antagonistic activity. Ongoing studies will validate the chemical screening hits in additional breast cancer in vitro and in vivo models.
Conclusions: Based on two independent approaches, compounds used to inhibit DNA topoisomerase, CDK, mTOR/PI3K and AKT synergize with (Z)-endoxifen to induce cell death in MCF-7 cells when compared to either agent alone. Taken together, these data suggest that combination therapies with (Z)-endoxifen and agents targeting topoisomerase, CDK, mTOR/PI3K and AKT hold great therapeutic potential and should be further validated in additional model systems.

P4-05-23: Quantitative Diffusion Weighted Imaging for Predicting Response to Neoadjuvant Therapy in Patients with Inflammatory Breast Cancer

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Background and Purpose: Inflammatory breast cancer (IBC) is a rare and aggressive disease, accounting for 2-4% of all breast cancers. One third of the patients have distant metastases at initial presentation [1]. Trimodality therapy beginning with neoadjuvant systemic therapy (NAST) is associated with the best local control and survival (2). The ability to determine tumor response early in the NAST course is essential in optimizing treatment and predicting prognosis (2,3). MRI (Magnetic Resonance Imaging) is suggested to be the best modality for assessing response. Cellular changes such as cell membrane destruction or tumor lysis lead to increased water diffusivity and can be measured on MRI (4). Apparent diffusion coefficient (ADC) is a measurement of the change in water diffusivity, calculated from the diffusion weighted imaging (DWI) scans. In this study, we investigated quantitative DWI for differentiating pathological complete response (pCR) from non-pCR in IBC patients after NAST.

Methods and Materials: All patients were selected from an IRB-approved prospective IBC registry at one tertiary academic center and treated between December 2019 to November 2022. A retrospective chart review was performed to identify IBC patients with analyzable imaging and pathology records. Patients with available pre-treatment and mid-treatment MRI with DWI scans were included. Of 262 scans, 149 pre-treatment MRI, 13 outside films (OSF) without DWI, and 5 biopsy MRIs were excluded from analysis. Of the remaining 97, 27 had both pre-treatment and mid-treatment DWI with same b-values (diffusion coefficient value applied to images) available for evaluation.
All MRI exams were performed on 3T scanners (either GE Healthcare or Siemens Healthineers) using phased-array dedicated breast coils. Typical scan parameters for DCE scans were: flip angle = 12°, TR/TE ~6/2 msec, acquisition matrix = 320 x 320 x 112, FOV = 30 x 30 x 18 cm, and temporal resolution = 10 secs. Contrast agent was administered intravenously via an MR-compatible injector and flushed with 20 mL saline solution. DWI was acquired with two b-values of 100, 800 sec/mm2 and scan parameters as follows: TR/TE = 4000/70 msec, and FOV = 16 x 16 x 6.4 cm. Quantitative ADC maps were computed using a mono-exponential model. Image analysis was performed on a DynaCAD system (Invivo Corporation, Pewaukee, WI). Three regions-of-interest (ROIs) of the index breast tumor, axillary node, and thickened skin were segmented on DCE by an experienced breast radiologist (24 years). ROIs were transferred to the ADC maps and co-registered with DCE images. The quantitative ROI histogram values (median, mean, standard deviation, minimum, maximum, skewness, and kurtosis) were extracted. Statistical analyses were performed to determine the association between these measurement and the patients' pCR status and PFS.

Results: Median follow up was 14.5 months. 19 patients had non-pCR and 8 had pCR. At the time of this analysis, 20 patients were alive and 7 deceased. The median and mean ADC values of tumor was 1.326 and 1.333, respectively. Both median and mean ADC values were correlated to pCR, with p-value of 0.0074 and 0.0071, respectively. None of the remaining values associated with DWI scans of the index tumor, axillary node, and skin ROIs (standard

deviation, minimum, maximum, skewness, and kurtosis) was significantly correlated to pCR. No statistically significant correlation was demonstrated between ROI measured and PFS (correlation coefficients = -0.35 to 0.48).

Conclusion: DWI is sensitive to changes in water cellularity and has the potential to characterize early tumoral response during NAST. Histogram analyses of ADC in IBC reflects tumor heterogeneity. Our pilot study shows promise of the median and mean ADC values as imaging biomarkers for predicting pCR at mid-NAST in IBC patients.

P4-05-24: Point mutations (PM), gene amplifications (GA) and variants of unknown significance (VUS) detected by next-generation sequencing (NGS) in a real-world sample of metastatic breast cancer (MBC)

Marcus Lee, Jules Cohen

Background: The management of MBC requires NGS of tissue biopsies or ctDNA. Certain changes in the tumor genome, predominantly PM in ESR1 and PIK3CA, are targetable with FDA-approved drugs. GA, with the notable exception of ERBB2, is a common but not actionable finding on NGS. Commercial NGS report amplification of multiple genes within a sample but do not always indicate they are amplified en bloc from the same chromosomal region or amplicon. We evaluated NGS results from MBC to (1) report the prevalence of PM across a real-world cohort; (2) organize individual GA within their respective amplicons; and (3) identify VUS that may show unexpected functional significance.
Methods: We evaluated NGS from 44 tissue (Foundation One) and 17 liquid (Guardant 360) biopsies. We correlated NGS results with histological type and ER/HER2 status. We analyzed PM in oncogenes vs. tumor suppressors and compared known cancer-related PM with VUS. We analyzed co-amplified GA and grouped them into amplicons based on their genomic location.
Results: 76% (35/46) cases were ductal (IDC) and 24% (11/46) were lobular (ILC). 59% (27/46) were luminal, 32% (15/46) were TNBC and 9% (4/46) were HER2-pos. 48% (22/46) of patients had a TP53 mutation, including 93% (14/15) of TNBC, 75% (3/4) of HER2-pos and 19% (5/27) of luminal. Individual TP53 PM were rarely repeated among patients. 39% (18/46) of patients had a PIK3CA mutation, including 20% (3/15) of TNBC, 75% (3/4) of HER2-pos and 44% (12/27) of luminal. 45% (9/20) of PIK3CA were H1047R. 82% (9/11) of ILC had a PM in CDH1. ESR1 (8/27), GATA3 (6/27) and AKT1 (3/27) PM were seen in luminal but not TNBC. 7/8 ESR1 PM were Y537 or D538. GATA3 (6/35) and AKT1 (3/35) PM were seen in IDC but not ILC. RB1 PM (but not PTEN) were more common in TNBC (4/15) than luminal MBC (1/27). 25% (11/44) of patients had a VUS in KMT2D (MLL2). PM were more common in liquid (20/17) than tissue (20/40) biopsies, with 65% of liquid biopsies showing PM in TP53 (vs. 48% in tissue) and 71% showing PM in PIK3CA (vs. 39%). Multiple PM in TP53 or PIK3CA were only seen in liquid biopsies.

11q13 amplification (CCND1, FGF3, FGF4, FGF19) was seen in 9 patients, 8q24 (MYC, RAD21, LYN) in 7 and 8p11 (FGFR1, ZNF703, NSD3) in 5. 4 patients each showed amplification in 20q13 (AURKA, ZNF217, GNAS, ARFRP1) and 17q12 (ERBB2, CDK12). 11q13, 8p11 and 20q13 were seen mostly in luminal MBC. 8q24 and 12p13 (CCND2, FGF6, FGF23, KDM5A, KRAS) were seen mostly in TNBC. The most common (11q13, 8q24, 8p11) were enriched in ILC (27% of cases). 20q13 and 12p13 were only seen in IDC. 15% (6/40) were TMB-high (>=10), 23% (9/40) TMB-int (8-9) and zero MSI-high.
Conclusions: NGS of real-world patients reveals a broad spectrum of genomic abnormalities in MBC. TP53 and PIK3CA associate with TNBC and luminal MBC, respectively. Mutations in tumor suppressors are mostly distinct since there are many ways to induce loss-of-function. Mutations in oncogenes are restricted to a few hotspots associated with ligand-independent activation. Liquid biopsies detect more PM and are the only modality to detect multiple PM in the same gene. Small molecule inhibitors may demonstrate increased efficacy more against PM than GA.
Increased expression of individual amplicons is frequently related to histology or ER/HER2 expression. Commercial NGS reports do not clearly organize individual genes into distinct amplicons. Tissue biopsies preferentially detect GA. mAbs/ADCs are likely the best therapeutic candidates against protein overexpression mediated by GA. Commercial NGS assays reveal descriptive and prognostic information alongside actionable mutations and novel potential targets. Careful evaluation of NGS will improve our understanding of the genotype/phenotype relationship in real-world patients.

P4-05-25: Impact of Estrogen Receptor Targeting Drugs on a Novel Epithelial-Mesenchymal Transition Pathway in Endocrine Resistant Breast Cancer: Implications for Metastasis

Nivida Shete, Debra A. Tonetti

Background: Approximately 10% of breast cancer patients are metastatic at the time of diagnosis with a 27% survival rate. Currently, endocrine therapy, including tamoxifen, aromatase inhibitors (letrozole, anastrozole, and exemestane), Selective Estrogen Receptor Degraders (fulvestrant and elacestrant), is the standard of care for patients with estrogen receptor-positive (ER+) breast cancer. The major treatment challenge is endocrine therapy resistance and metastasis. Epithelial Mesenchymal Transition (EMT) is the process in which cells lose epithelial properties and acquire motility and mesenchymal phenotype, which leads to migration and invasion. EMT is characterized by the destabilization of adherens junctions by loss of E-cadherin at the plasma membrane. We previously reported a novel signaling pathway that is present in tamoxifen-resistant (TR) breast cancer cells. This pathway involves transcriptional repression of p120catenin by upstream regulators PKCa and FOXC2, resulting in the loss of E-cadherin at the plasma membrane (Pham, 2017). EMT features were characterized by decreased expression of epithelial markers and increased

migration and invasion.
Initially, estrogen stimulates breast cancer growth but becomes inhibitory after long-term estrogen deprivation. A novel Selective Human Estrogen Receptor Partial Agonist (ShERPA) TTC-352 is an estrogen mimic that induces unfolded protein response and shrinks the TR tumors (Molloy, 2014; Abderrahman, 2021). In this study, we investigated the impact of various ER-targeting drugs including 17b-estradiol (E2), tamoxifen, fulvestrant, and TTC-352 on the novel PKCa-FOXC2-p120catenin signaling axis. We hypothesize that ER-targeting drugs modulate the novel EMT pathway in TR breast cancer cells.
Materials and Methods: TR cell lines used in this study include PKCa overexpressing cells MCF-7/PKCa, and MCF-7:5C and represent migratory ER+ breast cancer. Migration assays were performed in response to ER ligands using Falcon Permeable inserts. Migrated cells in response to chemoattractant were stained and counted after 24 hours. Luciferase reporter assays were performed to evaluate the effect of ER ligands on p120catenin transcription. Cells were transiently co-transfected with a p120catenin promoter-reporter plasmid using renilla vector for normalization. The reporter system readout is based on FOXC2 binding to the p120catenin promoter resulting in repression of luciferase transcription. Luciferase luminescence was measured on a plate reader corresponding to p120catenin transcription. Chromatin immunoprecipitation was performed using a FOXC2 antibody (Abcam-308055) to assess FOXC2 binding to the p120catenin promoter after ER ligand treatment.
Results: E2 and TTC-352 treatment lowered FOXC2 binding on the p120catenin promoter, increased its transcription, and decreased the migration of MCF-7/PKCa and MCF-7:5C breast cancer cells compared to vehicle. E2 and TTC-352 treatment does not activate the PKCa-FOXC2-p120catenin EMT signaling axis and reduces the migratory potential of TR cells.
Conclusion and Discussion: Our findings suggest that patients with endocrine-resistant ER+ breast cancer may benefit from E2 and TTC-352 therapy by inhibiting the activation of the PKCa-FOXC2-p120catenin signaling and cell migration identified in TR cell lines. Results of the Phase I clinical trial of TTC-352 reveal a reduced side effects profile and improved progression-free survival (Dudek, 2020). Strategizing less toxic treatment options may potentially reduce metastasis and prolong patient survival with a better quality of life.

P4-05-26: MamaTrace - A cell-free DNA methylation plasma only assay for minimal residual disease detection in breast cancer

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Background:Metastatic breast cancer (MBC) is an incurable disease, affecting 10-15% of breast cancer (BC) patients, with a 5-year overall survival of less than 25%. MBC arises when small populations of disseminated BC cells remain in the body after primary therapy, otherwise known as minimal residual disease (MRD). MRD is currently difficult to detect following primary therapy. Despite, improvements, there remains a need for more effective and sensitive clinical tools to identify patients with MRD following primary treatment who may benefit from additional treatment or increased surveillance. In the current study, we

aimed to develop a plasma only MRD test, based on cfDNA methylation markers specifically associated with developing breast cancer metastasis, rather than markers that may prioritize markers of tumor burden, making this approach a partially-informed MRD assay.

Methods: Blood was collected under informed consent from multiple commercial and academic sources. We constructed 6 pools of cfDNA comprising a total 43 samples from MBC patients, and 3 pools comprised of cfDNA from 39 healthy individuals. In addition, we sequenced 21 cfDNA libraries from treatment-naïve patients with stage I-III breast cancer who either developed a future recurrence (REC, N = 12) or remained disease-free survivors (DFS, N = 9). The 6 MBC pools represented all major BC subtypes (ER+/HER2-, ER-/HER2+, ER+/HER2+, TNBC). To improve model performance and represent DNA methylation patterns associated with underrepresented populations, we also included 19 samples from African American patients with triple-negative breast cancer (TNBC) across varying stages. Whole genome bisulfite sequencing (WGBS) was performed on the 9 pools and 21 individual cfDNA samples. Differentially methylated regions (DMRs) were identified between MBC versus healthy pools and between REC versus DFS samples. A total of 5077 DMRs were then used to generate a targeted methylation assay using hybridization probe capture, which was used to analyze 72 individual cfDNA samples from patients with MBC (5 stage I-III), and 83 healthy cfDNA controls. Next, we developed a binary classification model called MammaTrace to distinguish between MBC and healthy samples. To evaluate the model for MRD, we subsequently applied the classifier to assess its accuracy in distinguishing REC from DFS in patients with early-stage breast cancer prior to recurrence. Specifically, cfDNA was sequenced using the MammaTrace targeted panel at multiple timepoints from 107 patients with early-stage breast cancer who underwent neoadjuvant chemotherapy. Of these patients, 51 had blood collected 9–27 months after definitive therapy and was determined as the most predictive time point and used for landmark analysis. At the time of writing, 11 patients developed metastasis, while 40 remained disease-free. Blood samples were collected prior to recurrence for all patients.

Results: The classifier model, constructed primarily using samples from MBC patients and healthy individuals, demonstrated excellent performance in distinguishing MBC from healthy samples, achieving a ROC AUC of 0.96. For assessing MRD detection in early-stage breast cancer samples collected at least 9 months after the completion of therapy, MammaTrace achieved an ROC AUC of 0.88, correctly classifying 10 of 11 REC patients as MRD positive and 33 of 40 DFS patients as MRD negative (sensitivity = 0.91, specificity = 0.83). The 10 positive REC landmark samples were collected an average of 457 days prior to clinical recurrence.

Conclusion: MammaTrace, a plasma-only cfDNA methylation-based classifier, effectively distinguishes MBC patients from healthy individuals, and can detect MRD before clinical

recurrence, identifying high-risk patients in need of additional therapy. Prospective studies are ongoing to validate MammaTrace.

P4-05-27: Comparative analysis of TROP2 expression in tumor tissues and circulating tumor cells (CTCs) in the peripheral blood of patients with triple negative breast cancer

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Background: Trophoblast cell surface antigen 2 (TROP2) promotes breast cancer (BC) development, invasion and metastasis, with promising role as a biomarker and therapeutic target in triple negative BC. Due to the dynamic tumor evolution during disease progression, a significant discordance in tumor cell profiles is frequently observed among primary tissues and distant metastases. In this regard, analyses of circulating tumor cells (CTCs) in the peripheral blood (PB) can inform on the expression of biomarkers in real-time. In the current study we assessed in parallel the expression of TROP2 on CTCs and matched primary tumors and metastatic sites from patients with triple negative BC.

Methodology: PB was collected from 54 patients and CTCs were enriched by ficoll-density gradient centrifugation. Cytospins were immunofluorescently stained using antibodies for cytokeratins (Clones: AE1/AE3 & C11), CD45 and TROP-2 (Enzo Life Sciences); TROP2 expression on CTCs was defined as high, low or negative, by using the high TROP2-expressing MDA.MB.231 triple negative BC cell line as internal control. Matched primary (n=51) and metastatic (n=7) tumor tissue samples were evaluated for TROP-2 expression by immunocytochemistry (IHC); H-score was calculated as follows: (3 × % cells with strong intensity staining) + (2 × % cells with moderate intensity staining) + (1 × % cells with mild intensity staining), ranging from 0 to 300, and the following expression categories were defined: H-score 0 to <100: TROP2 low; H-score 100-200: TROP2 medium; H-score >200-300: TROP2 high.

Results: CTCs (CK+/CD45- cells) were identified in 12 out of 54 patients evaluated (total CTC counts: n=80; mean CTC counts per patient: n=6.7). TROP2-expressing CTCs were detected in 75% of CTC-positive patients and represented the 95% of total CTCs. Specifically, high and low TROP2-expressing CTCs were identified in 66.7% and 41.7% of patients, representing the 81.3% and 13.7% of total CTCs, respectively. Differential TROP2 expression levels (high, medium and low) were also observed in both primary and metastatic tumors, showing a great intra-tumoral heterogeneity. High TROP2 expression was identified in 58.8% and 57.1% of primary and metastatic tissues, respectively. When matched primary and metastatic tissues were analyzed, a decrease in TROP2 expression was observed [median H-Score: 172.5 (range: 11-300) versus 87.5 (range: 5-150) in primary and metastatic tissue, respectively, p=0.068)]. CTC detection in the PB was not

associated with TROP2 expression levels in primary or metastatic tissue (CTCs were identified in 13.3% and 50% of patients with high TROP2-expressing primary and metastatic tumors, respectively). Finally, there was no concordance in TROP2 expression pattern among CTCs and the respective tumor (high, low and negative TROP2-expressing CTCs were identified in 50%, 25% and 25% of patients with high TROP2-expressing primary tumors, respectively; high and low TROP2 -expressing CTCs were evident in all patients with high TROP2-expressing metastatic tumors).

Conclusions: Herein we demonstrate for the first time a significant discrepancy in TROP2 expression among CTCs, primary and metastatic tumor tissue samples in triple negative BC. A lower TROP2 expression was observed in metastatic as compared to primary tissue, while no concordance was demonstrated among CTCs and the respective tumors. The results suggest the dynamic change in TROP2 expression status among different disease sites, thus highlighting the value of using liquid biopsy as a tool for real-time biomarker assessment in triple negative BC.

P4-05-28: The cytokine profile correlates with less tumor-infiltrating lymphocytes in luminal A breast cancer

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Background: Tumor-infiltrating lymphocyte (TIL) levels are prognostic and predictive factors for breast cancer. Contrary to other subtypes, most luminal A breast cancers are immune deserts; however, the underlying mechanisms are poorly understood. As the immune-related molecules cooperate in a complex network, experiments that evaluate the immune status of breast cancer need to be performed by exploring multiple molecules rather than a single molecule. In this study, we focused on cytokines, chemokines, and growth factors, which modulate immune reactions, using multiplex assays to identify cytokine profiles linked with TILs in operated breast cancers, especially focusing on the luminal subtype.
Methods: A total of 103 patients with breast cancer who underwent surgery between September 2019 and October 2023 and had available blood samples coupled with resected tumor samples were constitutively recruited. Immune-related cytokines, chemokines, and growth factors were measured in the sera of the 103 patients using a multiplex panel. TILs were evaluated in the hotspot lesions and the TIL levels were divided into high (≥50%), intermediate (≥10%, <50%), and low (<10%). All statistical analyses were performed with JMP® Pro Version 16 (SAS Institute Inc., Cary, NC, USA), and statistical significance was set at $p < 0.05$.
Results: We compared expression levels of cytokines, chemokines, and growth factors according to the TIL levels, and found that the TIL levels were significantly associated with IL-1 receptor antagonist (IL-1ra) ($p = 0.0185$), IL-8 ($p = 0.0068$), IL-12 ($p = 0.0469$), IL-17 ($p = 0.0319$), macrophage inflammatory protein-1β(MIP-1b) ($p = 0.0398$), and homodimer

of platelet-derived growth factor B (PDGF-bb) (p = 0.0360). Cluster analysis using these six variables identified six clusters related to TIL levels. Breast cancers with high TILs were most frequent in cluster 3 (9 out of 15 cases, 60.0%), followed by cluster 1 (8 out of 34 cases, 23.5%), and fewest in cluster 6 (1 out of 21 cases, 4.8%), whereas only one or three cases were present in clusters 2, 4, and 5 (p = 0.0064). The distribution of the subtypes in each cluster was also significantly different (p = 0.0009). In cluster 6, 19 of 21 (90.5%) were luminal A, and 16 of 20 (80.0%) luminal B tumors were in cluster 1. Moreover, TIL levels in each cluster were significantly different (p = 0.0064). High expression levels of IL-12, IL-17, and PDGF-bb with low levels of MIP-1b, a characteristic of cluster 6, were prominent in luminal A but not in luminal B subtypes.
Conclusion: We identified a luminal A-associated immunosuppressive cytokine signature in the circulation. The results suggest that a tumor microenvironment with high levels of IL-17 and PDGF-bb, and low levels of MIP-1b in luminal A breast cancers resulted in low induction of TILs. Our data may partially explain the low TIL levels in luminal A breast cancer.

P4-05-29: ctDNA and serum thymidine kinase activity as tools to stratify ER+/HER2- metastatic breast cancer patients treated with endocrine therapy and CDK4/6 inhibitors: preliminary results of the TIRESIAS trial

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Background: Patients (pts) with ER+/HER2- metastatic breast cancer (MBC) have heterogeneous response and outcome when treated with standard first-line endocrine therapy (ET)+CDK4/6 inhibitors (CDK4/6i). We and others have previously shown that either circulating tumor DNA (ctDNA) or serum thymidine kinase activity (sTKa) may represent useful tools for the prognostic stratification of these pts according to the pattern of biomarker response during the first cycle of therapy. Here, we report a preliminary analysis of the TIRESIAS trial (NCT04660435) exploring both sTKa and ctDNA and their correlations in this setting.

Methods: TIRESIAS is an ongoing multicenter trial enrolling pts receiving ET+CDK4/6i as first-line therapy for ER+/HER2- MBC per standard clinical practice. Liquid biopsy samples are taken at different timepoints including Baseline (Bas), Cycle (C) 1 Day (D) 15, C2D1, C2D15, C3D1, end of treatment of first- and second-line therapy (EOT1, EOT2). ctDNA analysis, including low-pass whole-genome sequencing (lpWGS) and mutational profiling, was done at Bas, C1D15, EOT1 and EOT2, when available. Tumor content (TC) was estimated from lpWGS by ichorCNA, using a 3% threshold for detection (TC+). Mutational profiling was obtained with a custom enrichment-based panel of 43 breast cancer related

genes integrating unique molecular identifiers; somatic variant calls (Single Nucleotide Variants and Insertions/Deletions) were detected using three tools, including Mutect2, Strelka and LoFreq, and then validated by the tool ABEMUS. maxVAF (Variant Allele Frequency) was defined as the maximum mutant allele frequency. sTKa was measured at all time-points by DIVITUM TKa using 250 DuA as cut-off for Bas and 100 DuA for the subsequent time-points. sTKa patterns (P) were defined according to C1D15/C2D1 status (+, - or indifferent): P1 -/-; P2 -/+; P3 +/indifferent.

Results: 64 pts with ctDNA and sTKa data available were analyzed, (113 timepoints with matched data). Pts had either de-novo metastatic (53%) or secondary endocrine resistance (44%) and mainly received either ribociclib or abemaciclib in combination with an aromatase inhibitor; only 5% received a CDK4/6i + fulvestrant.
At Bas, 33% (N=21) of the pts were TC+, 44% (N=28) had at least 1 protein-affecting variant detected, and 36% (N=23) had sTKa + status. At Bas, detection of a ctDNA variant was significantly more frequent in TC+ vs – (53% vs 21%, p = 0.002), with sTKa being significantly higher in pts with TC+ (p<0.001) and in those with at least one variant detected (p 0.002). At C1D15, a parallel and significant reduction in TC and sTKa, was observed, with TC and maxVAF showing a positive correlation (R= 0.77, p< 0.001). During cycle 1, 37% of the pts were categorized as sTKa P1, 38% P2 and 25% P3. At C2D15, median sTKa was significantly lower for pts in P1 vs P3 (p 0.05). At EOT1 and 2, a parallel increase in the three biomarkers was observed with the detection of 18 new variants in 10 pts.

Conclusions: ctDNA and sTKa determination give complementary information throughout the pts treatment journey. TC estimation may give useful information on the potential detectability of ctDNA variants in a given sample, potentially informing biomarker search strategies. sTKa is a reliable marker which gives a result in every patient. Dynamic assessment of sTKa during treatment is concordant with ctDNA changes and offers a cost-effective and easier alternative to ctDNA for non-invasive monitoring of early response to therapy. Dynamic patterns of sTKa response in cycle 2 may complement sTKa response in cycle 1.

P4-05-30: MUC4 downregulation by TNF blockade enhances trastuzumab deruxtecan antitumor effect in a HER2-positive breast cancer model

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Background: Trastuzumab deruxtecan (T-DXd) is indicated for the treatment of adult patients with unresectable or metastatic HER2-positive (HER2+) breast cancer who have received a prior anti-HER2-based regimen. Results from DESTINY-Breast03 demonstrated that the median overall survival in this setting was 52.6 months, showing a need for novel biomarkers and efficient agents that may improve T-DXd outcome. Furthermore, it causes adverse effects that lead to dose reduction or treatment discontinuation. We have

demonstrated that mucin 4 (MUC4) is an independent biomarker of poor disease-free survival in early HER2+ breast cancer patients treated with trastuzumab and reflects an immunosuppressive tumor microenvironment (TME). In addition, we have shown that TNF induces MUC4 expression and that soluble TNF (sTNF) neutralization with a dominant negative protein to TNF, INB03 (DN), downregulates MUC4 and overcomes trastuzumab resistance in preclinical models. Previously, we observed that administration of 1.25 mg/kg, 2.5 mg/kg or 5 mg/kg T-DXd in nude mice reduce JIMT-1 tumor growth by 37%, 61% and 83%, respectively. Administration of 1.25mg/kg + DN induces tumor reduction that mimics the effect of the 5 mg/kg dose. In this work, we explored the precise contribution of TNF-induced MUC4 expression on T-DXd antitumor effect.

Methods: The HER2+, trastuzumab-resistant JIMT-1 cells were engineered to express a doxycycline (Dox)-inducible shRNA targeting human MUC4, rendering JIMT-shMUC4 cells. These cells were injected in nude mice and, once tumors were established, animals were administered with Dox (Dox+) or not (-Dox), and treated with IgG 2.5 mg/kg, T-DXd 2.5 mg/kg (T-DXd 2.5), or 1.25 mg/kg (T-DXd 1.25), DN 10 mg/kg or the combined therapies (n=6-8/group). T-DXd and IgG were administered i.v. on days 0, 7 and 14. DN was administered i.p. twice a week for 3 weeks. Tumor growth was monitored and differences among groups were analyzed using two-way ANOVA coupled with a Bonferroni post hoc test.

Results: In JIMT-1-shMUC4 Dox- tumors, T-DXd 1.25 inhibited tumor growth by 55% vs IgG ($p<0.0001$) at day 21. Addition of DN significantly enhanced this effect (73%, $p<0.05$ vs T-DXd 1.25). T-DXd 2.5 decreased growth by 72% ($p<0.0001$) and T-DXd 2.5 + DN by 74% ($p<0.00001$) vs IgG. Of notice, T-DXd 1.25+DN inhibits tumor growth to a similar extent to T-DXd 2.5 alone. In Dox+ tumors, with silenced MUC4 expression (30-50%), T-DXd 1.25 inhibited tumor growth by 61% and T-DXd 2.5 by60% ($p<0.0001$ each, vs IgG). Addition of DN did not further increase these effects.

Conclusion: Our results suggest that MUC4 interferes with T-DXd antitumor effect when this drug is administered at a low dose (1.25 mg/kg). This detrimental effect can be tackled by blocking sTNF, which downregulates MUC4 expression and enhances-DXd therapeutic effect. MUC4 expression seems not to affect T-DXd effect when administered at a higher dose (2.5 mg/kg). As bioavailability of anti-cancer drugs can be affected by the TME, we propose that MUC4 would be a suitable biomarker to evaluate T-DXd resistance in HER2+ breast cancer, Therefore, addition of sTNF blocking agents would be an option to reinstate tumor sensitivity to T-DXd. In addition, dose reduction of T-DXd in combination with DN could be a feasible therapeutic option to reduce adverse effects.

P4-06-01: Comprehensive Analysis of Breast Cancer Treatment and Patient Characteristics in a Nationwide Cross-Sectional Study

Bela Mrinakova, Martin Suchansky, Dalibor Ondrus, Martina Ondrusova

Background: The Medi-LINE project is a nationwide multicenter cross-sectional epidemiological study focused on analyzing the medical treatment of breast cancer (BC) in Slovakia and describing the population characteristics of patients undergoing active oncological treatment. Recommended treatment guidelines for BC often vary significantly from the treatments used in clinical practice. Real-world data based on medical oncologists' decisions reflect guideline implementation, regional practices, patient preferences, and drug availability. Studies like this are essential to measure the quality of oncological care and inform pharmacoeconomic decisions, providing a clear picture of current treatment landscapes and identifying areas for improvement.
Methods: The study collected data from medical oncologists treating breast cancer patients across all regions of Slovakia. Inclusion criteria required patients to be undergoing active oncological treatment during the data collection period. Data were gathered through an online protocol designed to capture comprehensive patient information. This included demographic data, tumor characteristics (such as year of diagnosis, histological type, multifocal/multicentric status, clinical stage, hormone receptor status, HER2, PD-L1, BRCA1/2 status), and detailed treatment histories (type of treatment, treatment sequences, and history of previous breast or other cancers). Separate analyses were conducted for the male patient population. This robust data collection methodology ensured the acquisition of detailed and high-quality information reflective of real-world clinical practices.

Results: The study analyzed data from 995 patients, providing a representative cohort of the disease prevalence. The median age at diagnosis was 60 years. Simultaneous multifocal/multicentric tumors were present in 12.06% of patients. Ductal carcinoma was the most common histological type (83.71%). HER2 negative status was observed in 78.35% of patients, with 71.96% having ER+, PR+ status. PD-L1 testing was conducted only in 2.99% of patients, with a 17.24% positivity rate. BRCA1/2 testing was performed on 25.36% of patients, with 15.04% testing positive. Previous BC was found in 3.20% of patients. The average duration of medical treatment was 2.85 years post-diagnosis. Paclitaxel was the most frequently used neoadjuvant treatment, while letrozole monotherapy and the combination of letrozole and ribociclib were most prevalent in adjuvant and palliative treatments, respectively. Significant variations from recommended treatment protocols were observed. Among male patients, 20% had a history of cancer of another origin before their BC diagnosis. All male patients were equally distributed between adjuvant and palliative treatments.

Conclusions: This study offers valuable insights into real-world oncological practices and emphasizes the importance of continuous data collection. By gathering data yearly, the results can track changes in clinical practice and serve as a sensitive indicator of the efficacy

of interventions and guideline implementation. This dataset is a vital resource for research, policy-making, and pharmacoeconomic analyses, aiming to enhance patient outcomes and optimize treatment strategies.

P4-06-02: Integration of simulated triple assessment clinics into undergraduate education improves preparedness for clinical practice

Rebecca Murphy Lonergan, Anoushka Samanta

According to the Outcomes for Graduates, the General Medical Council requires students be able to safely diagnose, investigate and manage clinical presentations, however newly graduated UK doctors have shown uncertainty in practicing autonomously and confidently in clinical settings.
Undergraduate clinical attachments provide the setting for students to learn through engagement with the clinical team and supervised participation in patient care, although well-respected barrier to this include the busyness of a student's firm, the availability of adequate supervision and the quality of feedback provided. In surgery, there are additional challenges: while the majority of UK foundation doctors will rotate through at least one surgical specialty, students report fewer opportunities to engage clinical activity in surgery than in medicine, yet are expected to develop the same standard of clinical skill and competency within surgical disciplines. Simulation is one strategy to create authentic learning opportunities adjacent to the clinical environment, allowing students to integrate their knowledge with the clinical skills required in their daily practice as junior doctors in a safe, controlled environment.
UK students may have as little as one day's exposure in breast surgery. Although considered a specialist surgical field, breast examination and history-taking are skills required not just of general surgeons but also general practitioners who make up the largest proportion of the medial workforce. In our study, we investigated the use of simulated triple assessment clinics to improve undergraduate confidence and competence with diagnosis, investigation and management of breast pathology and aid preparedness for practice.
Third year students (n=20) took part in a 4- station simulated triple assessment clinic. A prerequisit was that students had not received any formal breast examination teaching prior to participation. Participants' self-reported confidence complete eight clinical tasks were assessed pre- and post-session using anonymous questionnaires, utilising Likert scales and free text questions. We also collected data on students' preferred learning environments for clinical examinations and skills: simulation versus classroom teaching.
Overall, global confidence increased significantly post-session according to statistical analysis performed using a Wilcoxon signed-ranked test ($W = 1292.5$, p-value $< 2.2e-16$).
Most marked improvements were seen in the ordering of appropriate investigations, examining patients with breast lumps and taking a focused history of a breast-related case.
Students reported a strong preference for simulated learning environments due to relevance to practical examination, links to future practice, active learning process, and increased understanding and confidence when on clinical placement.

Integrating simulated breast clinics into the undergraduate curriculum aids development of specialist and generalist clinical skills through active learning and confidence in applying clinical knowledge in, which students value highly. Student are enthused to participate when the relevance to future practice is clear. Students reported a stronger interest in a clinical career in breast surgery after attending the session.

P4-06-03: Barriers and Goals of Care Expressed by Patients Living with Advanced Breast Cancer during a Nurse Practitioner-Led Visit

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Significance: Advanced Breast Cancer (ABC) is incurable with limited life expectancy. The ABC Program aims to improve the quality and quantity of life for ABC patients through navigation, education, and support. During an ABC Advanced Practice Registered Nurse (APRN)-led visit, patients are guided through the cancer center resources and provides education, resources, support, managed care between departments, and helps with the clinical trial process. Before COVID-19, an ABC APRN conducted longer in-person visits at a comprehensive cancer center in the southern US. Post-COVID-19, the program held visits via video and telephone, enhanced data collection, proactively contacted patients living within 75 miles of the center, collaborated on a clinic to bridge the gaps in oncology care, and led provider education about ABC resources. Additionally, registered nurse patient navigation was implemented for all new ABC patients.
Purpose: Understand barriers to care, goals of care, and time spent addressing barriers post- COVID-19. Compare patient characteristics including demographics, barriers to care, time spent addressing barriers for patients seen by an ABC APRN prior to COVID-19 (June 2018 to February 2020).
Methods: Data were collected as part of an ABC APRN-led visit between March 2020 to May 2024. Patients were referred by clinicians or by self. Data were analyzed utilizing descriptive statistics.
Findings: The ABC APRNs saw 253 unique patients in 586 visits. The visits were held via video (85%) and telephone (11%). Most participants were female (n=251) with a diagnosis of ABC (metastatic), and a mean age of 56 years; 69% identified as White and 83% as non-Hispanic. Most patients (53%) were diagnosed one to five years ago, married (70%), employed full-time (35%), time from first ABC diagnosis to time of navigation visit was one to five years (53%), and lived within 75 miles of the cancer center (63%). Barriers identified by ABC patients: education (90%), coordination of care (81%), emotional concerns (53%), and physical concerns (32%). Of the 586 encounters, education (77%) and coordination of care (73%) were more likely discussed at each encounter. Goals of care expressed were life prolongation (39%), symptoms (28%), and treatment (27%). For 85% of visits, 40 to 60 minutes were spent assessing and addressing barriers to care. Patients seen post-COVID-19 were more likely to be African American (16% vs 11%), Asian (5% vs 2%), be employed (35% vs 30%) and be disabled due to illness (26% vs 23%). The patients

were more likely to live within 75 miles (63% vs 37%) and have a new progression of ABC (62% vs 52%). Discussion and Implications: ABC patients have significant barriers to care, necessitating additional time at visits to assess and address these barriers. An APRN-led visit allows for time to address barriers to care and promote the patient's goals of care for inclusion in their treatment plan. Education and coordination of care barriers were more likely to be found over multiple encounters. Embracing video and telephone visits not only expanded access to appointments with the ABC APRN clinic but also reduced the need for physical trips to the cancer center. As a result, patients experienced minimal disruptions to their daily lives. Longitudinal studies are needed to further describe these barriers and better articulate goals of care.

P4-06-04: Exploring Unmet Dental Care Need and Delay Due to Cost between Breast Cancer Survivors and the General Population in the United States

Jincong Freeman, Victoria Umutoni, Xinyi Li, Yong Gun Lee

Background: Breast cancer survivors have various health needs but often encounter financial challenges or barriers to care and supportive services. Oral health is an essential component of overall health and quality of life. However, research is scarce concerning dental care specifically among breast cancer survivors. This study sought to evaluate unmet dental care need and delay due to cost comparing breast cancer survivors to the general population in the US.
Methods: This study analyzed data from the 2022 National Health Interview Survey that used multistage probability sampling. Adults with a history of breast cancer were categorized as breast cancer survivors, and the general population included adults without a cancer history. To assess unmet dental care need (yes/no), participants were asked if there was any time when needed dental care but did not obtain it due to cost in the past 12 months. Dental care delay (yes/no) was defined as whether or not participants have delayed getting dental care or services due to cost in the past 12 months. We tabulated unweighted frequencies and weighted proportions. Groups were compared using Rao-Scott Chi-squared tests, followed by multivariable weighted logistic regression controlling for sociodemographic factors. Adjusted odds ratios (aOR) and 95% confidence intervals (95% CI) were calculated. All analyses accounted for complex design and survey weights.
Results: Of 24,828 adults, 644 (1.8%) were breast cancer survivors, representing a weighted sample of 4,234,520 US survivors. Survivors were older than adults in the general population (mean age: 68 vs. 46 years). Most (60.1%) identified as White, 18.2% as Hispanic, 12.5% as Black, 6.4% as Asian, and 2.9% as Other. Compared with the general population, breast cancer survivors reported a lower proportion of coverage for dental services (34.8% vs. 37.3%) but a higher percentage of having had their last dental exam or cleaning within the past year (72.6% vs. 63.5%). Overall, 12.0% (95% CI: 9.0-15.0%) of survivors reported unmet dental care need compared to 15.7% (95% CI: 15.0-16.4%) of the general population (P=0.036). The proportion of dental care delay was lower among survivors than in the general population (15.3% [95% CI: 12.0-18.7%] vs. 19.2% [95% CI: 18.5-19.9%]; P=0.039). After covariate adjustment, the odds of unmet dental care need were similar between breast cancer survivors and the general population (aOR 0.96, 95% CI: 0.70-1.31). Breast cancer survivors were numerically more likely than the general population to have delayed dental care (aOR 1.10, 95% CI: 0.83-1.56), though the difference was not statistically significant. In the same adjusted models, compared with White adults, Black adults had greater odds of unmet dental care need (aOR 1.51, 95% CI: 1.31-1.74); Hispanic adults also had greater odds of unmet dental care need (aOR 1.77, 95% CI: 1.56-2.00) and dental care delay (aOR 1.51, 95% CI: 1.35-1.69). Smoking, drinking, and having public insurance were significantly associated with greater odds of having unmet dental care need or delayed dental care due to cost.
Conclusions: In this population-based sample of US adults, unmet dental care need and

delay due to cost were prevalent in breast cancer survivors, with similar prevalence rates to the general population. Nearly two-thirds of breast cancer survivors lack coverage for dental services. There are racial/ethnic and socioeconomic disparities in dental care need and delay. Strategies and policies should consider reducing dental care costs and addressing coverage barriers to dental services among breast cancer survivors and socioeconomically disadvantaged populations. Future research should examine the impact of unmet dental care needs and delays on breast cancer survivors' quality of life and survival outcomes.

P4-06-05: Telehealth and cancer care delivery: Evaluating the impact of telehealth on patient access and treatment utilization at a comprehensive cancer center using real world data

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introduction: Telehealth has persisted post-COVID 19 due to its convenience, potential to improve access to care, and changes in telehealth reimbursement incentivizing its use. Data are needed to examine telehealth's impact on care utilization and patient (pt) access among diverse cancer types to inform long-term telehealth policies.
Methods: We used a deidentified clinical data warehouse to develop 2 cohorts of pts treated at our center from 2017-2019 (pre-telehealth) and 2021-2023 (post-telehealth). Data from 2020 was excluded, given peak pandemic irregularities. We included pts ≥18 yrs who completed ≥1 medical, surgical or radiation oncology visit with an associated cancer diagnosis. We strategically selected 3 disease groups with varying telehealth use: breast (low), GI (medium), GU (high). This allowed us to control for external factors (eg COVID) and better isolate the impact of telehealth. We obtained pt data (age, sex, race, zip code), visit data (new/follow-up, in-person/video, provider, department) and identified pts receiving cancer treatment (tx) at our center. We compared changes in visit distribution, sociodemographics, and tx patterns within and between disease groups pre- and post-telehealth using Chi-square and ANOVA tests.
Results: A total of 102,516 encounters and 25,123 pts pre-telehealth vs 139,309 encounters and 47,814 pts post-telehealth were analyzed. The # of unique pts seen pre- and post-telehealth increased significantly in all groups (+101% breast, +60% GI, +108% GU). Post-telehealth, the proportion of video visits (VVs) increased in breast from 1.6% to 28.2%, with most visits remaining in-person (IP; 71.5%); GI increased VVs from 1.9% to 55.3% of total visits; GU increased VVs from 3.1% to 81.4%. The distribution of visits by department, visit and provider type were similar pre- and post-telehealth for all groups. Among all groups, the proportion of pts identifying as White decreased among aggregated visit types, while the proportion of Asian and LatinX pts increased post-telehealth. In breast and GU, there was a statistically significant change in the geographic distribution of pts post-telehealth, with an increase in unique pts seen who lived in San Francisco (+7.4% breast, +3.5% GU). Post-telehealth, the % of pts who received radiation increased in breast, GI, and GU (+3.7, +2.3, +1.3). In breast and GI, the % of pts receiving infusions increased (+3.4, +1.6), but

decreased in GU (-0.6). The % of pts who underwent surgery decreased among all groups (-6.6, -4.4, -6.7).
Discussion: We evaluated 3 oncology disease-based groups with varying telehealth use to characterize the impact of widespread telehealth adoption on pt demographics and tx patterns. Total pts seen increased in all 3 groups. Notably, more pts with breast and GU cancer were seen from San Francisco post-telehealth. Given similar increases in pt volume and geographic shifts between low and high telehealth groups, this cannot be attributed to telehealth alone. Additional analyses to characterize telehealth's impact on pt access are ongoing. Regarding tx, the % of pts receiving infusions and radiation increased most in breast, which maintained the highest % of IP visits, highlighting the importance of IP visits during active tx. Limitations of our study include an inability to capture whether pts were seen via telehealth for 2nd opinions and treated locally or pts who received oral medications. This will be explored in future analyses. Study strengths include the large sample size and study design that leverages differences in telehealth use among disease groups to better isolate telehealth's impact on care patterns and pt demographics. Overall, we observed significant increases in pt volume and shifts in geographic distribution post-telehealth. Further analyses will be reported.

P4-06-06: The Etiologic Causes of Diagnostic Delay in Locally Advanced Breast Cancer: _x000B_A Multicentric Nationwide Trial

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Purpose: Breast cancer is the most common type of cancer in women worldwide, and early detection in advanced stages is the most crucial factor in reducing mortality and improving quality of life. The aim of the presented study is to investigate patient and region-specific factors causing delays in the diagnosis and treatment of breast cancer in different geographical regions in Türkiye.
Method: A survey study has been designed to be conducted between August 2023 and October 2024 in every region, involving women who have been diagnosed with locally advanced breast cancer. The survey consists of two parts, filled out by both patients and physicians, focusing on possible delays in diagnosis and treatment. The survey form, designed in the data collection section, was filled out during face-to-face interviews conducted by trained doctors/nurses. The questions cover socio-demographic factors (age, education level, first year of marriage, marital status, occupation, menopausal status, residence, health insurance, daily exercise duration, body mass index (BMI), smoking habits, X-ray history, chronic illness, delay duration, family history of breast cancer, age range of first pregnancy, history of benign breast disease, breast self-examination,

knowledge, and regular performance status) and clinical factors (lymph node status, first symptom type, tumor location, tumor type, date and type of first symptoms noticed by patients, date of first detection, date of first medical consultation, and socio-economic factors during that period).
Results: The study included 37 centers from 7 regions of Türkiye involved in survey creation and implementation. In the initial phase, a total of 886 surveys were evaluated. The median age was 46 (22-89 years). 47% of the patients had primary school education, 18% live in rural areas, 63% knew how to perform breast self-examinations (BSE) and 51% rarely do BSE. Of the patients, 87% initially visited hospitals. 425 patients had the disease detected during screening and 543 sought medical attention within 1 month of detecting a lump. 42 cases tried alternative medicine. Factors causing diagnostic delays were reported as 21% ignorance and 17% fear of death delaying medical consultation. The examination time by specialists varied, with 574 patients examined within 1 month after detecting a symptom, 183 within 1-3 months, and 129 taking over 3 months. Mammography was performed within 1 month in 78% of patients, within 1-3 months in the rest of the cases. Other radiological imaging (ultrasonography-magnetic resonance imaging) was done within 1 month for 561 (76%) patients, and tissue biopsy for 509 (70%) patients. The most significant factor in delaying the adjuvant chemotherapy and radiotherapy process was identified as treatment side effects. In the Black Sea region, most common factors causing diagnostic delays were inability to get medical appointments and fear of losing the breast, while in the Mediterranean and Eastern Anatolia regions, fear of death, ignorance, and inability to find time due to daily tasks were prominent. In the Southeastern Anatolia, Marmara, Aegean, and Central Anatolia regions, ignorance, economic reasons, fear of death, and fear of losing the breast were listed as the primary factors.
Conclusion: More than half of the women diagnosed with locally advanced breast cancer do know how to perform BSE, but only 29% of the patients perform BSE monthly. Across geographical regions, the most common factor causing delays among patient-based factors is fear of death following ignorance.

P4-06-07: The Impact of a Multidisciplinary Quality Assurance Conference on the Management of Inflammatory Breast Cancer

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Introduction: Inflammatory breast cancer (IBC) is rare but aggressive breast cancer. With the variability in presentation, making prompt diagnosis is challenging even for the experienced physicians. Only in the last several years has a dedicated diagnostic and management algorithm been agreed upon among multidisciplinary teams at one academic institution. A weekly multidisciplinary management conference (MTC) was developed at our institution three years ago specifically for IBC patients, to provide a common platform for reviewing cases, discussion of challenging diagnoses and treatments, sharing of clinical

trial protocols, and ultimately optimizing patient care. In this abstract, we investigate the impact of the conference discussion on IBC patient management.
Methodology: All patients who were presented at the IBC multidisciplinary conference from one tertiary academic center were identified from an IRB approved registry protocol, between June 2020 to May 2024. The chart review, imaging studies, associated pathology, and conference notes were retrospectively reviewed from the institutional medical record system EPIC.
Results: 205 patients were presented at the IBC multidisciplinary team conference during the study period. The patients' age at the time of IBC diagnosis was: mean age of 50.9 years and median age of 52 years (range 26-83). Patients' demographics are : 152 (74.1%) white, 28 (13.7%) black, 10 (4.9%) Asian, 1 (0.5%) native Hawaiian or other pacific islander, and 14 (6.8%) others. Reasons for presenting at MTC include newly diagnosis or suspicion of IBC 40.8%, treated or being treated at outside facility for second opinion 24 %, treated or being treated 19.2%, and interesting case by a clinician 16%. Diagnostic imaging modalities presented or discussed included: mammography 47% of cases, breast ultrasound 58%, biopsies 13%, CT chest abdomen pelvis 20%, PET/CT 51%, brain MRI 17%, bone scan 9%, and other tests (eg. non-breast ultrasound, abdominal MRI, nuclear medicine exams, etc.) 25%. Repeat or additional biopsies were recommended in 4% of cases. At the conclusion of the conferences, recommendations were document in EPIC. Confirmation of IBC diagnosis was reported in 61% (126/205) while in 1% (2/205) diagnoses were changed or consensus was not IBC. In 45% (92/205) patients, treatment recommendation or management change were made. 47% (97/205) patients were recommended for enrollment in clinical trials.
Discussion: Multidisciplinary team conference even in rare disease such as IBC is impactful and beneficial for patients' care resulting in almost half of the patients having recommendation for enrollment in clinical trials, as well as confirmation of accurate diagnosis by a multiteam approach.

P4-06-08: Regional Disparities in Mammography Access in Brazil: The Impact on the Consolidation of Effective Breast Cancer Screening

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Introduction: Breast cancer (BC) is the most frequently diagnosed and lethal neoplasm among women globally and in Brazil, constituting a significant public health burden.

Mammography is the primary strategy for early detection of BC, but its effectiveness in reducing mortality is most evident in populations where adherence to the exam reaches at least 70% of the target population. In Brazil, unfortunately, there is a significant regional disparity in access to health services, raising concerns about the true effectiveness BC screening. In light of this, we aim to access the regional disparities in the consolidation of breast cancer screening, comparing the number of mammograms performed in Brazil by the five regions (North, Northeast, Southwest, South, and Midwest) between the years 2020 and 2024. Methodology: The DATASUS platform was used to collect data from the Cancer Information System (SISCAN), with analysis of the screening mammography variant according to the patient's state of residence and the year of the procedure. Additionally, data from the Brazilian Institute of Geography and Statistics (IBGE) was used to consult the target female population for screening between two thresholds: of 40 and 74 years old and 50 and 69 years old, according to the 2022 Demographic Census. Results: A total of 8,954,424 mammograms were performed in Brazil between 2020 and June 2024, with the Southeast region accounting for approximately 36.8% of this total. The Northeast region ranked second, with 30.8% of the exams, followed by the South (20.6%), Midwest (6.9%), and North (4.7%) regions. The proportion of mammograms per female population aged 40-74 years varied across regions, with the highest percentage found in the South (29.16%), followed by the Northeast (26.44%), Midwest (20.09%), Southeast (17.92%), and North (15.70%). Furthermore, when analyzing the proportion of mammograms in women aged 50-69 years, the South region achieved the highest mammography coverage rate (51.42%), followed by the Northeast (49.73%), Midwest (38.13%), Southeast (32.27%), and North (31.17%). Conclusion: The study revealed significant regional disparities in access to mammography screening across Brazil. The South and Northeast regions demonstrated the highest accessibility rates for the target population, while the North region faced alarmingly low rates. Regardless of the age range considered as the target population, the national percentages fell short of the recommended levels for adequate screening. This scenario can be attributed, primarily, to the lack of effective public policies that ensure equitable access to healthcare, a fundamental principle of Brazil's Unified Health System (SUS). This lack of equity hinders the consolidation of effective breast cancer screening nationwide.

P4-06-09: Does Age Impact Patient Involvement in Treatment Decision Making: Results from the Canadian Breast Cancer Network (CBCN) Assessment Project

Kathleen D. Swiger, Scott Richter, Bukun Adegbembo, Cathy Ammendolea

Assessing the information and support needs of Canadians diagnosed with breast cancer must be rooted in an understating of the patient. In 2022, the Canadian Breast Cancer Network (CBCN) initiated a project to identify the educational needs of Canadian breast cancer patients in order to develop tailored, focused programs and materials to fill educational gaps.
We conducted 45-minute key informant interviews (7) with patients and oncologists to

determine the needs and educational gaps of breast cancer patients. Five 90-minute patient focus groups (32 participants) were conducted to enhance the interviews. Findings from the interviews and focus groups were used to inform the questions for an online survey fielded between May 1 and June 10, 2022. Data analysis began in September 2022 and is on-going. A review of survey findings of patients under 50 years (U50) (n=131) and those 70 years plus (70+) (n=124) revealed little difference between the age groups regarding their involvement in the treatment decision making process. Both groups were more likely than not to have a healthcare provider (HCP) review their pathology report with them (71.0% of U50 vs. 73.2% of 70+); be included in the treatment decision making process (87% of U50 group vs. 86.1% of 70+) and be provided with information before treatment (72.5% of U50 vs.75.4% of 70+). The above findings are all significant at less than 0.001. In making their decision, both groups (75% of U50 vs. 80% of 70+) reported that "Efficacy of Treatment" was their primary consideration.
Age should not be a factor in whether to include patients in the treatment decision-making process. Patients, whether under 50, or over 70, can be equally involved in this process, if they choose to be. Communication between the HCP and the patient during the treatment decision-making process may build trust and understanding of the differences in treatment and follow-up and instill confidence in the overall patient experience.

P4-06-10: Impact of Online Education in Improving Clinicians' Knowledge of the Hot Topics in Breast Cancer Clinical Research

Zhizhi Fiske, Stephen Dunn, Deborah Grainger, Eloise Ballard, Jamie Habib, Giuseppe Curigliano

Background: The treatment landscape for breast cancer is evolving rapidly, with many clinical trials investigating novel treatment strategies and promising new agents. The objective of this study was to assess the effect of an online continuing medical education (CME) activity on clinicians' knowledge in latest clinical trials investigating novel and emerging therapies in breast cancer and their confidence in identifying patients suitable for clinical trials of emerging therapies.
Methods: This CME activity consisted of a 15-minute video presentation with synchronized slides. Educational effect was assessed using a repeated-pair design with pre-/post-assessment. 3 multiple choice questions assessed knowledge, and 1 question rated on a Likert-type scale assessed confidence, with each individual serving as their own control. A McNemar's test assessed significance of improvement in the percentage of correct responses to knowledge questions from pre- to post-assessment. P values < .05 are statistically significant. The activity launched on 27th of September, 2023, with data collected through 19th January, 2024 being reported in the current study.
Results: 57 oncologists and 37 obstetricians/gynaecologists who answered all the assessment questions were included in this analysis. Analysis of pre- vs post-intervention responses demonstrated a significant improvement in overall knowledge of both physician learner groups, with 150% more oncologists answering all questions correctly after

education (30% post- vs 12% pre-CME) and 38% more obstetricians/gynaecologists answering all questions correctly after education (22% post- vs 16% pre-CME). Specifically, the knowledge regarding the latest clinical trials investigating novel and emerging therapies in breast cancer increased from 50% pre- to 68% post-CME for oncologists (P<.001) and from 38% pre- to 58% post-CME (P<.001) for obstetricians/gynaecologists, respectively. Additionally, 35% of oncologists and 11% of obstetricians/gynaecologists reported increased confidence in effectively managing adverse events associated with novel ADC therapy for breast cancer, and that increase was, on average, 48% and 44% among the two physician groups, respectively.
Conclusions: This analysis demonstrates the success of a 15-minute online educational activity on clinicians' knowledge of the evolving treatment landscape in breast cancer. As more clinical data become available, it will be important to provide ongoing education to clinicians on the implications of the new data and how to translate them into clinical practice.

P4-06-11: What do patients, doctors, and payers truly value? Developing a multi-criteria decision analytic tool that assesses the value of adjuvant hormonal plus CDK inhibitors in breast cancer

Omar Castillo-Fernandez, Cristiane Martin, Maria Lim, Taysser Sowley, Mario Guardia, Jonatan Quintero, Jaime Ordoñez

Background: Multicriteria Decision Analysis (MCDA) is a collaborative process that helps clinicians, payers, and patients consider various factors such as treatment effectiveness, potential side effects, cost, individual preferences, and quality of life outcomes. This study aims to evaluate the agreement among patients, doctors, and payers regarding the factors used to assess interventions for adjuvant CDK inhibitors and hormonal therapy in breast cancer in our country. Methods: To create the rating instrument, we formed three groups – The patient group: 15 patients with non-metastatic HR-positive Her2-negative breast cancer undergoing hormonal adjuvant treatment (patient panel), a payer panel (n=3) consisting of Institute personnel with budget responsibilities, and a group of 6 breast medical oncologist specialists. These groups discussed and selected the main domains and subdomains of the instrument in meetings held from September 2023 to June 2024. The group agreement was evaluated using Kendall's coefficient of concordance (W). Results: The agreed instrument has six main domains. 1) Health outcomes, 2) Safety, 3) Public Health,4) Financial and Economics, 5) Innovation, and 6) Patient/Society. A very strong agreement among their weight was observed W=.0.95 (p=0.014). There was a weak agreement in the Safety subdomain (tolerability and medication suspension W=0.11(p=0.56) mainly because of payer evaluation. A moderate agreement was observed in three subdomains: Public Health (Severity of disease, disease burden, and proportion of patients needing adjuvant treatment) W=0.33 (P=0.36). Innovation (Another alternative available, technological innovation, value of hope) W=0.53 (p=0.20) and Health Outcomes (iDFS, cost-effectiveness, Predictability of clinic benefit, Doses optimization, OS, and Number necessary

to treat) W=0.49 (p=0.19). Domains with a strong agreement: Financial/Economics (Budget impact, return to work) W=0.99 and Patient/Society (free treatment period, tolerance, quality of life, out-of-pocket expenses) W=0.81 (p=0.062). Most of the concordance was seen between doctors and patients. Conclusion: Using the MCDA in the adjuvant setting is feasible and requires an open and comprehensive discussion among the patients, doctors, and payers. A proof-of-concept application of this tool to new adjuvant options is currently underway at our institution.

P4-06-12: Breast Cancer Hub (BCH) Case Study – Addressing Breast Cancer Challenges in Villages in India with Grassroots Sustainable Solution

Ratna Basak, Sima Basak, Rimpa Biswas, Sabarna Saraswati, Lopamudra Das Roy

Background and Challenges: The death rate to Breast Cancer is significantly higher in India due to taboo, ignorance, inaccessibility to healthcare services, proper diagnosis, and treatment management. The situation in the villages is grimmer. This populace is not only in financial constraints, struggling for daily food, with health taking a back seat, but due to geographical barriers, the hospitals equipped with cancer screening & treatment are in towns/cities, needing hours of travel for the villagers as they live in the remote far-flung areas, investing the full day to go back and forth, adding to their transport charges. It is hard for them to miss on their daily wage, and since a family member needs to accompany the suspicious case, the monetary loss adds up, eventually delaying or skipping treatment.
Missions: Breast Cancer Hub (BCH), founded by Dr Lopamudra Das Roy, aims to bridge the healthcare gap between developed and developing countries by providing sustainable grassroots solutions with 100% Free services. Since its inception in 2017, BCH has improvised guidelines to meet community needs, considering cultural stigmas and socio-economic conditions.
Breast Cancer in underserved areas: In deprived regions, accessing specialized cancer treatment is challenging. Mammogram and ultrasound screenings are rare in these areas, contributing to delayed or avoided treatments. To address this, BCH developed Breast Self-Exam (BSE) cards in 24 languages for Her and Him making it inclusive, targeting underprivileged and conservative communities. These cards, along with awareness campaigns, screening camps, and providing a holistic approach by Adopting Villages for door-to-door Cancer Screening, Treatment Aid, Counseling, Support, and Food, are part of BCH's strategy to improve early detection and adherence to breast cancer screening guidelines.
Case Study from one of the BCH adopted villages in India: Dipali Basak, a 54-year-old agricultural laborer from Huda Village, Nadia District, West Bengal, India, represents a typical case of the struggles faced by women in remote areas. With a family income of approximately $60 per month, accessing healthcare is a significant challenge. Dr Das Roy conducted a cancer awareness camp in Huda Village on August 9, 2022, where Dipali learned about BSE. On December 7, 2022, BCH team visited Dipali's home during door-to-door Cancer screening, providing BSE cards in local language and contact information.

Healthcare Journey: In October 2023, Dipali discovered a lump while performing a BSE and reached out to a BCH field worker. Despite having a government-provided health card for underprivileged families, the process of getting proper medical care was arduous. The nearest primary health center lacked imaging facilities for cancer screening, requiring BCH team to take Dipali to various hospitals, involving multiple modes of transport (Toto, Auto, Bus, Train) and long travel times. At Krishnanagar Sadar District Hospital, Dipali received treatment, but with limited specialist availability and the need for private pathology tests due to inadequate hospital facilities. Dipali underwent surgery on January 17, 2024, at Krishnanagar Hospital. Post-surgery, BCH facilitated further testing and provided comprehensive support, including covering transport and remaining treatment costs, counseling, and follow-up care. Despite facing resistance from Dipali due to treatment side effects, BCH ensured she received ongoing chemotherapy and radiation.
Broader Implications: Dipali's case highlights the systemic issues in breast cancer care in developing regions, where lack of awareness, inadequate healthcare infrastructure, and financial constraints delay or prevent treatment. BCH's unique trendsetting efforts underscore the need for revising the guidelines to address these challenges effectively, necessitating policy changes and targeted healthcare strategies to better serve vulnerable populations.

P4-06-13: Empowering the clinician and patient communities through the development of an accessible, online tool to support diagnosis of Inflammatory Breast Cancer (IBC)

Lindsey Anstine, Alexandra Erwin, Hosea Baker, Sainath Vaidhyanath, Trevor Polischuk, Anjali Patel, Brady Kazar, Kimberly Sabelko, Glendon Zinser

Inflammatory Breast Cancer (IBC) is a rare and highly aggressive form of advanced breast cancer. Compared to other breast cancers, IBC is uniquely characterized by skin changes and breast swelling often resembling a breast infection. Additionally, IBC is largely a subjective, clinical diagnosis that is challenging to identify. These issues are further potentiated by a general lack of awareness in the clinical and patient communities, further contributing to delays in diagnosis and treatment. Together, these circumstances contribute to poorer outcomes for people with IBC, who have a 5-year relative survival rate of only 39%. Thus, there is an urgent need to educate health care providers and patients about IBC and to develop tools to aid in differentiating IBC from other forms of breast cancer, enabling more accurate and timely diagnosis. To begin to address these challenges, the IBC Scoring System Online Tool (IBC Online Tool) (komen.org/ibc-calc) was developed as an easy-to-use, web-based version to help health care providers recognize and more effectively diagnose IBC. The IBC Online Tool is based on a set of proposed common diagnostic criteria identified by a panel of experts within the Susan G. Komen-IBCRF IBC Collaborative in partnership with the Milburn Foundation (Jagsi et. al. Breast Cancer Res Treat. 2022) that has recently undergone scientific validation (publication in preparation). Prior to the online version, providers had to navigate to the publication and manually calculate a score. The

IBC Online Tool provides the proposed IBC diagnostic criteria in a more convenient form, allowing clinicians to quickly calculate a patient's possibility of having IBC in the clinic. Development of the IBC Online Tool required a highly collaborative effort with medical experts, patient advocates, in-house IT experts and others to provide insights to build and test the online platform. Components of the IBC Online Tool include a user interface, reference images of IBC, supporting text to aid with response selections and user feedback surveys. In addition, a results page can be downloaded to document breast changes and/or support patients in initiating discussions with their providers about the possibility of IBC. Available in both desktop and mobile versions, the online tool enables providers to more quickly and easily assess patients for IBC, which may result in more timely diagnosis and care. To drive and support the use of this important tool, additional IBC educational materials were created including A Health Care Providers Guide to IBC, A Patient's Guide to IBC and an IBC Collaborative webpage (komen.org/ibc) to house the tool, providing important IBC information and resources for clinicians, researchers and patients. A QR code linking to the IBC Online Tool was generated and included within these resources and the QR code has been presented by IBC experts at major conferences to drive access and usage of the IBC Online Tool. This approach has been highly successful in raising awareness of this important resource, driving its use across the United States and in over 60 countries worldwide (as of June 2024). By digitizing the IBC Scoring System into an easily accessible online version, health care providers can now easily access a critical tool for IBC diagnosis in the palm of their hand. The development of the IBC Online Tool will ultimately help increase diagnostic accuracy, guide treatment decisions and improve IBC patient inclusion in clinical trials. Furthermore, Komen's IBC resources can help to support patients in conversations with their doctors ultimately leading to more timely diagnosis and improved outcomes for this rare and understudied breast cancer.

P4-06-14: Closing gaps in the diagnosis and treatment journey by a collaborative process design in a social and non-profit association in Peru

Mauricio Leon Rivera, Franco Doimi Garcia, Jose Marin San Yen Man, Steffi González Bocanegra

Knowing that in our country, 6 women die per day from breast cancer, totally preventable diseases and that none should die and that the state has been totally overwhelmed in its work, is that we will manage to make the project is carried out by the Liga Peruana Contra el Cancer (LCC), ROCHE pharmacy and the Oncological Pathology laboratory become great allies to reinforce.
According to statistics from the LCC, this institution treats approximately 50,000 patients a year. Of these patients, 4,000 (8%) present abnormal cellular changes, of which 600 (15%) have a type of invasive cancer, and of these, invasive breast cancer occupies first place in incidence with nearly 300 cases annually.
To complete the diagnosis of breast cancer, the immunohistochemistry (IHQ) test must always be performed; however, the access to this test has been subject to the patient's

ability to pay for it, with a delivery time of 1 month for the results in average. A current alternative is for patients to be referred to public institutions; however, the time for referral and for carrying out this test until obtaining the results is too long.
This entire situation has resulted in only between 10 to 20% of the patients treated in the LCC, undergoing the IHQ test, and the remaining percentage is unknown if they did so. Likewise, this institution does not know the situation of all patients after being diagnosed with breast cancer because LCC only do diagnoses and not treatments.
The first objective of this project has been to close these gaps in complete diagnosis by providing patients with the IHQ test and, if necessary, the confirmatory in situ hybridization (SISH) test, free of charge and with results within 1 week. The second objective has been to achieve a referral of these patients in an accompanied manner to the public or private hospitals that correspond to them so that they can receive the appropriate treatment according to the sub molecular type of breast cancer they present and their clinical stage.
To date, after 1 year and 8 months of starting the project, IHQ tests have been performed on 385 patients, with a cumulative HER2 positivity rate of 28.8% (111 patients), of which 85 were HER2 +++ and 26 presented the amplified SISH test. The SISH positivity rate was 31%, with 26 amplified tests out of 84 SISH tests performed in doubtful HER2 patients (HER2 ++).
Regarding the design of the collaborative process, it has consisted of three stages: contact with the patient, affiliation to the program, and accompaniment to the respective hospital to receive the corresponding treatment.
Regarding contact with the patient, a rate of 100% has been achieved, that is, all patients diagnosed to date have been initially contacted by the staff of the LCC, and subsequently by the staff of the program's call center from pharmacy.
Next step, the affiliation of patients to the pharmacy support program has reached 60% after collaborative work between the LCC, the pathology laboratory, and pharmacy lab.
Of the total number of patients affiliated with the program, 34 patients, 25 (74%) have managed to receive treatment, and 9 (26%) are in the staging process to choose the corresponding treatment, being accompanied by the pharmacy´s patient program.
Of these patients treated in the LCC, 46% have public health insurance of the ministry of health (SIS program), 43% belong to social security (ESSALUD), and the rest have private insurance (EPS) or insurance from the armed forces.
Without this project, these patients would have been left with a incomplete diagnosis of breast cancer in a timely manner, and without the corresponding treatment.

P4-06-15: Resource Utilization by Patients Living with Advanced Breast Cancer (ABC) Who Had a Nurse Practitioner-Led Visit

Ginny Kirklin, Abbey Kaler, Akshara Singareeka Raghavendra, Bei Wang, Ashley Anderson, Cathy Harris, Isabella Masso, Debasish Tripathy

Significance and Purpose: The diagnosis of ABC is life-changing and requires greater support and resource utilization compared to early-stage breast cancer patients. The ABC Program aims to improve the quality and quantity of life for ABC patients through navigation, education, and support. At an ABC Advanced Practice Registered Nurse (APRN) led visit, patients are guided through the cancer center services and provided education, resources, emotional support, managed care between departments, and informed about clinical trial opportunities and eligibility. The program reviewed resource utilization of the ABC patients seen by an ABC APRN at a comprehensive cancer center in southern US to understand what additional multi-disciplinary interventions are needed. The specific resources included emergency room and inpatients visits, therapeutic clinical trials, and completed visits to the LIMBS Clinic (Linking Internal Medicine and Metastatic Breast cancer for Success), a subspeciality clinic providing access to internal medicine services coordinated with oncology care.
Methods: Data were collected as part of an ABC APRN-led visit between March 2020 to May 2024. Patients were referred by clinicians or by self. Data were analyzed utilizing descriptive statistics.
Findings: A total of 480 patients had a total of 1,769 visits with an ABC APRN via video (94%) or telephone (6%). Participants were female (99%) and male (0.63%) with a diagnosis of ABC and a mean age of 57; 69% self-identified as white, 16% as black, and 84% non-Hispanic. Most patients (53%) live within 75 miles of the cancer center followed by 23% of patients living between 151 to 500 miles of the cancer center. Most patients (68%) had an ECOG status of 0 or 1. Fifty-six percent of patients presented to the emergency room visit (n=1,052). Of those, 48% of patients had an inpatient admission (n=587). Sixteen percent of patients were active on a therapeutic clinical trial at the cancer center, and 25% of patients had a completed visit in the LIMBS Clinic. There were 425 internal referrals including the LIMBS Clinic (n=110), Integrative Medicine (n=78), Supportive Care (n=59), Nutrition (n=42), and Physical Therapy (n=28) being the top five referrals from the ABC APRN visits. Most patients (52%) had at least one internal referral. Most incoming referrals to the ABC APRN clinic were from the Texas Medical Center site (77%) followed by a regional campus (14%) 41 miles north of the cancer center.
Discussion and Implications: People living with ABC utilize multiple resources for management of disease and treatment. Limitations include only having access to data for resource utilization at the cancer center and not resources available through the community. Additional research needs to be completed to match ABC patient needs to the type and amount of services and resources. Robust programs are needed to streamline and proactively address ABCs patient's needs.

P4-06-24: The effects of two multimodal exercise programs (face-to-face vs online) on physical and functional outcomes in breast cancer survivors: A randomized control trial.

Monica Castellanos-Montealegre, Soraya Casla-Barrio, Helios Pareja, Laura Cantero

Introduction: Extensive evidence supports the numerous benefits of physical exercise programs in cancer patients. Physical exercise has been demonstrated to reduce many of the side effects of cancer and its treatments, primarily by addressing alterations in body composition, CRF, and fatigue, all of which are associated with quality of life and survival. Despite these benefits, it is estimated that only 36% of breast cancer survivors adhere to the exercise recommendations for cancer survivors set by the World Health Organization (WHO). In this Randomized Control Trial (RCT), we examined the impact of a face-to-face oncological exercise program compared with an online oncological exercise program on body composition, CRF, fatigue, and quality of life in patients with breast cancer in stage IA to IIIB.

Methodology: Women diagnosed with primary breast cancer (stages IA to IIIB); aged 18 years or older; no more than 10 years since cancer diagnosis; and without other comorbidities that would limit their capacity to engage in exercise were included in this study. Patients assigned to the intervention group underwent a multimodal exercise program intervention for 16 weeks. This intervention was conducted both in-person at the training center and online to enhance participant adherence.

Both oncological exercise programs (online and in-person intervention) included resistance, endurance, balance, and proprioception exercises for 16 weeks, with intensity ranging from 55% to 95% of heart rate reserve (HRR). Each session lasted 75 minutes and was structured as follows: 10 minutes of warm-up involving joint mobilities, balance and proprioceptive exercises, and aerobic exercise at an intensity ranging from 55% to 75% HRR. This was followed by two 20-minute bouts of combined activities (endurance activities combined with resistance exercises) at an average intensity of 70-75% HRR with 30 to 60 second bouts of high-intensity activity of 85% to 100% HRR. The resistance exercises were developed with free weights in circuits of 6 to 8 exercises of 3x10 or 3x15, combined with endurance activities of 60% HRR. Intensity perception in resistance exercises was assessed using the Borg Scale, and endurance exercise intensity was assessed by HRR. Finally, full-body stretching was performed. The only difference between the two types of programs was the remote or face-to-face format.

Demographic data were analyzed by descriptive methods and were presented by Media and standard deviation and frequencies. Baseline analysis comparisons between groups were developed by t-test for independent samples. Final group comparisons were developed with the ANCOVA test, adjusting results by baseline assessments in each variable. Cohen's d was performed to find the effect size in the final group comparison results.

Results: 40 patients in the intervention group (mean 50.2±1.05 years old) and 34 in the control group (mean 49.48±1.88 years old) completed the study. In the intervention group, 14 (35.9%) patients participated in the online program, while 25 (64.1%) attended the in-

person sessions. The attendance rates for the intervention group were 89.5% in both instances. Specifically, for the online group, 84.02% of the sessions were conducted online, while 15.98% of the attendance occurred in person. Conversely, for the in-person intervention, 76.64% of the sessions were face-to-face, and 23.35% of the attendance was through online classes. No differences in attendance between interventions were observed (p=0.997) (table 1). In addition, no significant differences between interventions were observed in physical (cardiorespiratory fitness, functional capacity, and body composition) or emotional (fatigue and quality of life) variables (table 2).

Conclusions:
The multimodal physical exercise interventions (online and face-to-face) in this study prove to be effective not only in improving fitness and body composition, but also in promoting different exercise strategies, and improving the accessibility of exercise programs for patients far from sports centers. This accessibility is crucial in ensuring high levels of adherence to these programs and adapting the intervention to the personal situation of patients.

P4-06-25: The impact of multimodal exercise program on physical and functional outcomes in breast cancer survivors: A randomized controlled trial.

Soraya Casla-Barrio, Mónica Castellanos-Montealegre, Helios Pareja, Laura Cantero

Introduction: Despite there are considerable number and variety of physical exercise programs having demonstrated benefits in breast cancer patients (BCP), exist certain factors such as the lack of awareness and difficulty in accessing oncology exercise programs and the scarcity of qualified professionals in this field difficulted the adherence of the breast cancer survivors to the exercise recommendations by the World Health Organization (WHO). This study aimed to evaluate the impact of a multimodal oncological exercise program on these parameters in breast cancer patients at stages IA to IIIB.
Methodology: Patients contacted the research team to schedule baseline assessments at Centro Ejercicio Y Cáncer, in Madrid. Inclusion criteria for participation were women diagnosed with primary breast cancer (stages IA to IIIB); aged 18 years or older; no more than 10 years since cancer diagnosis; and without other comorbidities that would limit their capacity to engage in exercise (recent surgery or functional limitations) were included in this study. Patients with metastatic disease, undergoing chemotherapy and radiotherapy were excluded from the study. Afterward, all women were randomly assigned to one of the two groups. The intervention comprised a multimodal exercise program (online and face-to-face) for 16 weeks that included endurance, resistance, balance, and proprioception exercises, with an intensity ranging from 55% to 95% of Heart Rate Reserve (HRR). Each session lasted 75 minutes and was structured as follows: 10 minutes of warm-up involving

joint mobilities, balance, and proprioceptive exercises, and aerobic exercise at an intensity ranging from 55% to 75% HRR. This was followed by two 20-minute bouts of combined activities (endurance activities combined with resistance exercises) at an average intensity of 70-75% HRR with 30 to 60-second bouts of high-intensity activity of 85% to 100% HRR. The resistance exercises were developed with free weights in circuits of 6 to 8 exercises of 3x10 or 3x15, combined with endurance activities of 60% HRR. Intensity perception in resistance exercises was assessed using the Borg Scale, and endurance exercise intensity was assessed by HRR. Finally, full-body stretching was performed.

Results: 40 patients in the intervention group (mean 50.2±1.05 years old) and 34 in the control group (mean 49.48±1.88 years old) completed the study. Related to the program adherence was 95.23% in the intervention group and 82.93% in the control group, and the assistance rate was 85.13%. BCP in the intervention group showed significant improvements in cardiorespiratory fitness (DifMean (ΔM): 7.19 ml.kg.min; CI95% 4.50 – 9.60; p=<0.0001); fat mass (ΔM: -1.81%; CI95% -3.47 - -0.15; p=0.033); lean mass (ΔM: 5.24%; CI95% 3.74 – 6.74; p=<0.0001); functional capacity through sit -to-stand (ΔM: 5.26 number of repetitions; CI95% 2.01 – 5.26; p=0.002) and 6-minutes walking test (ΔM: 89.58 meters; CI95% 36.78 – 142.37; p=0.001); level of PA such us total METs (ΔM: 946.85 METs (min/week); CI95% 212.08 - 1681.64; p=0.012) and high intensity METs (ΔM: 419.65 METs (min/week); CI95% 37.93 - 801.36; p=0.032); Fatigue (ΔM: -3.20; CI95% -5.75 - -0.65; p=0.014)

Conclusions:
This RCT has demonstrated a multimodal exercise program is feasible and effective in significantly improving cardiorespiratory fitness, body composition, and physical function in BCP. The implementation of these new models of exercise interventions not only allows BCPs to access supervised exercise programs but also, presents high levels of adherence. These findings advocate for integrating structured exercise programs into the treatment plan for breast cancer patients, with potential long-term benefits for survival and quality of life.

P4-06-26: Fertility Preservation in Young Women with Breast Cancer in Brazil: Patient Profiles and Challenges in a Real World Scenario

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Introduction: Breast cancer, whose incidence among adolescents and young adults (AYA) has increased in recent years, harbors many global challenges and unmet needs. Among them, concerns regarding fertility preservation stand out. Many barriers, such as financial support, lack of timely referral and availability of cryopreservation have been identified,

which may be exacerbated in developing countries. In this study, we accessed real-world data regarding fertility preservation in Brazil among AYA breast cancer patients.
Methods: This retrospective cross-sectional observational study was conducted in a private referral network cancer center in Brazil. Breast cancer premenopausal patients aged 15 to 39 years undergoing systemic treatment between 2019 and 2023 were identified. Clinical data was obtained retrospectively from medical records. We searched for demographic and clinicopathologic characteristics and data regarding fertility counseling, funding and the practices of fertility preservation groups, aiming to map out the care among this population. Descriptive statistics were used to summarize data.
Results: In this cohort, 60 AYA breast cancer patients were identified, representing 10.9% of the disease diagnosis. Of them, 49 (81.6%) underwent chemotherapy, most with anthracycline-containing regimens (82.0%). However, only 23 (38.3%) of the patients had fertility counseling documented in their medical records. Among these referred patients, 12 (52.1%), in fact, proceeded to evaluation by the reproductive medicine team. Oocyte collection for cryopreservation was performed in 7 patients, representing 14.2% under systemic treatment. When this technique was not feasible, some patients (n=3) received LHRH agonists in monotherapy, considered a less effective method. The average time between diagnosis and the initiation of oncologic treatment was also analyzed. Among patients who underwent fertility counseling, it was 25 days (range, 7-34), a similar timeframe to those who did not (19 days, range 3-37). Considering only the patients who underwent oocyte collection, the median time was 26 days (range, 9-34). Regarding fertility preservation funding, procedures and medical expenses were not covered or reimbursed by health insurance but solely by the patient.
Conclusion: The present study shows a low rate of cryopreservation among AYA breast cancer patients who underwent systemic treatment in Brazil, although this procedure did not lead to clinically relevant delays in the initiation of chemotherapy. Potential barriers include a poor rate of fertility counseling and funding concerns. Given Brazilian economic and sociodemographic inequalities, this scenario may be even worse in the public setting and outside referral centers. This study highlights the need for standardized protocols to ensure that all AYA patients are counseled on fertility preservation from initial diagnosis. This includes integrating multidisciplinary teams, providing specific training for oncologists and other healthcare professionals and establishing automatic referrals for consultation with fertility specialists. Additionally, providing psychological support is crucial to help patients make informed decisions aligned with their future maternity desires. Thus, further studies would support national cancer care improvements among this population.

P4-06-27: "The decrease in survival of breast cancer patients during the COVID-19 pandemic"

Arely Gonzalez, Diana Vilar, Nancy Reynoso, Rafel Franco, Juan Medina, Paula Cabrera

COVID-19 tested the health system and its processes in the world, modifying primary and secondary health care for many diseases. Among the most affected were breast cancer, who

faced a delay not only in detection, but also in the start of treatments and the follow-up during this contingency.

In Mexico, the National Cancer Institute (INCan) was the only hybrid center that treated patients with cancer and COVID-19.

Cancer and COVID-19 management were a challenge from different angles, with consequences that are still difficult to evaluate. Breast cancer patients were affected by the pathology itself (COVID and cancer), by the hospital re-organization (no more hospitals in Mexico City look for patients with both diseases) and the shortage of oncological drugs (by political decisions and because the global quarantine. With this in mind, the following paper is presented.

We carried out a case-cohort study of women with breast cancer treated at the INCan between January 1, 2020, and December 31, 2021 (2,153 women). During this period, there were 151 cases of COVID-19 (defined as a patient with cancer and a positive test for SARS-CoV-2, by polymerase chain reaction (PCR) or an antigenic test). A pairing was performed with a case control ratio of 1:3, adjusted by clinical stage, molecular biology and month of care, with a total of 453 women studied.

Of our population, 20.4% of patients did not follow the indicated treatment. This had an impact on survival of the patients despite a follow-up of just 2 years, with 10.9% of deaths (66, of which 36 were secondary to the presence of COVID 19). Furthermore, there were patients who were never able to start oncological treatment after SARS-CoV-2 infection.

Regarding treatment compliance, 114 women (20.4%) did not follow the indicated treatment, 19 (17.12%) patients with COVID 19 presented disease progression, greater than that observed in the group without COVID-19 (3.01%).

Another relevant point of the findings in this study is that one of the main reasons for the delay in treatment was not directly attributable to COVID-19, but to the lack of medications. Although the worldwide stillness decreased the distribution of medicines and the demands for other medical supplies such as gloves and masks increased, this was aggravated in our country by a significant shortage of oncological medicines, derived in part from the health policies implemented in this six-year term. In this cohort, 20.4% of patients did not receive optimal treatment (due to lack of medications or lack of time). When reviewing this information, it is paradoxical that there were more women affected by the shortage of medicines than by the COVID-19 infection itself. These delays and changes in regimens due to lack of medications may have a high impact on the 5-year survival, and very possibly, on the quality of life of these women.

It is important to highlight that in this study the presence of COVID-19 was an important factor for non-compliance with oncological treatment despite not being significant, finding a trend at two years of follow-up or at the end of the initial treatment ($p=0.052$). In a subsequent study we intend to analyze the impact of COVID-19 on 5-year survival.

In our study, the prevalence of COVID-19 in patients with breast cancer was 7.11%, with a mortality of 23.8%.

The interruption, delay and multiple changes on the breast cancer treatment due to COVID-19 pandemic, modified negatively the outcome of these patients on a short term by decreasing the surveillance and arising progression case.

P4-06-28: Income and Geography: Key Drivers of Disparities in Breast Cancer Mortality

Emily McGovern, Wade Swenson, Ingrid Jacobson, Abigail Swenson, Zack Schroeder, Aquino Williams, Andrew Armstrong, Brooke Gully, Sahil Prakash, Sean Kelso

Background: Disparities in breast cancer outcomes between rural and urban populations are influenced by socioeconomic status and access to healthcare. This study examines the rural cancer gap in breast cancer survival rates, focusing on county-level geographic and income-related disparities.
Methods: Data from the SEER database (2001-2020) were analyzed, focusing on breast cancer cases. We compared observed 5-year survival rates between metropolitan (Metro) and non-metropolitan (Non-Metro) counties, as well as between counties with median household incomes above and below $50,000.
Results: Our analysis revealed a statistically significant rural cancer gap in breast cancer outcomes. Non-Metro counties consistently had lower 5-year survival rates compared to Metro counties. Additionally, counties with median household incomes below $50,000 showed significantly worse outcomes than those above this threshold. The mortality difference persisted over the entire observation period.
Conclusions: The findings highlight substantial disparities in breast cancer survival based on both county-level geographic location and income, emphasizing the need for targeted interventions and policies to improve outcomes in underserved populations.

P4-06-29: The impact of cardiovascular disease on breast cancer stage at diagnosis

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Background: Cardiovascular disease (CVD) and cancer are the leading causes of mortality in the United States. Large-scale population-based and mechanistic studies support a direct effect of CVD on accelerated tumor growth and spread, including in breast cancer. Our objective was to test the hypothesis that individuals with prevalent CVD will present with more advanced breast cancers at the time of diagnosis compared to those without CVD.
Methods: We conducted a retrospective cohort study in the Surveillance, Epidemiology, and End Results (SEER)-Medicare linked databases from 2009-2020. Participants were female patients aged 66 years or older at diagnosis of invasive breast cancer. We utilized a case-control design, based on breast cancer stage at diagnosis, propensity score matched on factors know to be related to delayed cancer diagnosis. Our full analytic cohort included 19,292 matched individuals, with median age 73 years (interquartile range 70-79), of which 86% were white and 49% had prevalent CVD. The exposure was prevalent CVD prior to breast cancer diagnosis. Our a priori hypothesis tested the odds of locally advanced (T3-4 or N+) or metastatic (M+) breast cancer at diagnosis based on prevalent CVD status.
Results: In propensity score matched, multivariable adjusted models we found that

individuals with prevalent CVD had a statistically significantly increased odds of locally advanced or metastatic breast cancer at diagnosis (OR, 1.10; 95% CI, 1.03-1.17; p=0.007). This association was observed among hormone receptor positive (OR, 1.11; 95% CI, 1.03-1.19; p=0.006), but not hormone receptor negative (OR, 1.02; 95% CI, 0.86-1.21; p=0.834) breast cancer. Our results were directionally consistent when separately examining locally advanced (OR, 1.09; 95% CI, 1.02-1.17; p=0.017) and metastatic (OR, 1.20; 95% CI, 0.94-1.54; p=0.152) disease, among all receptor subtypes.
Conclusions: Our study provides evidence that prevalent cardiovascular disease is associated with more advanced breast cancer at diagnosis. This finding may be specific to hormone receptor positive disease. Future studies are needed to confirm our findings and investigate interventions to improve patient outcomes, including personalized screening.

P4-06-30: Comprehensive Approach to Prevention: Cancer Risk Reduction and Education (CaRE) Program

Jessica Jones, Virginia Kaklamani, George McCann, Ismail Jatoi, Rachel Darling

With overall breast cancer incidence increasing, and a shift towards higher rates of premenopausal breast cancer (BC), effective risk reduction strategies are paramount for Latinx women. UTHealth San Antonio MD Anderson Mays Cancer Center (MCC) serves a majority minority community of Latinx patients, and Latinx women are more likely to hold BC adjacent diseases such as obesity and diabetes. Premenopausal Hispanic women are more likely to develop HR-negative BC. The healthcare systems within UTHSA holds a high number of Hispanic, Medicaid, low-income, and uninsured (MLIU) patients, and MCC provides care to both San Antonio but surrounding medically underserved areas (MUA). The newest guidelines recommend mammography every 2 years for women starting at the age of 40 have the potential to negatively impact Latinx MLIU since mammography preferentially detects more indolent HR-positive than higher mortality HR-negative tumors. False positives associated with breast MRI screening can lead to scan-anxiety and over-diagnosis. The novel and innovative Cancer Risk Reduction and Education (CaRE) program is a multispecialty endorsed precision prevention clinic which coordinates higher level BC surveillance for patients at higher risk of BC and integrates prevention strategies for modifiable risk factors. Herein, the following processes are shown: (i) Mission statement of program, (ii) Stakeholder engagement, (ii) BC Risk assessment models used, (iii) Site Specific treatment plan strategies for non-modifiable risk factors, (iv) Site specific treatment plan strategies for modifiable risk factors. Taken together, combined efforts from multiple stakeholders demonstrates a comprehensive program that provides personalized cancer risk assessments and will effectively reducing cancer incidence and improving long-term health outcomes. This program also sets the stage for future research opportunities to include patient outcome measures, adherence, cardio-oncology, weight loss studies, genetics, psychosocial interventions, medical education, patient education, and patient advocacy.

P4-07-01: Comparative 10-Year Survival Analysis of Breast-Conserving Surgery Versus Mastectomy in Early-Stage Breast Cancer for Young Patients

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Abstract: Comparative 10-Year Survival Analysis of Breast-Conserving Surgery Versus Mastectomy in Early-Stage Breast Cancer for Young Patients

Introduction:Pivotal studies established that breast-conserving surgery (BCS) followed by radiotherapy is as effective as mastectomy for breast cancer treatment. Recent retrospective population-based studies suggest a potential overall survival benefit for BCS over mastectomy, particularly in older patients. In younger patients this benefit was less clear. Therefore, we evaluated 10-year overall survival and distant metastasis-free survival in patients with early breast cancer, aged up to 55 years, comparing BCS with radiotherapy to mastectomy.
Methods:We conducted a retrospective cohort study using data from UZ Leuven including female patients aged up to 55 years, diagnosed with early invasive breast cancer (pT1-2, N0-1; for those receiving neo-adjuvant therapy cT1-2, cN0-1 and ypT0-2, N0-1) between 2008 and 2012. Patients underwent either mastectomy (with or without radiotherapy) or BCS with radiotherapy. Decision for type of surgery was made based on tumor and patient characteristics, as well as patients' preference. All received systemic adjuvant therapy according to prevailing standard of care at that time. Outcomes included overall survival and distant metastasis-free survival. The Kaplan-Meier method estimated crude survival rates 10 years after diagnosis. Multivariable Cox proportional hazard regression, incorporating propensity scores, was used to correct for confounding factors (age, cT, cN, pT, pN, molecular subtype, grade, and multifocality).
Results:Of 986 eligible patients, 407 underwent mastectomy (73.5% postop radiotherapy) and 579 received BCS (100% postop radiotherapy). Average age at diagnosis was 46,4 years and median follow-up time was 12,7 years. Patients who received a mastectomy had larger tumors and were more frequently node positive. Furthermore, these tumors were often of higher grade and multifocal. Of the patients in the mastectomy group 14.0% had triple negative tumors, compared to 15.7% in the BCS group. However, the patients in the mastectomy group had more HER2-positive tumors (15.2% vs 8.6% for BCS). Adjuvant chemotherapy was administered to 60.1% of patients who underwent mastectomy compared to 41.6% of patients who had breast-conserving surgery (BCS). Alternatively, neo-adjuvant chemotherapy was given to 5.6% of patients in the mastectomy group, whereas 2.2% of patients in the BCS group.
Overall unadjusted overall survival rates (crude survival, not corrected for confounding factors) were 85.5% for mastectomy and 92.1% for BCS. Adjusted analysis showed no significant difference in overall survival, though it did seem to show a trend favoring BCS (HR 0.52 [95% CI 0.36-0.77]; p=0.001; adjusted HR 0.67 [0.43-1.06]; p=0.09). This trend

was less clear for distant metastasis-free survival (DMFS HR 0.65 [95% CI 0.46-0.92]; p=0.01; adjusted HR 0.88 [95% CI 0.59-1.32]; p=0.56).
Conclusion:Consistent with previous cohort studies, our single-center study suggests a trend favoring BCS over mastectomy in terms of overall survival for patients up to 55 years old. This trend, however, was not statistically significant after adjusting for confounding factors, highlighting the need for further investigation of this population using larger cohorts.

P4-07-02: Concordance rate of 99mTc and ICG/near-infrared light detected sentinel-symphnodes in primary breast cancer

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Background: Sentinel lymph node biopsy (SLNB) by 99mTechnetium +/- patent blue has been long-time standard in SLN detection. However, the lack of nuclear sources, limited availability of nuclear medicine departments in some health providers and cost issues have led to the development of other techniques. ICG labelling and detection by near-infrared light is one of these techniques which empower surgeons to schedule their surgery independently from these prerequisites. Patients and methods: We conducted a prospective study to evaluate the concordance rate of ICG-fluorescence and 99mTc for sentinel lymph node detection of primary breast cancer. Patients, aged 18 - 80 years, with unilateral or bilateral, unifocal or multifocal/ multicentric primary breast cancer without signs of metastases were eligible for this study. ECOG status of 0-2 and life expectancy > 1 year was required. Tumor stages included are a) Tis (>= 4 cm) b) T1 c) T2 and d) T3. We applied both sentinel lymph node labelling (99 mTc and ICG) in the same patient and compared the concordance of both methods. Results: 275 pateints were elgible for interims analysis. For the first sentinel lymph node, we detected a concordance rate for both methods of 91,7 % , for the second sentinel lymphnode 81 %. 3 and 6 additional SLN were detected as first and second SLN by ICG which would not have been detected by 99mTc. After neoadjuvant chemotherapy, all sentinel lymphnodes have been detected by ICG. Conclusion: SLNB by ICG near-infrared light is a valid alternative for radioactive labelled SLN, however does reveal different SLNs as the second SLN: After neoadjuvant chemotherapy, ICG was able to detect all SLNs.

P4-07-03: LONG TERM PATIENT REPORTED OUTCOMES FOLLOWING THERAPEUTIC MAMMAPLASTY

Katherine Fairhurst, Harriet Cook, Joseph Vane-Daniel, Alison Hunter-Smith, Richard Sutton

Background: Therapeutic Mammaplasty (TM) is a safe oncoplastic surgical technique aiming to extend the boundaries of traditional breast conserving therapy (BCT) by removing larger breast volumes to reduce and/or lift the breast, without compromising cosmetic or oncological outcomes. Several systematic reviews have emphasised the paucity

of Patient Reported Outcome Measures (PROMs) following TM. This study aimed to assess the long-term PROMs following TM performed in our centre over a 17-year period.
Methods: Eligible women who underwent TM between 2005 and 2021 were invited to participate by returning the BREAST-Q questionnaire (combination of BCT and Reduction/Mastopexy modules). Surveys were returned by post or online from August to December 2023 (minimum 20-months post TM). Raw responses were transformed using Rasch conversion tables (0=worst;100=best) and descriptive summary statistics generated. Clinical outcome data was collected from digital hospital records/databases and analysed using descriptive summary statistics.
Results: Of 246 patients who underwent TM, n=21 (8.5%) subsequently required completion mastectomy, n=15 (6.1%) developed recurrence/metastatic disease and n=22 (8.9%) died. Questionnaires were returned by n=103/188 (53.4%) participants. Only n=4/103 (3.9%) participants were current smokers at the time of surgery; n=10/103 (9.7%) were ex-smokers. Neoadjuvant treatment was given to n=31/103 (30.1%) patients. Most tumours were T1 (n=53/103, 51.5%) or T2 (n=36/103, 34.9%). Only n=19/103 (18.4%) patients had immediate contralateral symmetrizing surgery at the time of TM, but a further n=34/103 (33.0%) had delayed symmetrization surgery. Few women suffered complications (n=15/103, 14.6%), all were minor and there were no peri-operative deaths. Most women received adjuvant radiotherapy (n=95/103, 92.2%); around two thirds received adjuvant endocrine treatment (n=63/103, 61.2%).
Overall, patients reported median scores of 69 (IQR 53-83.5) and 70 (IQR 54-86) for satisfaction with breasts for BCT/breast reduction modules respectively. Median wellbeing scores were physical (chest wall) 82 (IQR 66-100), physical (reduction) 72 (IQR 59-82) and psychosocial 77 (IQR 62-93) with lowest scores for sexual wellbeing 59 (IQR 36-79). Comparison was made with published long-term PROMs 12 years following immediate breast reconstruction (IBR) (Johnson et al doi:10.1093/bjs/znad276) using a minimal clinically important difference in mean scores of 4-points for satisfaction with breasts/psychosocial/sexual wellbeing and 3-points for physical wellbeing. TMs performed from 2005 to 2015 (n=40, 8-18 years previously) had significantly better mean long-term satisfaction with breasts scores (TM 72.3(BCT)/74.5(reduction) than all forms of IBR (Implant/expander 54.7; Latissimus Dorsi flap (LD) 59; Abdominal flaps 67.6). Sexual wellbeing scores were also significantly higher for TM (57.4) than for all forms of IBR (Implant/expander 44.7;LD 47.4;Abdominal 51.2). Psychosocial wellbeing scores for TM (78) were higher than both implant/expander 72.2 and LD 73.3 IBR, but comparable to abdominal flap IBR (77.6). TM physical (chest wall) wellbeing scores (80.6) were comparable to implant/expander (82.1) and LD (79.5) IBR, but significantly lower than abdominal flap IBR (87.8).
Conclusions: TM has a long-lasting positive impact on quality-of-life following breast cancer treatment. Patient satisfaction with breasts, sexual and psychosocial wellbeing may be significantly higher in the long term than all types of IBR. Physical wellbeing outcomes are comparable to both implant/expander and LD flap IBR but worse than abdominal flaps in our series.
With the now growing body of evidence suggesting BCT may also confer a survival

advantage over mastectomy, this data suggesting that overall, quality of life is better in the long term, further supports offering oncoplastic BCT preferentially whenever it is oncologically feasible.

P4-07-04: Impact of Involved Margin Status and Radiotherapy on Local Recurrence and Regional Recurrence in Patients Who Underwent Nipple Sparing Mastectomy or Skin Sparing Mastectomy with Immediate Reconstruction

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Backgrounds: A superficial margin less than 2mm from tumor is considered a risk factor for local recurrence (LR) after breast cancer surgery. For the prevention of LR and RR, radiation therapy (RT) is acknowledged as the principal adjuvant therapy. However, its role following NSM/SSM is less defined in contrast to the clear indication for RT following conventional mastectomy.
Purpose: The objective of this study was to investigate the factors associated with LR and RR to assess the impact of an involved margin and RT on LR and RR in patients who underwent NSM/SSM with immediate breast reconstruction for breast cancer.
Method: We conducted retrospective analysis of the medical records from 1675 patients who underwent NSM/SSM with immediate reconstruction for breast malignancies between 2004 and 2019. Patients who underwent NSM/SSM for recurrent disease, palliative surgery, prophylactic surgery, or delayed reconstruction were excluded. Uni- and multivariable analyses were used to assess the association of margin status, RT, and other clinicopathologic factors with LR, RR, local recurrence-free survival (LRFS) and regional recurrence-free survival (RRFS).
Result: Out of all patients, 28 experienced LR. Among the 28 LR cases, one patient had an involved margin, six patients had close resection margin (<2mm), and one patient received RT. Multivariable analysis revealed that HER2-positive subtype was a significant risk factor for LR (P=0.039) while adjuvant RT was identified to be protective (P=0.027). An involved margin status (P=0.009) and pathologic subtype including luminal B and triple-negative subtype (P<0.001) were significant risk factor associated to RR. In the multivariable model for survival, RT was the sole significant factor for LRFS showing protective effect (P=0.037). An involved margin status (P=0.021), luminal B and triple-negative subtype (P<0.001) were identified as a risk factor associated with shorter RRFS. Notably, RT did not exhibit a statistically significant association with LRFS in univariate analysis (P=0.245).
Conclusion: Tumor subtype is more strongly associated with LR compared to margin status. Additionally, RT showed protective effect against LR and LRFS. Although RT did not exhibit a direct correlation with RR, RT can be considered for LR prevention in patients with HER2-positive breast cancer or those with involved superficial margin.

P4-07-05: Breast Cancer and Local Therapy: Breast Conservation and Oncoplastic Surgery are Associated with Improved Quality of Life

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Introduction: Breast cancer is one of the leading causes of cancer in women worldwide, and local treatment can be distressful to patients. We aimed to evaluate how different types of local treatment impact the quality of life of Breast Cancer patients.

Methods: This is a retrospective cohort study from 2017 to 2022. All Breast Cancer patients treated in our institution are followed and voluntarily invited to answer Breast-Q Satisfaction with Breasts questionnaire pre and post-operatively. Of 711 eligible patients, 349 female patients answered both pre and post-operative questionnaires, and were included in the final analysis. Patients were divided in two groups: breast conservation and mastectomy. Use of oncoplastic surgery and types of breast reconstruction were recorded. Breast-Q Satisfaction with Breasts scores collected one year after a breast oncological surgery was used as a surrogate for Quality of Life. Linear regression was used to estimate the impact of breast conservation, use of oncoplastic surgery, types of breast reconstruction, and use of radiation therapy on Breast-Q scores. All analyses were adjusted by age, education, marital status, body-mass index, T stage, N stage, tumor subtype, presence of bilateral cancer, radiation therapy, axillary surgery, presence of complications, and pre-operative Breast-Q scores.

Results: In total, 237 (68%) patients received breast-conserving surgeries, and 112 (32%) received mastectomies. All mastectomy patients received breast reconstruction and 176 (74% of breast-conserving surgeries) received concomitant oncoplastic surgery. After multivariable analysis, mastectomy was associated with lower scores compared to breast-conserving surgery (-21.3; 95%CI: -36.2, -6.4, p=0.005), oncoplastic surgery was associated with higher scores (9.2; 95%CI: 0.8, 17.6, p=0.032). There was a tendency of higher scores with the use of flaps in breast reconstruction, and a tendency of lower scores with the use of radiation therapy, but not significant. Interestingly, bilateral cancers were associated with higher scores (25.8; 95%CI: 3.6, 47.9, p=0.023). Of note, there were 6 bilateral cancers, 5 treated with bilateral mastectomies.

Conclusions: Breast-conserving surgery is associated with better quality of life compared to mastectomy. Additionally, oncoplastic surgery is associated with better quality of life compared to standard breast-conserving surgery. Patients should be counseled whenever multiple options of surgery are possible, and efforts should be made to increase the availability of trained surgeons in oncoplastic techniques.

P4-07-07: Comparative analysis of breast-conserving surgery using a 3D-printed breast surgical guide versus standard breast-conserving surgery in breast cancer patients treated with neoadjuvant chemotherapy

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Introduction: Breast cancer remains a major health concern for women globally. During breast conserving surgery (BCS), tumor involvement in the resection margins is closely related to recurrence and prognosis. To achieve precise BCS, accurate imaging tools are essential, with MRI being the most accurate. However, for patients who have received neoadjuvant chemotherapy (NACT), determining the extent of breast cancer remains challenging. MRI has limitations in predicting the location of cancer during surgery. In this context, a 3D-Printed Breast Surgical Guide (3DP-BSG) can effectively identify the extent of breast cancer in NACT patients, enhancing surgical accuracy.
Material and Methods: This retrospective single-institution cohort study focused on female patients diagnosed with invasive breast cancer who underwent NACT. Between November 2015 and October 2021, patients received BCS with the aid of a 3DP-BSG. Personalized 3DP-BSG targeted tumors by tracking changes in breast and tumor anatomy on MRI before and after NACT. The 3DP-BCS (using 3DP-BSG) group was matched 1:2 with conventional BCS (cBCS) group.
Results: 591 patients were enrolled in this study. 197 people in the 3DP-BSG group and 394 people in the cBCS group were compared and analyzed. The median follow-up period was 43.4 months (range: 1.2-99.6 months). Pathologic complete remission with NACT was confirmed in 221 patients (37.4%). Positive resection margins were observed in 1.5% of the 3DP-BSG group and 2.8% of the cBCS group. Recurrence occurred in 8.6% of the 3DP-BSG group (0.5% of local recurrence) and 12.9% of the cBCS group (3.3% of local recurrence). The distribution of age, BMI, stage, and subtype proportions is identical between the two groups. Pathological stage and subtype are statistically significant factors that increase the recurrence rate. ($P < 0.05$) There were 3 deaths (1.5%) in the 3DP-BSG group and 11 deaths (2.8%) in the cBCS group.
Conclusion: The application of MRI-based 3DP-BSG is effective in achieving low positive margins and local recurrence in patients undergoing BCS after NACT, offering a promising approach for improving surgical outcomes.

P4-07-08: Targeted Axillary Dissection using magnetic seed placement in the axilla (mag-TAD) combined w/ dual tracer sentinel node procedure after neo-adjuvant systemic therapy (NST) in primary node pos breast cancer. Real-world evidence study in the UAE.

Ernest Luiten, Tallal Younis, Aydah Al-Awadhi, Hussam Mousa

Targeted Axillary Dissection using magnetic seed placement in the axilla (mag-TAD) combined with dual tracer sentinel node procedure after neo-adjuvant systemic therapy (NST) in primary node positive breast cancer.
A real-world evidence study of patients treated in a tertiary breast center in the United Arab Emirates (UAE).

Introduction: Patients with primary node positive breast (BC) are most often treated with NST followed by breast surgery and axillary lymph node dissection (ALND). Axillary pCR is achieved in up to 80% post NST1 . Less invasive axillary surgery as opposed to ALND is a promising surgical strategy post NST to detect remnant nodal disease while reducing morbidity and preserving oncologic safety. In a large Dutch clinical trial targeted axillary dissection (TAD) was associated with a 3.5% False Negative Rate (FNR) and a 92.8% Negative Predictive Value (NPV)2. A recent multicenter retrospective cohort study showed that in patients found to be ypNo - either sentinel lymph node biopsy (SLNB) or TAD - axillary recurrence following omission of completion-ALND was low (1.0%)3. Implementation of TAD is increasing in western parts of the world. The performance of TAD relative to ALND outside clinical trials, however, remains unknown, particularly in jurisdictions such as the United Arab Emirates (UAE) where BCs are characterized by younger age of onset, poorer biological features and more advanced disease stages4.

Methods: A retrospective cohort analysis was done, involving patients with primary node-positive BC treated with NST followed by mag-TAD plus ALND from August 2021 until March 2024 at the Breast Surgery Department of Tawam Hospital. A magnetic seed was inserted in the biopsy proven clipped primary positive node preceding surgery. Following completion of NST, surgery was carried out by a single breast surgeon, experienced in TAD. Dual tracer sentinel node procedure was carried out using either Technetium (Tc-99) or a magnetic tracer and combined with Patent Blue injection. (Neo-)Adjuvant treatment decisions were taken in accordance with NCCN guidelines. The performance of mag-TAD relative to mag-TAD plus ALND was examined.

Results: 60 patients with node positive BC underwent mag-TAD following NST. Completion ALND was omitted in 6 patients and mag-TAD yielded inconclusive results in 1 patient due to incorrect placement outside a lymph node (1.7%). Of the remaining 53, ALND was carried out during the same (n=52) or second (n=1) surgery. All patients received neo-adjuvant chemotherapy +/- targeted therapy, except for 1 patient (neo-adjuvant endocrine). Median age was 50 year (range 30-68). Clinical T-stage was cT1-2 in 56.6% (n=30) vs cT3-4 in 43.4% (n=23) while clinical N-stage was cN1 in 83.0% (n=44) vs cN2-3 in 17.0% (n=9). Breast conserving surgery was performed in 45 out of 53 patients (84.9%). Of these, 2 (4.4%) had incomplete resection necessitating additional resection in 1 patient while the other opted for completion mastectomy. Of the 53 patients, 52.8% (n=28) had HER2 positive expression and 11.3% (n=6) had triple negative disease. The median number of lymph nodes removed by mag-TAD and the consecutive ALND was 4 (range 1-16) and 10

(range 1-18) nodes, respectively. Axillary pCR was achieved in 20 out of 53 patients (37.7%). In 2 out of 22 patients with mag-TAD ypNo, a positive lymph node was found in the ALND. In 21 out of 31 patients (67.7%) with mag-TAD ypN+, additional positive nodes were found in the ALND; median 2 (range 1-11). The mag-TAD was associated with FNR 6% (95% CI: 2 - 23 %) and NPV 91% (95% CI: 72-98%).

Conclusion: In this RWE study – unique in the GCC - involving patients with high-risk node-positive BC, mag-TAD was feasible in our population with a high success rate and comparable FNR and NPV to those observed in the relevant clinical trial2. Recurrence and survival analyses will be planned. Further confirmatory studies in other jurisdictions in the region, with larger sample sizes, are warranted to confirm the favorable performance of mag-TAD observed in this single center study.

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P4-07-09: Does energy device improve operative procedure and postoperative management of total mastectomy with axillary lymph node dissection?

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Background: Axillary lymph node dissection (ALND) is still necessary in breast surgery, although it is now less frequently employed. Although it is often experienced that increased surgeon experience and better performance of the surgical equipment used reduces operative time and blood loss, there are still few reports on the extent to which these improvements are reflected in the recent introduction of recent ENERGY devices. Furthermore, the vessel sealing effect of energy devices is expected to reduce the volume of

discharge after ALND, which is expected to improve postoperative management by reducing hospitalization period until drain removal and the number of hospital visits after discharge. In the present study, we compared cases in which surgery was performed using an electrocautery scalpel, thermal scalpels and energy device to find a difference in the operative procedure and post-operative management.
Methods: The subjects were 300 patients who underwent a total mastectomy with ALND between October 2018 and December 2022 at Tokyo Metropolitan Cancer and Infectious Center, Komagome Hospital. Their electronic medical records were retrospectively reviewed to collect data on the patient's sex, age, BMI, history of sentinel lymph node biopsy, history of neoadjuvant chemotherapy, number of lymph nodes removed, number of metastatic lymph nodes, surgical devices, and postoperative complications. The clinical path at the study center includes drain removal on postoperative day 7 and discharge on the following day. In the present study, if a seroma was detected post-discharge, it was punctured in the outpatient clinic. The total volume of drainage during hospitalization was calculated, as were the total number of seroma puncture and the total volume of punctured seromas. Surgical devices were divided into a normal device (ND) group, consisting of conventional electrocautery machines and thermal scalpels, and an energy device (ED) group, consisting of LigaSURE EXACT and Harmonic FOCUS Plus. Surgical device selection was primarily due to surgeon preference, with no differences by timing or patient factors.
Results: Operative time, blood loss, and surgical complications were examined in 266 patients (226 in the ND group and 40 in the ED group) after excluding patients with bilateral surgery or a level III dissection. The median operative time was 140 minutes and 137, and the median blood loss was 70 mL and 65 mL, in the ND and ED group, respectively. Surgical complications comprised two cases of hemorrhage and four cases of wound infection in the ND group, but the difference was non-significant. The volume of drainage and punctured seromas were then examined in 212 and 39 patients in the ND and ED group, respectively, after excluding post-discharge follow-up at other clinics, drop-outs, and patients with surgical complications. No significant difference was found between the groups in terms of BMI, sentinel node biopsy or neoadjuvant chemotherapy. The median total volume of drainage during hospitalization was 655 mL and 730 mL in the ED and ND group, respectively. The difference was non-significant ($p=0.11$), but the median total volume of punctured seromas following hospital discharge was 108 mL and 235 mL in the ED and ND group, respectively, indicating a significantly lower volume in the former ($p= 0.0114$). Hence, the median sum of total volume of drainage and punctured seromas was 763 mL and 965 mL in the ED and ND group, respectively, indicating a significantly lower volume in the former ($p= 0.020$). Furthermore, the mean number of seroma puncture after discharge differed significantly, at 2.04 in the ED group versus 2.86 in the ND group ($p= 0.0091$).
Conclusion: Surgical devices had little effect on operative time, blood loss or complications. The volume of drainage during hospitalization was also unaffected, but the volume of punctured seromas post-discharge decreased significantly when energy devices were used. This decrease was likely also to have reduced the burden on the patient as well as the surgeon.

P4-07-10: Ensuring the precise interpretation of intraoperative specimen 3D imaging is crucial in avoiding the need for additional surgery for patients.

Hannah Jeffery, Mohamed Attia, Ravindran Karthigan, Rachael Manning, Ashutosh Kothari

Introduction: 1 in 7 women develop breast cancer over their lifetime risk. 70% of women undergoing surgical intervention will undergo breast-conserving surgery (BCS). 20-25% of women who undergo BCS require at least a second operation due to positive margins on histopathology. In keeping with the guidelines of the Association of Breast Surgeons in the UK, our institution defines a positive margin as less than 1 mm of healthy tissue for invasive cancer with associated DCIS and 2mm for pure DCIS.
Intraoperative specimen radiography has become standard practice in the UK. Surgeons review 2D or rarely 3D images of the specimen intraoperatively to assess if further excision is necessary. Advanced 3D imaging using a Kubtec machine with tomosynthesis allows the evaluation of the entire height of the specimen via 1mm slices, although the 3D function is not always utilised. Some centres have specimens assessed by radiologists who opine on probable specimen margin positivity, to guide surgeons.
This retrospective review of specimen tomosynthesis images by a Kubtec 3-D system looks at whether a meticulous review of all tomography slices by the surgeon, a radiologist or a combined assessment could prevent the need for subsequent operations.

Method: A senior surgeon and a senior radiologist reviewed intra-operative specimen images in 54 patients who had undergone BCS with intra-operative specimen tomosynthesis using the Kubtec system over a year and required re-operation for positive histopathological margins. The operating surgeon initially assessed the margins as clear. The surgeon and radiologist (blinded to the pathology report) used the slice function to independently measure the shortest distance from the tumour to the edge of the specimen; their assessments were correlated with histopathology.

Results: The review included 54 specimens from 54 patients, including invasive and pure ductal carcinoma in situ (DCIS) patients, primary surgery patients, and post-neoadjuvant-chemotherapy (NACT) patients.
81 margins out of 216 (37.5%) were positive on histopathology, 44 (54.3%) of these were assessed as having involved margins by radiologists and 25 (30.9%) by surgeons using the 3D tomosynthesis function. The radiologist individually reported margins with better sensitivity (54.3% vs 30.9%) but poorer specificity (95.6% vs 100%) than the surgeon; however, there was a fair agreement between their individual interpretation (Kappa agreement 78.3%, Kappa statistic 0.26, $p<0.0001$). Dual assessment showed overall a better accuracy of 85.2% (Radiologist 80.1% and Surgeons 74.1%).
Overall, 27 (50%) patients could have avoided returning to the theatre if the images had been reported intra-operatively by a breast radiologist alongside the surgeon. At our intuition, a day case procedure for re-excising margins costs circa £3,638 per patient, saving approximately £200,000 a year.

Conclusion: If radiologists and surgeons use 3D images intraoperatively to undertake a dual assessment, the re-excision rates can be reduced by 50%. This would significantly improve patient outcomes and reduce healthcare provision costs.

P4-07-11: Intraoperative sentinel lymph node evaluation in patients with breast cancer undergoing surgery after neoadjuvant systemic therapy

Saskia Leonard, Shirley Cheng, Brendan Seto, Ashley Marumoto

Background: Current literature recommends performing an axillary lymph node dissection (ALND) for patients undergoing neoadjuvant systemic therapy (NAST) for breast cancer who have residual disease in the sentinel lymph node (SLN). Pending the results of Alliance 110202, accurate intraoperative lymph node evaluation is imperative to determine which patients are candidates for axillary dissection. There is some evidence that frozen section (FS) has a higher sensitivity than touch-prep (TP) for intraoperative evaluation of SLN for clinically node positive patients who convert to node negative in the post-NAST setting; however, the data is sparse. This study aims to compare the intraoperative techniques of touch-prep (TP) and frozen section (FS) in clinically node negative patients after NAST at a single institution.
Methods: This was a case series retrospective medical chart review of patients diagnosed with invasive breast cancer treated with NAST followed by surgery who underwent intraoperative evaluation of SLNs from 2013-2022. Patient demographics, AJCC clinical stage, tumor histology, biomarker status, lymph node features and size, surgery type and intraoperative lymph node evaluation technique by either TP or FS were analyzed for sensitivity, specificity, and false negative rate (FNR). Chi-squared or fischer's Exact test were used to determine significance.
Results: A total of 167 node-negative patients and 68 node-positive patients were included in the analysis. Overall, the sensitivity of TP was 0.46 versus 0.80 for FS (p=0.036). The specificity was 1 for both intraoperative preparations. Specimens with lymphovascular invasion (LVI) were 6.5 times as likely to have a false negative TP or FS result compared to those without LVI (46% with LVI versus 7% without LVI). Patients who were clinically node positive prior to NAST were 20 times as likely to have a false negative TP vs FS result compared to those who were clinically node negative prior to NAST (41% vs 2.7%). Age, sex, race, AJCC clinical stage, histology, biomarker status, lymph node features and size, surgery type, intraoperative lymph node evaluation technique were not associated with FNR.
Analysis of patients who were clinically node-positive prior to NAST demonstrated 13/68 patients who underwent both TP and FS evaluation, 47/68 patients underwent TP only, and 8/68 patients underwent FS only. Of the 13 who underwent both TP and FS, 38% (5/13) evaluated by TP were discordant with final pathology while 15% (2/13) evaluated by FS were discordant.
Discussion: FS of the SLN may be preferrable for clinically node-positive patients who

convert to clinically node-negative after NAST. There were no predictors of FNR. Further studies are needed to evaluate accuracy of intraoperative evaluation of SLNs after NAST.

P4-07-12: Attitudes on mastectomy in sub-Saharan Africa; a systematic literature review exploring womanhood and femininity

Mikaela Belsky

Introduction: Breast cancer mortality remains high in sub-Saharan Africa (SSA), which stands in contrast to the gradual mortality decrease in high income countries. This disparity is partly attributed to fears of mastectomy and its effects, however, mastectomy is the primary treatment modality of breast cancer in SSA. Thus, it is crucial to better understand the culture of mastectomy in these regions, so that appropriate interventions can allow for both timely treatment and sufficient support of women as they transition to a postmastectomy life. This review aims to examine existing literature on attitudes surrounding mastectomy in SSA and to further explore its impact on womanhood.

Methods: During March of 2024, a literature search was conducted on PubMed and EMBASE data sources, using key search terms such as: “mastectomy,” “breast removal,” “qualitative,” “questionnaire,” “interview, “africa,” “sub-saharan,” and the names of each sub-Saharan African country. This method was supplemented by hand searching. Inclusion criteria are as follows; 2000-2024 publication year, explicit discussion of attitudes surrounding mastectomy, sub-Saharan country study setting, and use of qualitative design or mixed methodology.

Results: The search yielded 83 nonoverlapping articles and 15 articles met inclusion criteria. The majority (n = 12) administered in-depth interviews as opposed to open-ended questionnaires. Two studies supplemented data with focus groups, and one utilized ethnography. Of note, all explicitly identified study settings were urban (n=11), and only six countries were represented. Only five studies exclusively worked with mastectomy patients; other studies often failed to differentiate between mastectomy patients and breast cancer patients in general. The most highly discussed themes fell under an overarching theme of womanhood; 1) poor body image and discomfort, 2) loss of femininity and uncertainty with one’s identity as a woman, 3) mixed impacts on intimate relationships and marriage, and 4) fears related to breastfeeding and motherhood.

Discussion: In SSA, there are many attitudes surrounding mastectomy that relate to one’s identity as a woman. These themes emphasize the idea that a breastless woman in SSA is disfigured to the point of losing her womanhood, thus imparting concerns on her capacity for intimacy and motherhood. Femininity is assigned only to those with breasts; this belief is not just held by community members, but also holds true for breast cancer patients both pre- and post-mastectomy. As supported by prior quantitative literature, the findings of this qualitative review imply that womanhood-related consequences of mastectomy are

associated with fears, delays, and even refusal of breast cancer treatment, thus likely worsening morbidity and mortality. Limitations of this review include a qualitative framework, which precludes definitive outcomes, and a dearth of broad SSA representation. Future areas of research should explore long-term impacts of mastectomy, attitudes around breast conservation and reconstruction, and postmastectomy breastfeeding trends, and the knowledge base of male partners.

P4-07-13: FACTORS ASSOCIATED WITH THE PRACTICE OF ONCOPLASTIC SURGERY AMONG BREAST SURGEONS IN BRAZIL: A NATIONAL SURVEY

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Introduction: Breast oncoplastic surgery (BOS) is an important tool in breast cancer treatment. In Brazil, since 2008, various actions endorsed by the Brazilian Society of Mastology (SBM) have stimulated the dissemination of these techniques over the years, including: (1) BOS in National and regional Congress; (2) organization of a specific oncoplasty congress; (3) support for hand-on courses and fellowship; (4) inclusion of BOS in medical residency. However, its results and the proportion of surgeons whose performs BOS, including the level of complexity, is unknown.
Materials and Methods: A structured cross-sectional web-push survey, containing 30 questions focused on the attitudes and skills of Brazilian mastologists regarding oncoplastic surgery, was sent in a hybrid way (mail, email, telephone and during the Brazilian Mastology Congress) to SBM members between July and December/2023 (n=1,759). All associates were considered eligible since the SBM list was updated in 2023, and no prior screening was conducted to assess eligibility. The response rate was calculated by the number of complete responses (80% of questions) returned. Questionnaires with a response proportion of less than 80%, but above 50%, were considered partial and did not enter the response rate calculation but were considered with sufficient information for analysis. Responses less than 50% were considered "breakoff." The questionnaire was divided into general and demographic questions, followed by questions related to breast surgery and the last part for those performing oncoplasty. The degree of practice in oncoplasty in this group was evaluated. The characteristics of professionals performing oncoplasty were compared to those who did not. For this purpose, the chi-square test was used. In variables where differences were observed and $p<0.10$, logistic regression was performed.
Results: 1089 (Response rate: 61.9%) professionals returned the questionnaire, of which 530 (48.6%) performed oncoplasty. Factors associated with performing oncoplasty were: primary surgical training (56.5% General Surgery versus 43.6% Gynecology; $p<0.001$),

secondary surgical training (51.9% Oncologic Surgery versus 47.8% Mastology versus 12.5% without other secondary training; p=0.007), male gender (53.7% Male versus 40.4% Female; p<0.001), region of Brazil (55.9% South / 53.1% North / 51.0% Midwest / 49.8% Northeast / 41.5% Southeast; p=0.009), exclusive dedication to breast surgery (53.5% 100% practice; 51.6% breast and general oncologic surgery; 26.4% breast and gynecology; p<0.001), number of breast cancer cases performed by year (65.6% >100 cases/year; 56.0% 51 to 100 cases/year; 38.2% 11 to 50 cases/year; 26.4% <10 cases/year; p<0.001). On the other hand, city size (p=0.288), practice in a teaching hospital (p=0.305), age (0.128) and private or public hospital (p=0.08) were not associated with oncoplasty. Among those practicing oncoplasty, most professionals were skilled in multiple procedures: nipple-sparing mastectomies (99%), therapeutic mammoplasty/symmetrization (96%), implant reconstruction (93%), skin-reducing mastectomies (84%), extreme oncoplasty (81%), thoracoabdominal flaps (71.5%), latissimus dorsi flap (66%), lipofilling (61.5%), breast augmentation (54.6%), and rectus abdominis flap (28.2%). Most breast surgeons developed their techniques in hands-on courses (36.7%), medical residency (32.8%) and dedicated fellowships (21.3%).
Conclusion: A high proportion of Brazilian mastologists perform oncoplastic techniques, even those with higher complexity. These results show that the SBM actions were effective and could serve as a model to be used in other countries. Knowledge of the factors associated with non-training in oncoplasty is essential for intervention strategies aiming to enhance the use of oncoplasty.

P4-07-15: Minimally Invasive Mastery: A Comparative Analysis of Endoscopic Mastectomy and Conventional Nipple-Sparing Mastectomy with Immediate Reconstruction in Patients with Early-Stage Breast Cancer - A Paradigm Shift in Breast Cancer Surgery

Voratape Kijtavee, Korawan Chandrachamnong, Anakkawee Hsiung

Introduction: Endoscopic mastectomy (EM) has emerged as a minimally invasive alternative to conventional nipple-sparing mastectomy (cNSM) for the treatment of early-stage breast cancer. This study aimed to compare the surgical outcomes, aesthetic results, and quality of life (QoL) between EM and cNSM with immediate implant reconstruction, with or without acellular dermal matrix (ADM) augmentation, in a single-institution cohort.
Methods: A retrospective review of 50 patients with breast cup A-B who underwent either endoscopic mastectomy (EM, n=25) or conventional nipple-sparing mastectomy (cNSM, n=25) with immediate implant reconstruction at Police General Hospital between January 2022 and December 2023 was conducted. The study population included patients with early-stage breast cancer without pathological lymph node involvement or those undergoing prophylactic mastectomy. Primary endpoints were operative time, incision length, and pathology outcomes. Secondary endpoints included local recurrence rate (with 1-year follow-up for EM and 2-year follow-up for cNSM) and QoL (BREAST-Q) preoperatively and at 3, 6, and 12 months postoperatively. The BREAST-Q was used to

assess differences in aesthetic results, psychosocial well-being, and sexual health between the two groups. Multivariable binary logistic regression evaluated local recurrence risk.Results: The EM group demonstrated significantly longer operative times compared to the cNSM group (mean ± SD: 125 ± 60.5 minutes vs. 105 ± 35.4 minutes, p = 0.045). The EM group had significantly smaller incision lengths (mean ± SD: 4.5 ± 0.4 cm vs. 10.7 ± 3 cm, p < 0.001). In the EM group, removed breast tissue weight ranged from 120 to 380 grams, with a mean of 220 grams and a median of 250 grams. This was not significantly different from the cNSM group (p = 0.065). All surgical margins were free of tumor in both the EM and cNSM groups. No significant differences were observed in other pathology outcomes, including tumor size, lymph node involvement. Local recurrence rates were similar between the groups at their respective follow-up time points.Patients in the EM group reported significantly higher BREAST-Q scores for satisfaction with breasts (mean ± SD: 56.6 ± 6.4 vs. 39.5 ± 8.6, p < 0.001) and psychosocial well-being (mean ± SD: 79.5 ± 11.0 vs. 58.1 ± 10.9, p < 0.001) at 12 months postoperatively. There was no significant difference in sexual well-being scores between the two groups (p = 0.034).Conclusions: Endoscopic mastectomy offers a safe and effective alternative to conventional nipple_x0002_sparing mastectomy for the treatment of early-stage breast cancer in patients with breast cup A-B, without pathological lymph node involvement or those undergoing prophylactic mastectomy. EM is associated with significantly longer operative times, smaller incisions, and comparable surgical and oncologic outcomes to cNSM. Furthermore, EM may provide superior long-term aesthetic satisfaction and QoL compared to cNSM, particularly in patients with smaller breasts or those who prioritize minimizing scarring. The use of porcine or human ADM did not significantly impact the surgical or aesthetic outcomes in either group, consistent with findings from other studies.Keywords: endoscopic mastectomy, nipple-sparing mastectomy, breast reconstruction, immediate implant reconstruction, acellular dermal matrix, surgical outcomes, aesthetic outcomes, quality of life, early-stage breast cancer, prophylactic mastectomy.

P4-07-16: First line CDK4/6 inhibitors plus endocrine therapy versus chemotherapy for HR+/HER2- metastatic breast cancer patients in real world: a multicenter YOUNGBC-30 study.

Biyun Wang, Yifan Chen, Yizhao Xie, Yuan Peng, Ning Xie, Die Sang, Xinhua Han, Yanxia Zhao, Juanjuan Li

Background: The combination of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) and endocrine therapy (ET) is widely utilized as the first-line treatment for hormone receptor (HR) positive and human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer (MBC) patients. However, there has been long debates on the choice between CDK4/6i plus ET and chemotherapy (CT), especially those with visceral metastasis or visceral crisis. Several prospective trials suggested a suspected superiority of CDK4/6i over CT, however, conclusions vary among different studies due to the complexity of treatment lines and different inclusion criteria. Here we report CDK4/6i plus ET versus CT in first line

treatment of HR+/HER2- MBC in a real-world setting.
Methods: The medical records of patients diagnosed with HR+/HER2- MBC from 2020 to 2023 in 6 institutions were retrospectively evaluated. And patients received first-line CDK4/6i plus ET or first-line CT were included in this study.
Results: A total of 339 patients were available for analysis. 244 (72%) patients received CDK4/6i plus ET and 95 (28%) patients received CT. The median PFS of CDK4/6i plus ET cohort was significantly superior to CT group (17.2 months versus 9.1 months, hazard ratio = 0.49; 95% CI, 0.36 to 0.66; P < 0.0001). Subgroup analysis showed similar results, except subgroup of patients with visceral crisis (hazard ratio =0.73; 95% CI, 0.37 to 1.43; P = 0.36) or primary endocrine resistance (hazard ratio =0.59; 95% CI, 0.33 to 1.06; P = 0.08). The median overall survival was not reached. No significant difference of grade 3 or worse adverse events was observed in two groups (25.8% vs 26.3%, P = 0.93).
Conclusions: In a real-world setting, first line CDK4/6i plus ET showed better efficacy than chemotherapy in patients with HR+/HER2- MBC, while CT and ET showed similar results in patients with visceral crisis or primary endocrine resistance. Safety profile was similar between two groups. This study provides real-world data for future clinical decision.

P4-07-19: Quality of life with palbociclib plus tamoxifen in hormone receptor-positive, HER2-negative advanced breast cancer: results from NCCH1607/PATHWAY, an Asian international double-blind randomized phase 3 trial.

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Background: In the PATHWAY trial, palbociclib plus tamoxifen demonstrated improved progression-free survival compared with placebo plus tamoxifen in hormone receptor-positive, HER2-negative (HR+/HER2−) advanced breast cancer. Quality of life (QOL) data was also collected and evaluated as a secondary objective. This analysis compared QOL between the two treatment groups.
Methods: Pre-, peri-, or postmenopausal women with locally advanced or metastatic HR+/HER2− breast cancer who were candidates to receive tamoxifen as first-line or second-line endocrine treatment for advanced disease were randomly assigned 1:1 to receive palbociclib–tamoxifen or placebo–tamoxifen in Japan, Republic of Korea, Taiwan, and Singapore. Patients continued to receive the assigned treatment until progressive disease (PD), clinically diagnosed symptomatic deterioration, unacceptable toxicity, death, or patient's refusal, whichever occurred first. The study was conducted as a Clinical Research Collaboration with the National Cancer Center Hospital as the regulatory study sponsor and Pfizer providing drug and financial support (NCT03423199). Patient-reported

outcomes were assessed on Day 1 of Cycles (C) 1 (baseline), 4, and 7, and at the end of treatment (EOT) using EORTC QLQ-C30 and EORTC QLQ BR23. Least-square means (LSM) changes from baseline were calculated. Kaplan-Meier Plot was used to calculate time to deterioration (TTD). A composite definition of deterioration based on death, PD, clinically diagnosed symptomatic deterioration, and minimally important difference (MID) was used for TTD. MID was defined as the increase in QOL score of 10 points or greater from baseline. Results: The EORTC QLQ-C30 and the EORTC QLQ-BR23 were completed by 88 patients in the palbociclib plus tamoxifen arm and 93 patients in the placebo plus tamoxifen arm at baseline, and by 64 (72.7%) to 80 (90.9%) patients in the palbociclib plus tamoxifen arm and by 59 (63.4%) to 79 (84.9%) patients in the placebo plus tamoxifen arm, from C4 to EOT. In the EORTC QLQ-C30, the global QOL subscale, the LSM changes from baseline for C4, C7, and EOT were -0.22 (Standard error [SE]: 1.99) and -0.92 (SE: 2.09), 2.83 (SE: 2.21) and 0.66 (SE: 2.30), and -12.31 (SE: 2.40) and -7.12 (SE: 2.21) in the palbociclib plus tamoxifen arm and in the placebo plus tamoxifen arm, respectively. The difference in the LSM between treatments at C4, C7, and EOT were 0.70 (95% CI: -5.00, 6.41), 2.17 (95% CI: -4.14, 8.48) and -5.19 (95% CI: -11.64, 1.26). For either the functional subscales or symptom scales, no notable trends or differences between the treatment arms were found in the LSM changes from baseline. In the pain symptom scale, a total of 68 patients (77.3%) in the palbociclib plus tamoxifen arm and 81 patients (87.1%) in the placebo plus tamoxifen arm had documented PD, clinically diagnosed symptomatic deterioration, or MID at the data cutoff as of 15 Sept 2022. The median TTD was 10.3 months (95% CI: 5.8, 24.4) for the palbociclib plus tamoxifen arm and 5.5 months (95% CI: 3.6, 7.6) for the placebo plus tamoxifen arm with the hazard ratio of 0.601 (95% CI: 0.433, 0.834). In the analysis of the EORTC QLQ-BR23: no notable trends or differences between the treatment arms were found in the LSM changes from baseline for either the functional subscales or symptom scales.
Conclusion: The addition of palbociclib demonstrated a prolongation of TTD in the pain symptom scale of EORTC QLQ-C30 compared with placebo. No differences were found between treatment arms in other QOL analyses. Palbociclib plus tamoxifen allowed patients to maintain QOL while experiencing delayed disease progression.

P4-07-20: Is Distance to Treating Oncologist a Potential Prognostic Real-World-Factor for Patients with HR+/HER2- Advanced Breast Cancer? Results from the Non-Interventional Study PERFORM

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Background: Real-world evidence (RWE) is generated to explore the safety and effectiveness of approved agents in a broad real-world population in regionally specific

health care structures. The distance to treating oncologist (DTO) is a rarely analyzed real-world factor of high interest. This real-world exploratory analysis investigated the possible influence of the DTO on patient characteristics, adverse events (AEs), therapy modifications and effectiveness.
Methods: The prospective non-interventional study PERFORM is carried out in 320 urban and rural study sites across Germany and Austria. Up to 1,900 patients with HR+/HER2- advanced breast cancer (aBC) treated with palbociclib plus endocrine therapy (ET) in the first-line setting (1L) will be enrolled. Three years after 1st patient enrollment, the 3rd interim analysis (IA3) was conducted with data cutoff in September 2023. Demographic and disease characteristics as well as socioeconomic information, including DTO, are documented at baseline. AEs and therapy modifications are continuously documented. Disease progression is evaluated according to routine clinical practice. Here, we focus on subgroups with DTO <20 km and ≥20 km with additional age-stratification (<75 and ≥75 years). Patient-, disease- and socioeconomic characteristics, AEs, therapy modifications and real-world PFS (rwPFS) rates at 6, 12, 18 and 24 months are included in this analysis. The median rwPFS and rwPFS rates are estimated using the Kaplan-Meier method. Descriptive statistics are used to summarize results. Limitations are the descriptive, exploratory, hypotheses generating character of the results, that are not necessarily transferable to other regions.
Results: An exploratory analysis of IA3 (990 patients) evaluated 854 patients who reported DTO. For 612 patients (71.7 %), DTO is less than 20 km and 242 patients (28.3 %) have a DTO of ≥20 km. The latter tend to have a slightly lower ECOG-PS (ECOG 0: 46.6% vs. 50.4%) and are younger at inclusion (median: 68.6 vs. 66.5 years). Interestingly, patients with a shorter DTO are more likely to live alone, irrespective of age (41.0 % vs. 29.5 %). Patients with a greater DTO present more often with de novo aBC (<20 km: 36.4% vs. ≥20 km: 42.1%), which is mainly driven by patients ≥75 years (<20 km: 39.3% vs. ≥20 km: 58.3%). Patients with a DTO of <20km are more likely to have visceral metastases at inclusion (<20 km 46.7% vs ≥20 km: 43.8%). In the age-stratified analysis this was more pronounced in patients <75 years (<20 km: 48.2% vs. ≥20 km: 42.9%). AEs and therapy modifications are comparable, regardless of DTO. Median rwPFS in the subgroup with ≥20 km DTO was 22.8 months (95% CI 19.2, NA). For the subgroup with <20 km DTO, the median rwPFS could not (yet) be determined (NA (95% CI 23.5, NA)). This tendency is consistently confirmed in the 6-, 12-, 18-, 24-month rwPFS rates compared to the <20 km subgroup (<20 km: 85.9 %, 73.2 %, 64.2 %, 55.1 %; ≥20km: 82.7 %, 71.0 %, 59.8 %, 46.7 %).

Conclusions: This exploratory analysis shows a trend towards poorer rwPFS rates in patients with DTO of ≥20 km compared to <20km although they had a lower rate of visceral metastases and a higher rate of de novo aBC. These results indicate that the DTO might be a relevant real-world factor influencing outcome, that warrants further analyses including PROs with a longer follow-up.

P4-07-21: Prognostic analysis of operable HR-positive HER2-negative loco-regional recurrent breast cancer; A multi-institutional Retrospective Cohort Study

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Background: Locoregional recurrence (LRR) after surgery in early-stage breast cancer patients is not uncommon。 Several reports suggest that LRR in hormone receptor-positive HER2-negative breast cancer (HR+HER2- BC) has a better prognosis compared to other subtypes, however, studies on the prognosis of LRR in this subtype and factors affecting it are limited.
Methods: Multicenter retrospective cohort study was conducted [te2] within the JCOG study group to assess the prognosis of HR+HER2- LRR and the factors affecting it. The study included patients diagnosed with HR+HER2- LRR at one or more sites, such as the ipsilateral breast, skin, chest wall, or regional lymph nodes following primary breast cancer surgery. All participants were free of distant metastases at LRR diagnosis and underwent radical surgery from January 2014 to December 2018.
Results:Out of 967 enrolled patients across 41 centers, 946 were analyzed. There were 504 cases of ipsilateral breast tumor recurrence only (IBTR), while 442 had LRR at sites other than IBTR (oLRR). During a median follow-up period of 62.1 months, 239 invasive disease-free survival (iDFS) events and 80 deaths occurred. The 5-year iDFS rate for all LRR cases was 75.0%, and the 5-year overall survival (OS) rate was 92.3%. The 5-year iDFS was 83.4% for IBTR and significantly lower at 66.5% for oLRR. Multivariate analysis identified several independent prognostic factors for poorer iDFS: age over 60 years (HR: 1.43, 95% CI: 1.03-1.99) at LRR diagnosis, tumors with high Ki67 (>30) or high grade (HG3) (HR: 1.65, 95% CI: 1.19-2.27), and LRR diagnosis during adjuvant endocrine therapy (HR: 2.06, 95% CI: 1.30-3.66). For OS, poor prognostic factors included age over 60 (HR: 1.76, 95% CI: 1.04-2.98) or under 40 (HR: 2.31, 95% CI: 1.05-5.13) at LRR diagnosis, and tumors with high Ki67 or HG3 (HR: 2.25, 95% CI: 1.35-3.77).
Conclusion: In this study, we investigated the prognosis and influencing factors of LRR in HR+ HER2- breast cancer using a large cohort of 946 cases, which has not been previously reported. Our findings highlight the imperative of tailored treatment strategies based on recurrence risk for HR+HER2- breast cancer patients experiencing LRR. Further randomized controlled trials are warranted to validate these strategies and improve the outcome in high-risk populations.

P4-07-22: Real world effectiveness of overall survival (OS) with palbociclib (PAL) plus (+) endocrine therapy (ET) in hormone receptor-positive (HR+)/human epidermal growth factor receptor 2-negative (HER2-) advanced breast cancer (ABC) patients in Japan

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Introduction: PAL, cyclin-dependent kinases 4 and 6 inhibitor (CDK4/6i), + ET is a standard therapy for the treatment of HR+/HER2- ABC. Efficacy and safety of PAL + ET was shown by two randomized clinical trials, PALOMA-2 and PALOMA-3. Further, recent large real-world studies revealed effectiveness of PAL + aromatase inhibitor (AI) for HR+/HER2- ABC in routine United States clinical practice. To strengthen the real-world evidence (RWE) of PAL + ET for HR+/HER2- ABC treatment under different clinical practice and healthcare situations, we conducted observational study to evaluate the effectiveness of PAL + ET in Japanese real-world setting. Recently, we reported that the median (95% CI) OS were 68.2 months (60.8-not estimated (NE)), among patients who treated with PAL + ET in 1st line (1L) in Japanese routine clinical practice with longer follow-up after interim analysis. Here, we investigate the subgroup of real-world OS of Japanese patients treated with PAL + ET as 1L for HR+/HER2- ABC to understand the impact of OS.
Methods: In this multicenter, observational study, patients with HR+/HER2- ABC, who initiated PAL + ET from 15 December 2017 (launch date in Japan) to 31 December 2020 were enrolled. To reduce selection bias, all patients who started PAL during the above period were listed up and eligibility was confirmed. Key eligibility criteria were: 1) Diagnosis of HR+/HER2- ABC; 2) Patients received PAL + ET in 1L or 2L; 3) Patients had at least 6 months of follow up from start of PAL or died within that period. The primary endpoint was real-world progression free survival (rwPFS), and one of the secondary endpoints was OS, defined as the time from start of PAL + ET treatment to death due to any cause. We performed the further analysis, including subgroup analysis, about OS of 1L PAL + ET to investigate what factors could affect its clinical benefits.
Results: In this study, 693 patients from 20 study sites in Japan were enrolled. Among them, 426 and 267 received PAL + ET as 1L and 2L, respectively. The median age was 60.0 (range 29-87) and 482 patients (70.0%) were postmenopausal. 54.0% patients had visceral involvement and 43.3% had <12 months of treatment-free interval (TFI; defined as end date of adjuvant therapy to diagnosis date of recurrence). 295 patients received subsequent therapy out of 426 patients after PAL + ET as 1L. 60.7% (179/295) of patients received an endocrine-based regimen as first subsequent treatment; of those 23.1% (68/295) received CDK4/6i + ET, 19.3% (57/295) endocrine monotherapy, and 17.6% (52/295) ET + everolimus. After a median follow-up of 48.1 months, the following subgroup analysis was performed for OS in 1L, showing median OS (95% CI) and, survival rate at 60 months: pre-

/post-menopausal status was not reached (NR) (59.4-NE), 61.3%/68.0 months (58.5-NE), 55.6%, TFI<12 months/TFI12>months/De novo metastatic was 56.3 months (43.9-68.2), 46.1%/NR (NE-NE), 72.9%/NR (56.3-NE), 60.0%, visceral/non-visceral metastasis was 65.0 months (56.3-NE), 52.3%/NR (63.2-NE), 61.3%, liver metastasis was 46.4 months (37.2-NE), 42.8%, and bone only disease was NR (57.8-NE), 61.2%.
Conclusions: The findings of the study based on Japanese routine clinical practice further confirmed that the clinical benefits of PAL+ET is consistent regardless of patient characteristics in the real-world setting. In all subgroup other than TFI＜12 months or liver metastasis, median OS was >5 years with 1L PAL + ET, underlining the use of PAL + ET as 1L standard for ABC in the real-world setting.

P4-07-23: Preliminary safety in the inavolisib + fulvestrant + ribociclib/abemaciclib arms of MORPHEUS-pan breast cancer: A Phase 1b/2 study of efficacy & safety of multiple treatment combinations in patients with metastatic/locally advanced breast cancer

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Background: First-line (1L) standard of care for hormone receptor-positive, HER2-negative metastatic breast cancer (HR+, HER2– mBC) is endocrine therapy + a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i). However, crosstalk between the estrogen receptor, CDK4/6 and PI3K oncogenic pathways may lead to treatment resistance. PIK3CA mutations are not only associated with poor prognosis but also a predictive biomarker of response to PI3K inhibitors. The INAVO120 (NCT03006172) study showed that combining 1L inavolisib (INAVO; a highly potent and selective inhibitor of the p110 catalytic subunit alpha isoform encoded by PIK3CA) with fulvestrant (FUL) and palbociclib conferred a significant and clinically meaningful progression-free survival improvement, and numerically favorable trend in overall survival, in patients (pts) with PIK3CA-mutated, HR+, HER2– locally advanced (LA)/mBC. Abemaciclib (abema) and ribociclib (ribo) are other CDK4/6is used in clinical practice, and data showing combinability with INAVO are of great interest. We present preliminary safety data from the INAVO + FUL + abema/ribo arms of the MORPHEUS-pan BC (NCT03424005) umbrella study.
Methods: Eligible pts had PIK3CA-mutated, HR+, HER2– LA/mBC, prior therapy for metastatic disease, fasting glucose of <126 mg/dL or <7.0 mmol/L and hemoglobin A1c levels of ≤6.4%. Pts were randomized to receive INAVO (9 mg orally [PO] once daily on Days [D] 1–28 of 28-D cycles) + FUL (500 mg intramuscularly on D1 & 15 of Cycle 1, then on D1 of each cycle) + either abema (150 mg PO twice daily on D1–28 of 28-D cycles) or ribo (400 mg PO once daily on D1–21 of 28-D cycles).
Results: In the INAVO + FUL + abema arm (n = 4), one pt had received one prior line of therapy, one pt had three prior lines, and two pts had more than four prior lines. In the INAVO + FUL + ribo arm (n = 6), one pt had received one prior line, three pts had two prior lines, one pt had four prior lines, and one pt had more than four prior lines. The most

common metastatic sites (occurring in >1 pt in either arm) in the INAVO + FUL + abema arm were bone (n = 3) and liver (n = 2); in the INAVO + FUL + ribo arm, bone (n = 5), liver (n = 3), and lymph nodes (n = 2). At clinical cutoff (May 1, 2024) all pts were on study and receiving treatment. Median duration of safety follow-up was 2.9 months in the INAVO + FUL + abema arm and 2.5 months in the INAVO + FUL + ribo arm. All pts had at least one adverse event (AE). The most common AEs, occurring in >1 pt in either arm (INAVO + FUL + abema vs ribo), included hyperglycemia (4 vs 5 pts), diarrhea (3 vs 4), vomiting (2 vs 4), increased blood creatinine (2 each), nausea (2 each), cough (1 vs 2), fatigue (1 vs 2), musculoskeletal pain (1 vs 2), erythema (0 vs 2), headache (0 vs 2), and decreased platelet count (2 vs 0). Two pts in the INAVO + FUL + abema arm and one pt in the INAVO + FUL + ribo arm had grade 3 AEs. There were no grade 4 or 5 AEs, and no AEs leading to withdrawal of any treatment in either arm. All pts in the INAVO + FUL + abema arm and two pts in the INAVO + FUL + ribo arm had AEs leading to dose modification/interruption; the most common (>1 pt in either arm) were diarrhea (n = 2 in the INAVO + FUL + abema arm; n = 1 in the INAVO + FUL + ribo arm) and hyperglycemia (n = 2 in each arm). Two AEs of special interest were reported in the INAVO + FUL + abema arm (grade 2 pneumonitis, grade 3 stomatitis) and one in the INAVO + FUL + ribo arm (grade 3 hyperglycemia) per CTCAE 4.0.
Conclusions: No unexpected safety findings were observed at this early timepoint in either arm; pts remain on-study and follow-up is ongoing. Treatment-related AEs for each combination were driven by the known safety profiles of INAVO and each individual CDK4/6i. Updated data, including pharmacokinetics and data from additional participants, will be presented.

P4-07-24: Prospective Cohort Study of Abemaciclib in Combination with Endocrine Therapy for Chemotherapy-Treated HR+/HER2- Metastatic Breast Cancer

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Background: Combination therapy with cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors and endocrine therapy as the first-line and second-line treatments has already been standard therapy in patients with hormone receptor-positive (HR+) human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer (MBC) based on clinical trials. On the other hand, in clinical practice, CDK4/6 inhibitors are used not only as first-/second-line but also for patients receiving prior chemotherapy in metastatic setting. However, the efficacy and safety of combination therapy in these patients remain unclear.
Patients and Methods: We conducted a multi-institutional prospective cohort study to obtain real-world evidence about the clinical efficacy and safety of combination therapy with abemaciclib and endocrine therapy for chemotherapy-treated patients with

HR+/HER2- MBC. The eligibility criteria included a history of chemotherapy after diagnosis of MBC, no recent history of CDK4/6 inhibitor treatment as the previous line, and no history of abemaciclib treatment. The primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival and adverse events. The preplanned subpopulation analysis comprised prior treatment history with CDK4/6 inhibitors other than abemaciclib, the number of chemotherapy regimens for HR+ HER2- MBC (one or two vs. three or more), and menopausal status (pre vs. post). We also planned the subpopulation analysis on the PFS treated with abemaciclib as maintenance therapy: defined as response of the former chemotherapy was determined as CR, PR or SD, and switched to abemaciclib and endocrine therapy for a reason other than PD.
Results: 181 patients were registered from December 2019 to November 2022. a minority of patients discontinued abemaciclib because of toxicity (13.9%) and of patient request (6.9%) without progression. Median PFS for abemaciclib in this population was 10.3 (95%CI: 8.4-12.0) months and median overall survival was 25.2 (95%CI: 12.5-18.0) months. Median number of endocrine therapies for MBC was 1 regimen. Median PFS of the patients treated with abemaciclib as maintenance therapy after chemotherapy (n=71) was 13.8 months (95%CI: 11.8-20.2). Median PFS of patients with prior treatment history with CDK4/6 inhibitor (palbociclib) (n=59) was 6.6 months (95%CI: 4.8-8.5). Patients with one or two chemotherapy regimens in the previous line (n=143) had a significantly longer PFS than those with three or more regimen (n=30) (median PFS: 10.6 months versus 6.5 months, p = 0.039). Premenopausal patients (n=40) had a longer PFS, but not significantly, than postmenopausal patients (n=133) (median PFS: 14.9 months versus 9.4 months, p = 0.073). As for safety, severe adverse events including diarrhea (6.9%), fatigue (4.6%), appetite loss (2.9%), nausea (1.7%), vomiting (1.2%), interstitial lung disease (1.2%), arthralgia (0.6%), infection (0.6%), up to grade 3, were observed during treatment with abemaciclib and endocrine therapy; these were consistent with known abemaciclib profile.
Conclusion: Abemaciclib, in combination with endocrine therapy, demonstrated considerable efficacy and manageable safety profiles in chemotherapy-treated HR+/HER2- MBC patients. Notably, the extended PFS of over one year, when abemaciclib is used as maintenance therapy after chemotherapy due to reasons other than disease progression, underscores its potential utility. Maintenance administration of abemaciclib could represent a significant advancement in MBC treatment, offering a promising option for patients transitioning from chemotherapy.

P4-07-25: Verification of a Highly Sensitive, Circulating Cell-Free DNA and Exosomal RNA RT-qPCR Assay to Monitor ESR1 Mutations from a Liquid Biopsy

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Introduction: At a rate of 90 new cases per 100,000 women per year, hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-) is the most

prevalent subtype of breast cancer. Mutations in the ligand binding domain of the estrogen receptor gene (ESR1) are frequently the cause of resistance to aromatase inhibitor therapy. With repeat biopsy collection rarely performed after starting therapeutic treatment, early identification of ESR1 mutations in metastatic breast cancer (mBC) in plasma is critical after progression on aromatase inhibitor therapy and is in alignment with recent NCCN guideline updates. The decision to switch to elacestrant, a second line therapeutic, hinges upon the presence of ESR1 resistance mutations, highlighting the importance of early detection in an easily accessible sample type. We report the performance of an assay that detects 11 ESR1 acquired resistance mutations associated with HR+/HER2- mBC from plasma liquid biopsies that utilizes RT-qPCR technology combined with a novel isolation method that captures exosomal RNA in addition to cfDNA in order to improve mutation detection sensitivity.
Methods: Samples were processed using a novel isolation method included with the kit, which is optimized to enrich for exosomal RNA in addition to cell-free DNA from as little as 2 mL starting plasma volume. The assay workflow includes a single reverse transcription step followed by a preamplification enrichment step and a three-tube multiplex qPCR. The accompanying software applies automated quality controls and summarizes allele calls for both the controls included in the kit and test samples. We characterized the assay for key performance metrics such as precision, sensitivity, and specificity using multiple sample panels comprising a combination of plasma from a cohort of mBC subjects and surrogate samples. All mutant-positive samples were verified against internal reference methods.
Results: Based on an equivalent of 5 mutant copies per 1 mL plasma (utilizing 2 mL plasma total), analytical sensitivity exhibited a percent positive agreement ≥90%. Analytical specificity results were ≥90% negative percent agreement in mutation-negative samples. Single-site precision revealed no significant variability contribution across experiment days, operators, or instruments. The entire workflow from sample to answer was completed within a single workday, including sample isolation and software analysis.
Conclusions: The multiplex RT-qPCR assay exhibited robust performance for 11 ESR1 variants following commonly utilized workflow techniques that enable sensitive and accurate single day sample-to-results. Achieving high specificity and sensitivity levels on qPCR instruments that are broadly accessible will facilitate quick, reliable, early detection from a minimally invasive liquid biopsy in breast cancer patients. This approach demonstrates the potential to improve outcomes by monitoring for the presence of ESR1 mutations to aid in selecting the most appropriate therapy to quickly respond to endocrine therapy resistance.

P4-07-26: CDK 4/6 inhibitor switching and associated dosing patterns in Swedish HR+/HER2- MBC patients

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Background: The combination of CDK4/6 inhibitors (CDK4/6i) and endocrine therapy is a first-line treatment standard in patients with hormone receptor-positive (HR+), human

epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer (MBC). Switching between CDK4/6i may be used to manage side-effects prior to progression, and the postMONARCH study recently showed evidence on improving efficacy when sequencing CDK4/6i post progression. Limited evidence on real-world switching and associated dosing patterns within this class of drugs exist. This study investigated the frequency and timing of CDK4/6i switching and dosing before and after switch in a real-world setting using a nationwide Swedish cohort of MBC patients.
Methods: This was a retrospective analysis utilizing the population-based Swedish Prescription Registry. The overall cohort included all patients with ≥ 1 dispensation of >1 of the available CDK4/6i (palbociclib, ribociclib and abemaciclib) from January 2017 – May 2024. Dosing before and after switch, overall frequency of CDK4/6i switching as well as time from the start of the first CDK4/6i to the subsequent were investigated. Only the first switch was considered. Information on treatment line of therapy and reason for switching was not available. Patient baseline characteristics and demographics were not available.
Results: Out of 5781 patients with ≥ 1 dispensation of a CDK4/6i, 606 patients (11%) receiving >1 CDK 4/6i were analyzed. The most common switch was from ribociclib; 18% of 1756 patients who started with ribociclib changed to another CDK4/6i: 75% to palbociclib and 25% to abemaciclib. The second most common switch was from palbociclib; 8% of 2905 patients who started with palbociclib changed to another CDK4/6i: 55% to abemaciclib and 45% to ribociclib. There were 58 patients who switched from abemaciclib to another CDK4/6i, representing 5% of 1120 patients. Notably, this may also include patients in the adjuvant setting; 64% changed to palbociclib and 36% to ribociclib.

Within 3 months of initiating CDK4/6i treatment, 201 patients out of the total 606 patients (33%) switched, with most changing from ribociclib; ribociclib-palbociclib (57%) and ribociclib-abemaciclib (15% patients). The remaining switches occurred 4-6 (27%), 7-9 (11%), or 10-12 (7%) months after treatment initiation. After more than a year on treatment, 136 patients (22%) switched CDK4/6i, with the majority changing from palbociclib; palbociclib-abemaciclib (37%) and palbociclib-ribociclib (35%).

Prior to the switch, 343 patients (57%) were on full dose of the initial CDK4/6i treatment, and this share was highest for patients who switched within 0-3 months (71%), and lowest for patients who changed CDK4/6i after more than a year (40%). Half of the patients began the subsequent CDK4/6i treatment on the label dose, and this proportion was highest for patients with a change after more than a year (57%) and varied between 51% for patients switching between 0-3 months to 35% for patients switching between 9-12 months. Overall, 80% of patients started their initial CDK4/6i treatment on the full dose.

Conclusion: Among Swedish patients who started treatment with a CDK4/6i, one in ten have switched to another CDK4/6i. The majority of patients switched to a subsequent CDK4/6i within 6 months of initiating their first CDK 4/6i. Most of these early switchers remained on full dose before the switch. The large proportion of early switchers may be related to tolerability issues. However, further investigation is needed to assess the clinical

reasons and outcomes for such treatment changes. Despite the limitations of this real-world data analysis, these findings provide a new perspective around the trends related to switching and associated dosing in patients treated with CDK 4/6i.

P4-07-27: Real-world observational study of patients with endocrine-resistant, hormone receptor+, HER2- metastatic breast cancer in the first-line setting: Patient characteristics, PIK3CA mutation prevalence, treatments, and clinical outcomes

Peter Lambert, Eirini Thanopoulou, Preet Dhillon

Background: Patients (pts) with endocrine therapy (ET)-resistant, hormone receptor+ (HR+), HER2– metastatic breast cancer (mBC) have a poor prognosis and may derive less benefit when treated with ET + a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) in the first-line (1L) setting. PIK3CA mutations occur in 35–40% of pts with HR+, HER2– mBC and are associated with poor prognosis. In the INAVO120 (NCT03006172) study, the addition of inavolisib, a PI3Kα inhibitor and mutant degrader, to fulvestrant plus palbociclib (a CDK4/6i) conferred a substantially longer progression-free survival (PFS) improvement in pts with PIK3CA-mutated (PIK3CAmut), HR+, HER2– mBC in the 1L, ET-resistant setting. This analysis aimed to evaluate baseline characteristics, PIK3CA mutation prevalence, and treatment outcomes in pts with HR+, HER2– mBC treated in the 1L, real-world (RW) setting, including those pts who met further INAVO120 criteria.
Methods: This is a retrospective, observational study of de-identified, electronic health record-derived data from the United States Flatiron Health Network (thus is not human subjects research, which would have required institutional review board assessment/approval).
Pts selected for inclusion in the study had recurrent HR+, HER2– mBC diagnosed between 2015 and 2023, and started 1L treatment within 90 days of mBC diagnosis. A cohort of pts with recurrent disease, prior ET, and any 1L treatment for mBC was defined ('broad cohort'), as well as 'INAVO120-like' populations (subdivided based on receipt of fulvestrant + palbociclib, fulvestrant + CDK4/6i, or ET + CDK4/6i) that included further criteria such as ET resistance, Eastern Cooperative Oncology Group Performance Status 0–2, no bone-only disease, and no elevated HbA1c or glucose levels at baseline.
Results: The broad cohort included 7096 pts, of whom, 922 met the further INAVO120 criteria (overall INAVO120-like population). All pts in the overall INAVO120-like population had received 1L CDK4/6i + ET; 514/922 pts received CDK4/6i + fulvestrant, of whom 339/514 received palbociclib as the CDK4/6i partner. 39–41% of the INAVO120-like populations (212/922, 120/514, and 82/339) tested positive for PIK3CA mutations. Fast progressors (relapse within ≤6 months [mo] of 1L treatment start) were more frequent in the INAVO120-like populations (35–38%) vs the broad cohort (25%).
Race/ethnicity distribution, body mass index at mBC diagnosis, duration of adjuvant treatment and use of different CDK4/6is were similar between the 'INAVO120-like' populations and the broad cohort.

Compared with the broad cohort, INAVO120-like pts were more likely to have visceral metastases (75–78% vs 48%), stage 3 disease at initial diagnosis (32–37% vs 29%) and a shorter time to mBC diagnosis (3.9–4.8 years vs 5.7 years).
The INAVO120-like populations also had shorter RW median PFS (8.2–8.5 vs 13.0 mo), shorter RW median time to chemotherapy (16.2–21.7 vs 33.0 mo), and shorter RW median overall survival (27.0–29.7 vs 36.8 mo) compared with the broad cohort.
PIK3CA mutation testing at any point was more frequent in pts in the INAVO120-like populations compared with the broad cohort (59–61% vs 45%); however, the proportion of pts tested for mutations prior to 1L treatment was numerically lower, but generally similar across cohorts: 9.5–11.8% (INAVO120-like) vs 12.4% (broad cohort). The PIK3CA mutation rates were similar across cohorts (39–41% vs 41%).
Conclusions: These RW data from a large US, community-based oncology network suggest that pts meeting the INAVO120 criteria have poor outcomes with 1L ET+ CDK4/6i treatment, regardless of the type of ET and CDK4/6i partner. Pts with PIK3CAmut, HR+, HER2– mBC may derive more benefit from therapies targeting the PI3K pathway (since PIK3CA mutations are a predictive biomarker of response), as demonstrated in the INAVO120 study.

P4-07-29: Analytical Validity of the Digistain Test in the Face of Variable Fixation Times: A Study from Charing Cross Hospital

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Background: In the setting of adjuvant therapy for breast cancer, the accuracy of quantitative real-time PCR-based risk profiling tests can be compromised by variable formalin fixation times, affecting the fidelity of genetic material. The Digistain test, using mid-infrared spectroscopy, potentially offers immunity to such pre-analytical variables. This study assesses the robustness of Digistain in maintaining consistent performance across a wide range of fixation times in breast cancer surgery samples.
Methods: We analyzed 233 breast cancer tissue samples collected post-surgery at Charing Cross Hospital, with fixation times documented between 5 to 144 hours. The primary objective was to investigate the consistency of the Digistain Index (DI) across varied fixation durations.
Results: The samples were stratified into three groups based on fixation time: 0-24 hours, 24-48 hours, and 48+ hours. An Analysis of Variance (ANOVA) was performed to compare Digistain Index (DI) values across these groups. The analysis revealed that DI values remained consistent across all groups, with no significant variation in mean DI values attributable to differences in fixation time ($p = 0.84$). This robust consistency of Digistain's results, irrespective of fixation duration, suggests potential advantages in analytical reliability, particularly in contexts where the fidelity of PCR-based tests may be influenced by pre-analytical variables.
Conclusions: These results validate the analytical robustness of the Digistain test,

confirming its suitability for use in clinical settings where fixation times can vary. This attribute positions Digistain as a dependable tool for breast cancer risk stratification in the adjuvant therapy context, enhancing its applicability in routine clinical practice.
Keywords: Breast cancer, Digistain, fixation time, analytical validity, adjuvant therapy, mid-infrared spectroscopy.

P4-07-30: The prognostic and predictive value of Oncotype DX® in ER+/HER2-/pT2N0, cM0 Breast Cancer Patients

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Background: The Oncotype DX® assay is a 21-gene assay used to predict the likelihood of breast cancer recurrence and benefit from chemotherapy in early-stage, estrogen receptor-positive (ER+), HER2-negative (HER2-) breast cancer patients. Based on AJCC Anatomic Stage Groups, breast cancer patients with ER+/HER2-/pT2N0, cM0 tumors are categorized as stage IIA. If the Oncotype Dx® test is performed in such cases and the Recurrence Score (RS) is less than 11, the case should be assigned Pathological Prognostic Stage Group IA. This study aims to evaluate the utility of Oncotype DX® in providing a better prognosis and predicting the benefit of chemotherapy in pT2N0, cM0 breast cancer patients.
Methods: We conducted a retrospective analysis of data from a Greek cohort of ER+/HER2-/pT2N0, cM0 breast cancer patients who underwent Oncotype DX® testing between 2008 and 2020. Patients were categorized based on their Oncotype DX® RS according to AJCC Prognostic stage guidelines into RS<11 and RS ≥11. Additionally, subgroup analysis for patients ≤50 years old was performed in reference to their Oncotype DX® RS and clinical risk status.
Results: A total of 524 ER+/HER2-/pT2N0, cM0 patients were included in the analysis. The mean RS was 17.3. RS 0-10 was reported in 105 (20%) of these cases. In the above cohort, 200 patients were ≤50 years old. RS 0-10 was reported in 39 (19,5%) and RS<16 in 99 (49,5%) of these cases. Further stratification of patients ≤50 years old according to their clinical risk status, identified 163 (81,5%) of them as high clinical risk (T2/G2, G3). RS 0-10 was reported in 25 (15%) and RS<16 in 74 (45,4%) of these cases.
Conclusion: Among breast cancer patients with pT2N0, cM0 ER+/HER2- tumors, 20% had a RS<11. All these patients based on the AJCC 8th Edition Cancer Staging Manual are assigned Pathological Prognostic Stage Group IA. These patients based on TAILORx data have 0,7% distant recurrence risk at 5 years, 3% distant recurrence risk at 9 years and no

chemotherapy benefit. Among younger patients (≤50 years) with pT2N0, cM0 ER+/HER2- tumors, the same percentage (19,5%) had a RS<11 (Pathological Prognostic Stage Group IA). Also, 49,5% out of them had a RS 0-15 and should be treated with only endocrine therapy based on NCCN Guidelines.Additionally, taking into consideration the clinical risk status of the younger patients (≤50 years), 45,4% of those with high clinical risk had a RS<16 and could safely forego chemotherapy.These findings highlight the critical role of Oncotype DX in enhancing risk stratification, predicting recurrence, and guiding treatment decisions for patients with ER+/HER2-/pT2N0, cM0 breast cancer.

P4-08-01: pionERA Breast Cancer: Phase 3 study of first-line giredestrant vs fulvestrant, + a CDK4/6 inhibitor, in patients with estrogen receptor+, HER2- locally advanced/metastatic breast cancer with resistance to prior adjuvant endocrine therapy

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Background: Patients (pts) with estrogen receptor-positive, HER2-negative, locally advanced/metastatic breast cancer (ER+, HER2– LA/mBC) who relapse on/after adjuvant endocrine therapy (adj ET) and have endocrine resistance have a high unmet need. For pts who relapse on/or <12 months after adj ET, fulvestrant plus a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) is first-line (1L) standard of care. However, fulvestrant has limited efficacy in pts with ESR1-mutated tumors and requires monthly intramuscular injections that negatively impact quality of life (QoL). New treatment options, such as combinations of an oral selective ER degrader (SERD) plus a CDK4/6i (beyond palbociclib) are needed to improve survival, QoL, tolerability, and adherence.
Giredestrant is a highly potent, nonsteroidal, oral SERD that achieves robust ER occupancy and is active regardless of ESR1 mutation status. Giredestrant was well tolerated with promising activity as monotherapy and in combination with any approved CDK4/6i in Phase I/II studies in ER+, HER2– BC. pionERA BC is the first study to investigate an oral SERD (giredestrant) plus investigator's choice of CDK4/6i in pts with 1L ET-resistant ER+, HER2– LA/mBC.
Methods: pionERA BC (NCT06065784) is a Phase III, global, randomized, open-label, multicenter study in pts with ER+, HER2– LA/mBC with resistance to prior adj ET. Pts will be randomized 1:1 to receive giredestrant (30 mg orally, once daily) or fulvestrant (500 mg intramuscularly on Days 1 and 15 of Cycle 1 [28-day cycles], then on Day 1 of subsequent cycles), in combination with investigator's choice of CDK4/6i (palbociclib, abemaciclib, or ribociclib administered per local prescribing information), stratified by: prior adj CDK4/6i (yes vs. no); choice of CDK4/6i; ESR1 mutation status by central testing (ESR1 mutated vs. ESR1 no mutation detected [ESR1nmd]); disease site (visceral vs. non-visceral). Pts will receive treatment until disease progression, unacceptable toxicity, death, or withdrawal of consent.

Eligibility: female/male pts with ER+, HER2– LA/mBC; relapse on prior standard adj ET on treatment after ≥12 months or off treatment within 12 months of completion (prior neoadj/adj CDK4/6i allowed); no prior systemic treatment for LA/mBC; documented ESR1 mutation status by circulating tumor DNA. Enrollment of pts with ESR1nmd tumors will be capped at 60% of the total study population.
Dual primary endpoints (EPs): investigator assessed progression-free survival (PFS) in the ESR1 mutated population and in the full analysis set (per RECIST v1.1). Secondary EPs: PFS (ESR1nmd population); overall survival (OS); investigator assessed confirmed overall response rate; duration of response; clinical benefit rate (per RECIST v1.1); time to chemotherapy; safety; pt-reported outcomes (time to confirmed deterioration in pain, physical and role functioning, global health status/QoL).
The dual primary EPs will be compared between treatment arms using a stratified log-rank test at an overall 0.05 significance level (two-sided). PFS will be assessed using a hierarchical fixed-sequence testing approach. An interim OS analysis is planned at the time of the primary PFS analysis; an independent data monitoring committee review the safety data.
Target enrollment: 1050 pts; recruitment ongoing.

P4-08-02: Identifying unique transcriptomes associated with HER2+ breast cancer recurrence at the single-cell level.

Paola A. Marignani, Jinhong Kim

Precision medicine aims to provide more effective treatments, detect disease earlier and improve patient outcomes. The development of more targeted therapies relies on advanced technologies like transcriptomics and machine learning to identify new and unique markers of a person's cancer. Breast cancer remains one of the leading causes of cancer-deaths amongst women world-wide, with 670,000 deaths world-wide in 2022, thus breast cancer is ranked as the second most common cancer globally, and the number one cancer amongst women. HER2 positive (HER2+) breast cancer accounts for 20-30% of all breast cancer cases and is highly aggressive. The mortality rates for HER2+ are higher than other breast cancer subtypes. When treated with Herceptin, survival rates are good, however approximately 20-30% of cases experience recurrence and metastasis. Our question is whether HER2+ breast cancers express transcriptomic signatures that are distinguishable between non-recurrence and recurrence.
Objective: The objective of our research is to interrogate breast cancers at the single cell level to identify new predictive biomarkers associated with HER2+ breast cancer recurrence using a single-cell microfluidics platform coupled with DNA barcoding genome-wide single-cell RNA-sequencing (scRNA-seq) and machine learning. Specifically, scRNA-seq allows one to study the heterogeneity of breast cancer cells to better understand molecular

mechanisms that support tumorigenesis and recurrence. Herein, we detail the analysis of scRNAseq analysis of resected primary HER2+ breast cancers from patients that received Herceptin adjuvant therapy.
Methods: Single-cell transcriptomes (10X Genomics) were characterized from 8 patients identified with HER2+ breast tumors that were treated with Herceptin adjuvant therapy. From these patients, four did not experience recurrence, while four did experience recurrence within 5 years of Herceptin treatment. Tumors were prepared for scRNAseq analysis, followed by computational analysis of data.
Results: From the 80,000 single nuclei analyzed, we obtained 5.9 billion reads and detected a total of 27,303 genes following quality processing and read mapping. We performed dimension reduction and clustering analysis at pseudo-bulk level merging normal (8 samples), recurred tumor (4 samples) and non-recurred (4 samples) data. We annotated individual cells in each of the three merged data with 14 different cell types. We found 5,147 DEGs (Avg. 514) are common between the recurred and non-recurred samples, while 62.8% (8,691; avg. 869) DEGs and 66.8% (10,344; avg. 1,034) DEGs were specific to recurred and non-recurred data, respectively. Next, we applied GSEA for transcriptomic characterization using multiple reference databases such as transcription factor (TF) collection, biological pathways, hallmark gene sets and gene ontology (GO) terms. We identified 683 (203 unique) and 1,025 (379 unique) TFs were enriched in recurred and non-recurred tumors, respectively. The number of enriched biological pathways revealed that 50 of 62 pathways were activated in non-recurred tumor cells compared with recurred tumor cells where 38 of 62 pathways were activated.
Summary: The outcome of our study reveals significant molecular distinction between recurrent and non-recurrent HER2+ breast cancers, suggesting potential biomarkers for predicting recurrence. Specifically, the identified DEGs and enriched pathways could serve as targets for developing new therapies aimed at preventing recurrence. Future studies will include the integration of scRNAseq data with other omics technologies for a comprehensive understanding of recurrence mechanisms.
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P4-08-04: Selective activity of rogaratinib in PIK3CA- and ESR1-wild-type, FGFR1/2-amplified hormone receptor-positive breast cancer (HR+/HER2- BC): a co-clinical trial

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Introduction: FGFR1/2 amplification is associated with poor outcomes and treatment resistance in HR+/HER2- BC. However, FGFR inhibitors have shown limited activity in this disease. Recently, we demonstrated that complete cell cycle arrest was only achieved if FGFR inhibitors were combined with CDK4/6i and endocrine treatment (ET) in FGFR-

amplified breast cancer models (PMID33579347). Co-clinical trials (different study designs involving concurrent patient treatment and model testing) aim to expedite and enhance the translation of laboratory discoveries to clinical practice. Thus, we conducted a co-clinical trial with patient-derived tumor organoids (PDTOs) resistant to CDK4/6i and ET and a phase-I clinical trial of fulvestrant, palbociclib and the pan-FGFR inhibitor rogaratinib in women progressing to first-line CDK4/6i combined aromatase inhibitor (AI).
Methods: PDTOs: 4 PDTOs, 3 FGFR1-amplified and 1 FGFR1-non-amplified. Among the amplified, one (H12-5) was PIK3CA mutant (H1047R); one (H12-31) was ESR1 mutant (Y537S); and one (H12-7) was wild-type for both genes; the FGFR1-non-amplified (H12-28) was double wild-type as well. PDTOs viability was explored in response to rogaratinib, fulvestrant, palbociclib, camizestrant, and alpelisib in monotherapy or in combinations. Trial: dose-escalation, multi-centric clinical trial. Women >18 years old with metastatic HR+/HER2- BC progressing to a combination of CDK4/6i plus AI in the first-line, candidates for second-line therapy. Patients were pre-screened for FGFR1/2 amplification (FISH) or overexpression (RNAScope) in a recent tumor sample. Treatment consisted on 28-day cycles of fulvestrant 500 ug in day 1 (and day 15 on cycle 1), palbociclib 100 mg in days 1-21, and rogaratinib bid in days 1-28. Rogaratinib started at 400 mg/bid and was escalated in 200 mg intervals; palbociclib could be escalated to 125 on cycle 2.
Results: PDTOs: the 4 PDTOs were refractory to fulvestrant, palbociclib, and the combination. Rogaratinib monotherapy only displayed modest activity in H12-31. The triple-combination of fulvestrant, palbociclib (1uM each) and increasing rogaratinib was highly effective in H12-7, but lacked activity in H12-5, H12-31 and H12-28 (an FGFR-amplified variant of the latter turned sensitive to the combination). PDTOs H12-5 and H12-31 were re-sensitized by replacing palbociclib for alpelisib and fulvestrant for camizestrant, respectively. Trial: 67 patients were screened; 29 (43%) were positive for FGFR1/2 amplification and/or overexpression. The RP2D was established at 500 ug fulvestrant, 100 mg palbociclib (days 1-21) and rogaratinib 600 mg/bid. Nine patients were treated. The most frequent grade 1/2 toxicity were hyperphosphatemia (77% of patients) and diarrhea (33%). Grade 3 neutropenia occurred in 66.7% of the patients; grade 3 asthenia, diarrhea and hyperphosphatemia were observed in 11.1% of the patients each. Median PFS was 3.5 months.
Five patients displayed PIK3CA and/or ESR1 mutations in the tumor or plasma sample, and 4 were wild-type for both. All double wild-type patients remained PFS for longer than 6-months (185-330 days), and the PFS time comparison between double wild-type against PIK3CA/ESR1-mutant patients favored the former by an almost 5-fold advantage: 274 vs 58 days; P=0.005.
Conclusion: A high percentage (43%) of second-line HR+/HER2- BC displays FGFR1/2 overexpression or amplification, of which approximately half are wild-type for ESR1 and PIK3CA. FGFR1/2 inhibition with rogaratinib in combination with palbociclib and fulvestrant is active in second-line, FGFR1/2-amplified/overexpressed HR+/HER2- BC, but the activity is restricted to wild-type PIK3CA/ESR1 patients. These results frame the population in which the development of this combination should be focused.

P4-08-05: A Phase Ib/II trial of Lenvatinib plus Pembrolizumab plus Fulvestrant in ER+/HER2- Metastatic Breast Cancer (MBC)

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Background: The prognosis for endocrine-resistant ER+/HER2- MBC remains poor and novel therapeutic strategies are urgently needed. Immunotherapy is FDA-approved in triple negative breast cancer but has not shown significant efficacy in ER+ BC. This is likely due to differences in immunogenicity, as ER+ tumors are characterized by low levels of tumor-infiltrating lymphocytes, lower mutational load, and lower PDL1 expression; furthermore, driver alterations associated with ER+ BC can contribute to suppressing immunity. Single-agent immunotherapy has yielded low overall response rates (ORR) in this subtype; priming strategies that improve immunogenicity are needed. Lenvatinib is a multitargeted tyrosine kinase inhibitor that inhibits VEGF, FGFR, PDGFRa, RET, and KIT, leading to modulation of the tumor microenvironment by decreasing regulatory T cells and tumor-associated macrophages. The combination of lenvatinib + pembrolizumab is superior to standard therapies in renal cell carcinoma and endometrial cancer and is FDA-approved in these settings.
Methods: This is a single-center, open-label, phase Ib/II trial of lenvatinib + pembrolizumab + fulvestrant for ER+/HER2- MBC. The objective of this study is to test the efficacy of this triplet combination. Enrolled patients undergo a pre-treatment biopsy and initiate a 2-week lead-in of lenvatinib 20mg PO daily + fulvestrant 500mg IM, after which they undergo an on-treatment biopsy and pembrolizumab 400mg IV q6 weeks is added. Key eligibility criteria include progression on at least 1 prior endocrine therapy combined with a CDK4/6 inhibitor, ≤2 lines of chemotherapy in the metastatic setting, measurable disease per RECIST v1.1, ECOG performance status 0-1, adequately controlled blood pressure of ≤150/90 mmHg with or without anti-hypertensive medications, and adequate organ function. Prior fulvestrant is allowed. Patients with autoimmune disease, prior immunotherapy exposure, or baseline proteinuria are excluded. During the phase Ib safety lead-in portion, a maximum of 6 patients will be enrolled to assess the safety/tolerability of the triplet combination at each dose level, with three dose levels of lenvatinib tested under a dose de-escalation design. Once the maximum tolerated dose (MTD)/ recommended phase II dose (RP2D) of lenvatinib is confirmed, the study will proceed to phase II using a Simon's optimal two-stage design. The primary endpoint of phase II is best ORR by week 24 using RECIST 1.1. Additional secondary endpoints include clinical benefit rate, progression-free survival, duration of response, and safety/tolerability of the triplet combination. With 43 patients, we will have 80% power to compare an estimated ORR of 25% to a historical control ORR of 10% under a Type I error of 5%. This trial opened to accrual January 2024. Clinical trial registry number: NCT06110793]

P4-08-06: Onvansertib enhances the anti-tumor efficacy of trastuzumab deruxtecan in CDK4/6 inhibitor-resistant HR+/HER2- breast cancer patient-derived xenograft models

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Background: The preferred first-line treatments for hormone receptor-positive/HER2-negative (HR+/HER2-) metastatic breast cancer is the combination of endocrine therapy and CDK4/6 inhibitors like abemaciclib or palbociclib. Although these regimens are effective, most patients eventually develop resistance leading to disease progression. At this stage, patients typically receive endocrine therapy with or without targeted therapy, followed by chemotherapy. Trastuzumab deruxtecan (T-DXd, Enhertu) is a HER2-directed antibody-drug conjugate with a topoisomerase I (Top1) inhibitor payload. T-DXd is approved for metastatic HER2-low (IHC 1+ or 2+/ISH-) breast cancer patients who have received prior chemotherapy in the metastatic setting or developed disease recurrence within six months of completing adjuvant chemotherapy. Combination therapeutic strategies may be able to extend the clinical benefit of T-DXd. Onvansertib is a highly potent, and selective polo-like kinase 1 (PLK1) inhibitor in clinical development. The combination of onvansertib and irinotecan, a Top1 inhibitor, has shown efficacy in colorectal cancer preclinical models and is currently under clinical evaluation. Here, we investigated the potential of onvansertib to increase the activity of T-DXd in HR+/HER2- breast cancer patient-derived xenograft (PDX) models resistant to CDK4/6 inhibitors and endocrine therapy.
Methods: Four PDX models were established from primary breast tumor (HBCx-3), metastatic bone biopsies (HBCx-180, HBCx-139palbo+fulvR5) and metastatic breast tumor (HBCx-246). The HBCx-246 PDX was resistant to the combination of fulvestrant and abemaciclib, while the other models were resistant to fulvestrant plus palbociclib. PDX tumors were engrafted in nude mice, and were treated with vehicle, onvansertib (oral, 45 mg/kg, 5 days a week), T-DXd (IV, 4 or 10 mg/kg, once every three weeks), or the combination. Tumor growth inhibition (TGI) was calculated as 100% x (Vcontrol-Vtreated)/Vcontrol).
Results: The combination of onvansertib and T-DXd was well tolerated and showed robust antitumor activity in three out of the four PDX models tested. In the HBCx-3 PDX model, the combination therapy caused tumor regression in all mice, whereas onvansertib treatment resulted in tumor progression and T-DXd monotherapy led to tumor stasis. In the metastasis-derived HBCx-139palbo+fulvR5 PDX model, the combination effectively inhibited tumor growth (TGI = 88%) compared to onvansertib (TGI = 54%) and T-DXd (TGI = 68%) monotherapies. Similarly, in the HBCx-246 model, the combination therapy induced profound anti-tumor activity, achieving an 89% TGI compared to 74% TGI with onvansertib and 54% TGI with T-DXd. The effect of the combination in the three responsive models was durable, with significant improvement in event-free survival compared to the monotherapies.
Conclusions: Our preclinical findings strongly support the rationale for combining T-DXd

with the PLK1 inhibitor onvansertib. The combination represents a promising therapeutic strategy for HR+/HER2- breast cancer patients resistant to first-line therapies and merits clinical evaluation.

P4-08-07: Survival outcome in patients with metastatic breast cancer with low hormonal receptor expression

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Purpose: In the treatment of hormonal receptor positive(HR+) metastatic breast cancer(MBC), various guidelines recommend endocrine therapy as the first-line drug for quality of life if there is no visceral crisis, and recommend an endocrine approach for subsequent therapy as well. However, cases with low hormonal expression have poor endocrine responses even if they are HR+ metastatic breast cancer by definition. In this study, we aimed to compare the survival outcomes of such low HR+ metastatic breast cancer patients.
Method: This study included the patients with metastatic/recurrent breast cancer who has been diagnosed from January 2000 to December 2022 in our institution. Patients were classified according to the level or percentage of estrogen receptor (ER) and progesterone receptor(PR) expression in tumor tissue using immunohistochemistry. Low HR+ was defined with Allred score 3~4 or 1%~10% of ER and PR expression. We classified HER2 negative patients into HR negative, HR low, and HR high groups and retrospectively analyzed their clinical characteristics and survival outcomes. Patient characteristics were compared among the patient groups using Pearson's chi-square test and Kruskall-Wallis tests. The statistical analysis was performed using Kaplan-Meier survival analysis.
Result: A total of 679 MBC patients were enrolled during the period, of which HER2- patients were 455 (67.0%), and of whom HR-, HR low, HR high and unknown were 134 (29.5%), 27 (5.9%), 274 (60.2%) and 20(4.4%), respectively. Median age of HER2-/confirmed HR status 435 patients was 47 years-old (40-55), 430 patients (98.9%) were female and 280 patients (64.4%) showed pre-menopausal status. There were 130 patients (29.9%) with visceral metastasis, 79 (20.3%) were overweight, and 132 (33.8%) were obese. During a median follow-up of 45.7 months, the median overall survival (OS) of the HR low subgroup was 47.9 (95% CI 2.5-93.4), which was lower than that of the HR high group (71.3 months, 95% CI 57.2-85.5), (Log rank p=0.134), and higher than that of the HR- group 28.9 (95% CI 15.7-42.0), (Log rank p=0.390). Median progression-free survival (PFS) for first-line endocrine therapy in the metastatic setting was lower in the HR low group than in the HR high group, but there was no statistical significance (6.0 vs. 16.5 months, Log rank p=0.234).
Conclusion: Endocrine therapy is an important means in HR positive MBC, but oncologic outcomes may be worse for HR low patients than for HR high patients. Therefore, it is

necessary to identify clinical and biologic factors that can guide endocrine therapy and subsequent therapies in HR low MBC patients.

P4-08-08: Real-world data on cyclin-dependent kinase 4/6 inhibitor switching in patients with first-line hormone receptor-positive, HER2-negative metastatic breast cancer

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Background: Standard-of-care first-line (1L) treatment for hormone receptor-positive, HER2-negative metastatic breast cancer (HR+, HER2– mBC) is endocrine therapy + a cyclin-dependent kinase 4/6 inhibitor (ET + CDK4/6i). There are limited data on switching between CDK4/6is in HR+, HER2– mBC after discontinuing a CDK4/6i due to toxicity, with a recent European real-world data (RWD) study suggesting that CDK4/6i switching is rare. We present RWD from a large US population to evaluate CDK4/6i switching frequency and patterns in a broad cohort of patients treated for HR+, HER2– mBC in the 1L, and in a sub-cohort of patients with ET resistance (ETR), to assess whether switching differs in the ETR population. We also assess whether choice of backbone ET impacts CDK4/6i switching patterns.

Methods: This retrospective cohort study used nationwide (US-based) de-identified electronic health record-derived data from the Flatiron Health database. Selection criteria included adult patients with HR+, HER2– mBC who started 1L ET + CDK4/6i treatment between March 1, 2018 and June 1, 2023, with no adjuvant abemaciclib (abema) ≤1 year prior to mBC diagnosis. Switching was defined as receipt of a new CDK4/6i after the 1L start date and before the earliest occurrence of any of the following: disease progression, start of new anti-cancer therapy that was not a replacement CDK4/6i, data cutoff, or death. In patients with recorded adjuvant ET information, ETR was defined as having a duration of adjuvant ET of 12 months or longer and developing metastatic relapses while on ET or within 12 months of ET completion.

Result: Baseline characteristics were similar across CDK4/6is in the overall cohort (N = 4,320); 70% of patients received 1L treatment with an aromatase inhibitor (AI) versus 30% with fulvestrant (FUL). In the ETR sub-cohort (n = 1,080), 39% of patients had 1L AI versus 61% who had FUL. 1L CDK4/6i segmentation was similar between the overall cohort/ETR sub-cohort: palbociclib (palbo) 74%/75%; abema 15%/15%; ribociclib (ribo) 12%/10%. CDK4/6i switching occurred in 3%, 11%, and 10% of 1L palbo-, abema-, and ribo-treated patients, respectively, in the overall cohort and in patients with ETR. The proportion of patients who switched was similar in FUL-treated patients (4%, 13%, and 13% switched from palbo, abema, and ribo, respectively) versus AI-treated patients (3%, 10%, and 10%). Switching prevalence remained similar throughout the 2018–2023 period. In patients who switched from 1L palbo, the majority (69%) received abema as the replacement CDK4/6i. In

patients who switched from 1L abema or ribo, palbo was the most used replacement (in 84% and 74% of patients, respectively). In the ETR sub-cohort, all patients in the 1L abema subgroup who switched CDK4/6is replaced it with palbo, versus 83% of the patients who switched in the 1L ribo subgroup. Median time from start of 1L treatment to switching was 3.3–5.0 months in the overall cohort and 1.5–4.7 months in the ETR sub-cohort.

Conclusions: These RWD show that CDK4/6i switching frequency is generally uncommon, with the frequency being higher in patients who received 1L abema or ribo versus palbo, consistent with discontinuation rates reported in the respective trials. Palbo was the most used option when switching CDK4/6is, and switching patterns were not impacted by the choice of backbone ET or by ETR. Limitations include the use of data from a single country only, the lack of available data on the use of dose reductions prior to CDK4/6i switching, and the emergence of newer data that may impact physicians' preferred choice of CDK4/6i.

P4-08-09: Phase 3, Randomized, Open-label TroFuse-010 Study of Sacituzumab Tirumotecan (sac-TMT) Alone & W/ Pembrolizumab vs. Treatment of Physician's Choice Chemotherapy in Patients With HR+/HER2- Unresectable Locally Advanced or Metastatic Breast Cancer

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Background: Additional therapies are needed to improve outcomes in patients with HR+/HER2- breast cancer that progressed on endocrine therapy (ET) plus CDK4/6 inhibitors (CDK4/6i). Trophoblast cell surface antigen 2 (TROP2) is commonly overexpressed in patients with HR+/HER2- metastatic breast cancer (mBC) and is associated with poor prognosis. Sac-TMT (also known as MK-2870/SKB264) is a novel anti-TROP2 antibody-drug conjugate composed of an anti-TROP2 antibody coupled to a cytotoxic belotecan derivative via a novel linker (average drug/antibody ratio, 7.4). In a phase 1/2 study, intravenous (IV) sac-TMT alone had antitumor activity in patients with previously treated HR+/HER2- mBC (ORR, 36.8%). TroFuse-010 evaluates sac-TMT alone or with pembrolizumab vs treatment of physician's choice chemotherapy (TPC) in patients with HR+/HER2- unresectable locally advanced or mBC who have not previously received chemotherapy.
Methods: This phase 3, randomized, open-label study (NCT06312176) is enrolling patients ≥18 y with HR+/HER2- unresectable locally advanced or mBC, ECOG PS ≤1, and a tumor sample for central assessment of TROP2 expression and HR, HER2, and PD-L1 status. Patients are candidates for chemotherapy and had either (a) PD after ≥1 line of ET, 1 of which was with a CDK4/6i, (b) PD ≤6 mo after starting 1L ET plus CDK4/6i where the CDK4/6i was discontinued before the PD, (c) PD >6 mo after starting 1L ET plus CDK4/6i (where the CDK4/6i was discontinued before the PD) and PD on an additional ET (either as monotherapy or combined with a PI3K or mTOR inhibitor), or (d) relapse during or ≤12 mo after completing CDK4/6i given as adjuvant therapy together with ET. Key exclusion criteria are prior treatment with chemotherapy in the metastatic setting and, if treated with prior

(neo)adjuvant chemotherapy, recurrence ≤6 mo after completion of chemotherapy. Patients are randomized 3:3:2 to receive IV sac-TMT 4 mg/kg Q2W, IV sac-TMT 4 mg/kg Q2W plus pembrolizumab 400 mg Q6W, or TPC (paclitaxel, nab-paclitaxel, capecitabine, or liposomal doxorubicin) until radiographic PD, unacceptable toxicity, patient withdrawal, or discontinuation criteria are met. Randomization is stratified by PD-L1 combined positive score (<1 vs 1-9 vs ≥10), TROP2 expression (low/medium vs high), and region (Western Europe vs North America vs Rest of World). Primary endpoints are PFS per RECIST v1.1 by blinded independent central review (BICR) with sac-TMT vs TPC and sac-TMT plus pembrolizumab vs TPC. Secondary endpoints include OS, PFS per RECIST v1.1 by BICR with sac-TMT plus pembrolizumab vs sac-TMT, ORR, duration of response, patient-reported outcomes, and safety. Tumor imaging occurs at baseline, Q9W through week 54, and Q12W thereafter. Recruitment began in April 2024.

P4-08-10: Correlation between prior treatments on CDK4/6 Inhibitors Survival in Hormone receptor-positive metastatic breast cancer

Elina Rodriguez-Melendez, Evelyn Valencia, Katherine García Matamoros, Mayra Santacruz, Felipe Campoverde, Ruth Engracia Vivanco

Background: Breast cancer (BC) is the second most common type of neoplasm wordwide. According to GLOBOCAN 2022, the incidence of breast cancer in Ecuador is 39.5 per 100,000 people/year, with a mortality rate of 11.2 per 100,000 people/year, and represents 12,6% of new cases.
Our health system provides coverage to approximately 60& of the population by th Ministry of Public Health (MSP), 30% by The Ecuatorian Social Security Institute (IESS), 5% by other entities (ISSFA, ISSPOL), and <3% of the population have private medical coverage. Medical care attentions for BC covered by the MSP was 7,134 consults in 2013, with an increase to 8,767 in 2018.
SOLCA Guayaquil, as a national reference center, provides 24,425 oncologic consults per year, 38% correspond to BC. The proportion of clinical stage IV BC varies from 5-10% with and average of 6% in urban areas, reaching up to the 50% in rural areas, so metastatic BC is a public health challenge, especially for countries with emerging economies like ours.
Methods: We conducted an observational, retrospective, case control single center study. All patients with Hormone receptor positive (HR+)/Her 2 negative metastatic BC who had been treated at SOLCA Guayaquil, in the period from 2016 to 2020 were included in the analysis. The clinical and pathological characteristics were recorded, and Pearson linear correlations were determined.
Results: A total of 1,113 patients with BC attended in the period 2016-2020 were included, of which 84 had de novo metastatic BC (7,6%). Of these patients, 37,1% (31 patients) were HR+/HER2 negative BC subtype, which were treated with various chemotherapy protocols. It was evidenced that 19 patients with luminal metastatic BC received cyclin inhibitors in some line of treatment. The average age of the patients in the iCDK4/6 group was 54 years vs. 52 years in the chemotherapy arm. The origen of the patients was mostly from Guayas,

with 61,9% in the iCDK4/6 arm vs 73,3% in the chemotherapy arm. The coverage of the patients in the iCDK4/6 arm was 76,2% by IESS, 19% MSP, and 4,8% institutional, unlike the chemotherapy arm which the coverage was 60% institutional, 26,7% IESS, and 10% MSP. Patients who received CDK4/6 inhibitors has a median indication in the third line of treatment, ranging from first to seventh line of treatment; with a DFS (disease free survival) of 9,05months, and Overal Survival 18,68 months. A Pearson linear correlation between line of CDK4/6 treatment vs. DFS was determined at -0.519, tralating to a moderate negative correlation. Similarly, the waiting time to start iCDK4/6 treatment vs. DFS reported a weak negative correlation of -0.181.
Conclusions: In our population metastatic BC occurs in 7,6%, similar to the reported worldwide. Among the subgroup of luminal metastatic patients, only 19 of them could access CDK4/6 inhibitors in some line of treatment, with an average indication in the third line. It was evidenced that the vast mayority of these patients had some type of health coverage, either IESS or MSP, unlike the chemotherapy arm, where 60% had no health coverage; implying that the patient self-financed the treatment, which could have limited access to iCDK4/6 treatment due to the high cost of medication. Pearson`s correlation analysis evidenced an inversely proportional relationship between the line of treatment and DFS, meaning the later the indication of iCDK4/6 reatment, the shorter the progression time of the disease, and consequently, the shorter OS. The results of the main studies of cyclin inhibitors such as PALOMA 3 TRIAL shows DFS of 9,2 monts vs 3,8 monts in placebo arm, OS 34,9 monts, vs 28 months; and MONALEESA 3 TRIAL shows DFS 20,6 months vs. 12,8 months with placebo, OS 67,6 months vs. 51,8 months, which drastically differ from our reality.
Our results differ from main trials, probably due to the lack of access to target medicine and difficulties in accessing health caer. Thus, better strategies for early access to adequate systemic treatment protocols should be developed.

P4-08-11: Linc01588-V4 is UPR-downregulated lncRNA in a PERK-dependent manner and estrogen-responsive gene mediated cell proliferation and migration of ER+ breast cancer cells

Wen Liu, Sanjeev Gupta, Ananya Gupta, Wenyuan Zhao, Qian Xu

Background: Physiological or pathological processes that disturb protein folding in the endoplasmic reticulum cause a state known as endoplasmic reticulum stress (ER stress) and then activate a set of signalling pathways termed the Unfolded Protein Response (UPR). This concerted and complex cellular response is mediated by three molecular sensors, PKR-like ER kinase (PERK), activated transcription factor 6 (ATF6), and inositol-requiring enzyme 1 (IRE1). UPR is mostly associated with adaptive signalling, allowing cancer cells to cope with adverse environmental conditions and conferring therapy resistance. Long noncoding RNA (lncRNA) belongs to a class of non-protein coding transcripts longer than 200 nucleotides. However, not much is known about UPR-regulated lncRNA and the causative effect of lncRNA in affecting ER stress responses and cancer development. Recent

studies have highlighted the role of interaction between the UPR and the estrogen signaling pathway in contributing to endocrine resistance. Here we have evaluated the role of UPR-regulated and estrogen-responsive lncRNA in breast cancer.
Method: Gene Expression Profiling Interactive Analysis, an interactive web server developed to analyze the RNA sequencing expression data, was used to analyze the Linc01588 expression across all cancers and overall survival in breast cancer patients. Expression of all Linc01588 variants V1-V5 in breast cancer cell lines was verified by conventional PCR. Additionally, UPR-induced and estrogen-responsive Linc01588 was assessed using RT-qPCR in ER+ breast cancer cell lines treated with UPR inducers Brefeldin A and Thapsigargin or estrogen (E2) and estrogen receptors-targeted Tamoxifen and Fulvestrant. Furthermore, sub-clones of MCF7 cells deficient in downstream UPR signalling were utilized to further validate the regulatory mechanisms of Linc01588 in response to UPR stimulation. Linc01588 knockdown clones in MCF7 and T47D generated by the lentivirus strategy were used to investigate the functional mechanism of cell proliferation and migration.
Results: Linc01588 expression is increased in primary and metastatic breast cancer as compared with normal breast tissue. Increased Linc01588 expression was associated with poor overall survival in ER+ and HER2-amplified breast cancer patients but not in TNBC. There are 5 isoforms of Linc01588 and variant 4 (Linc01588-V4) and variant 5 (Linc01588-V5) were two main predominantly expressed variants in breast cancer cell lines. Linc01588-V4 expression was downregulated upon treatment with UPR inducers in ER+ breast cancer cell lines in a PERK-dependent manner. Furthermore, the expression of Linc01588-V4 was markedly elevated in ER+ breast cancer cell lines upon E2-stimulation and 293T cells transiently transfected with estrogen receptors (ERα). Tamoxifen treatment increased Linc01588-V4 expression in MCF7 cells but not in T47D cells. The Linc01588-V5 was downregulated by UPR inducers but not affected by E2, ERα, Tamoxifen, and Fulvestrant. Knockdown of Linc01588-V4 in MCF7 and T47D cells reduced cell proliferation and migration but had no effect on the expression of ERα protein and induction of E2-responsive genes.
Conclusion: We have identified Linc01588-V4 as an estrogen-responsive lncRNA which can regulate cell proliferation and migration of ER+ breast cancer cells.

P4-08-12: Second-line, post-CDKi treatment of metastatic ER+ HER2-negative breast cancer (ER+mBC): The impact of a 25-minute CME video on treatment choices of community-based general medical oncologists (GMOs)

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Background: The recent introduction of oral selective estrogen receptor degraders (SERDs) and PIK3CA/AKT/PTEN inhibitors into clinical practice has added complexity to the rapidly evolving biomarker-based management of ER+mBC in patients with disease progression on

a CDKi and endocrine therapy (ET). For almost 40 years our CME group has utilized and evaluated practical, efficient mechanisms to inform GMOs. An important focus has been the use of audio content (cassettes > CDs > podcasts), which allows multitasking (exercise, commuting). Another valuable media tool is video which has evolved from a clumsy, costly analog base to a digital and online platform with profound potential impact. More recently videos are increasingly distributed by email and social media. Since the COVID-19 pandemic along with the widespread availability of telerecording and teleconferencing, our group has increasingly turned to short- and intermediate-form video to deliver oncology education content. For this project, we investigated the impact of a concisely produced video featuring a prominent clinical investigator (KJ) discussing her preferred treatment approach for ER+mBC as well as results of a related survey of the practice patterns of 20 US-based breast cancer clinical investigators. Key survey variables included age, biomarker profile, disease location and time on prior CDKi.

Methods: We recruited 24 US-based GMOs from geographically diverse locations who have presented cases in 1 or more of our CME programs. Participants filled out an online survey that included common clinical scenarios and then viewed a 25-minute highly edited video discussing second-line, post-CDKi treatment decisions specifically with respect to preferred choice of therapy, based on biomarker profile, disease burden and duration of prior CDKi/endocrine treatment (ET). After viewing the video, participants repeated the online survey.

Results: 19 of 24 GMOs stated that they planned to make significant changes to their practices after viewing the program. Overall, in 8 basic scenarios (4 featured in the table), the 24 GMOs answered 192 treatment-based questions and in 67 instances (35%) treatment changed after viewing the video.

Patient subsets of particular interest (see Table) included:

1. ESR1+; PIK3CA WT: For a patient with relapse at 18 months (4/24 GMOs switched to a SERD). For a patient with relapse at 6 months (12/24 switched choices).
2. ESR1+ and PIK3CA+: The split between Capi and E relates to preferred tolerability of a SERD but questions about benefits related to optimal sequencing. Again, for relapse within 6 months on a CDKi 13/24 (54%) changed their treatment decisions.

Additional data: For patients with PIK3CA mutations only, Capi was preferred to alpelisib 19/24 (79%). Of an additional 96 treatment-decision questions 30 GMOs changed their clinical approach after viewing the video (31%).

Conclusions: For this project, we specifically recruited informed GMOs who have been confident enough to present cases on our webinars. However, after being presented with 25 minutes of highly concise video content, almost a third of these very important decisions changed.

While many appreciate the rapid advances in endocrine therapy for breast cancers, the findings here support a quieter revolution not widely appreciated: the simultaneous technologic advances in digital media production and the instant post-pandemic cultural adaption of teleconferencing and recording. In coming months, we will evaluate social media platforms and other methods to reach other audiences, including patients.

P4-08-13: Evaluating CYP3A4-Mediated Drug Interaction Risks for Vepdegestrant, a PROteolysis TArgeting Chimera (PROTAC) Estrogen Receptor (ER) Degrader, in Combination With Cyclin-Dependent Kinase (CDK)4/6 Inhibitors and Everolimus

Stefanie Drescher, Weiwei Tan, Yuanyuan Zhang, Julia Perkins Smith

Background: Vepdegestrant (ARV-471) is an oral PROTAC ER degrader that has shown a manageable safety profile and signals of clinical efficacy as a single agent and in combination with the CDK4/6 inhibitor palbociclib in a phase 1/2 study in patients with ER+/human epidermal growth factor receptor 2 (HER2)- advanced breast cancer who had received prior treatments (NCT04072952). Ongoing studies are evaluating vepdegestrant in combination with other anticancer therapies in patients with ER+/HER2- advanced breast cancer, including with abemaciclib (NCT05548127) or ribociclib (NCT05573555) in the phase 1b/2 TACTIVE-U umbrella study and with everolimus in the phase 1b TACTIVE-E study (NCT05501769). We performed a clinical study in healthy female adults to evaluate the effect of multiple doses of vepdegestrant on the pharmacokinetics (PK) of midazolam, a sensitive index substrate of CYP3A4. Based on the clinical study results, we assessed the potential risk of CYP3A4-mediated drug interactions with anticancer agents currently being tested in combination with vepdegestrant.
Method: A phase 1, open-label, 2-period, fixed-sequence study was conducted in 15 healthy female adults of non-childbearing potential (NCT06256510). In period 1, participants received a single oral dose of midazolam 2 mg alone, followed by a ≥1 day washout period. In period 2, participants received vepdegestrant 200 mg orally once daily (QD) under fed condition on days 1-15, and a single oral dose of midazolam 2 mg on days 1 and 15 ≈1 hour after vepdegestrant dosing. Serial plasma samples were analyzed to estimate the effect of vepdegestrant on midazolam PK. Static mechanistic models1,2,3, incorporating findings of this clinical study and drug interaction mechanisms as victim of CYP3A4-mediated metabolism for each combination partner, were used to calculate the predicted effect of vepdegestrant 200 mg QD administration on the PK of palbociclib, abemaciclib, ribociclib, and everolimus, represented as the ratio of area under the concentration-time curve (AUC) in the presence and absence of vepdegestrant (AUCr).
Results: A total of 14 participants were eligible for evaluation of the effect of vepdegestrant 200 mg QD on the plasma AUC from time 0 extrapolated to infinite time (AUCinf) of midazolam. Midazolam AUCinf was approximately 74% higher when midazolam was given after multiple doses of vepdegestrant compared to when midazolam was given alone. Multiple doses of vepdegestrant were generally well tolerated by all participants; no serious or severe adverse events, and no discontinuations or dose reductions due to adverse events were reported. Based on the observed changes in midazolam AUC, the predicted effect of vepdegestrant on combination partners, expressed as AUCr, ranged from 1.13–1.29 for palbociclib, 1.37–1.55 for abemaciclib, 1.00–1.04 for ribociclib, and 1.38–1.63 for everolimus. These predicted effects based on CYP3A4 metabolism are considered negligible or minor and unlikely to have major impact in clinical combinations.

Conclusions: Vepdegestrant shows a weak inhibitory effect on CYP3A4-mediated metabolism in the clinical study with midazolam. The study results, combined with mathematical modeling, suggest low potential of meaningful drug interactions for vepdegestrant in combination with CDK4/6 inhibitors and everolimus. Clinical data are anticipated from ongoing studies of vepdegestrant in combination with other anticancer therapies in patients with ER+/HER2- advanced breast cancer.
Citations:
1. Fahmi OA, Maurer TS, et al (2008) Drug Metab Dispos, 36
2. Fahmi, OA, S Hurst, et al (2009) Drug Metab Dispos, 37
3. FDA Guidance for Industry (2020) In Vitro Drug Interaction Studies

P4-08-14: ALISertib in combination with endocrine therapy in patients with hormone receptor-positive, HER2-negative recurrent or metastatic breast CAncer: the phase 2 ALISCA-Breast1 study

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Background: Despite the wide range of available treatment options for patients (pts) with hormone receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) recurrent or metastatic breast cancer (MBC), optimal treatment after progression on CDK4/6 inhibitors (CDK4/6i) remains unclear. Several mechanisms of resistance to CDK4/6i have been postulated, including increased expression of Aurora kinase A (AURKA), a key regulator of mitosis associated with poor prognosis in multiple tumor types. Further implicated in CDK 4/6i resistance, high c-Myc or RB1 loss of function (LOF) have been associated with transcriptional co-regulation or synthetic lethality, respectively, with AURKA.
Alisertib is a highly selective, reversible, ATP-competitive, orally administered, small-molecule AURKA inhibitor with antiproliferative activity in HR+ BC-derived cell lines and BC xenograft models. Greater alisertib sensitivity has been reported in models with elevated AURKA or c-Myc expression, or RB1 LOF. Clinically, alisertib showed efficacy in phase 1 and 2 trials, including objective response rates (ORRs) of 19.6–20% and median progression-free survival (PFS) of 5.4–5.6 months alone or with fulvestrant in pts with HR+/HER2-, endocrine-resistant MBC. The most common treatment-related grade ≥3 adverse events (AEs) were neutropenia, anemia, and leukopenia.
Methods: ALISCA-Breast1 (NCT06369285) is a randomized phase 2 study. Primary objective: to determine the optimal alisertib dose level administered with selected endocrine therapy (ET) for use in future studies based on observed safety (AEs, serious AEs per CTCAE v5.0) and efficacy (ORR, duration of response, disease-control rate, PFS, overall survival). Secondary objectives: to identify biomarkers correlating with efficacy; to evaluate alisertib pharmacokinetics (PK). Key inclusion criteria: age ≥18 years; ECOG performance status 0 or 1; confirmed HR+, HER2–, recurrent or metastatic breast adenocarcinoma not

amenable to curative therapy; available tumor tissue for biomarker analyses; progression on or after ≥2 prior ET lines in recurrent or metastatic setting; prior CDK4/6i with ET in recurrent or metastatic setting; ≥1 measurable target lesion per RECIST v1.1. Pre-menopausal pts are eligible if amenable to treatment with a luteinizing hormone-releasing hormone agonist. Key exclusion criteria: prior chemotherapy in recurrent or metastatic setting; bone-only disease not meeting the measurability definition per RECIST v1.1; prior AURKA-specific or pan-Aurora-targeted agents; active infection; immunocompromised pts; unstable brain metastases; uncontrolled symptomatic visceral disease. Eligible pts will be randomized 1:1:1 to alisertib 30mg, 40mg, or 50mg orally twice daily on days 1–3, 8–10, and 15–17 every 28 days and combined with physician's choice of anastrozole, letrozole, exemestane, fulvestrant, or tamoxifen not previously used in the recurrent or metastatic setting or progressed upon in the adjuvant setting. Up to 50 pts will be enrolled per arm at multiple centers in the USA and Europe. Treatment will continue until disease progression, unacceptable toxicity, or consent withdrawal. Stratification factors: time to recurrence/progression after initiating CDK4/6i therapy (<12 mo or ≥12 mo); presence of visceral disease (yes/no). All pts will undergo sparse PK sampling. Tumor tissue will be centrally assessed for biomarkers, including but not limited to RB1, MYC, TP53, ESR1, PI3K/AKT pathway, HER2 and AURKA genomic alterations and/or expression levels. The study is expected to determine the optimal alisertib dose to combine with ET and may identify biomarker(s) that define pts deriving the greatest benefit from alisertib-containing therapy.

P4-08-15: Levels of Circulating Tumor DNA as a Predictive Marker for Early Switch in Treatment for Patients with Metastatic Breast Cancer: A Phase 2 Randomized, Open-Label Study

Frances Valdes, Carmen Calfa, Mauricio Escobar, Lawrence Negret, Aleksei Efremov, Dmitry Tabakov, Egor Savin, Kelley Lauziere, Alexander Bagaev, Elisa Krill, Alejandra Perez

The efficacy of a CDK4/6 inhibitor (i) with an aromatase inhibitor (AI) or fulvestrant (ful) as frontline therapy for hormone receptor positive (HR+) human epidermal growth factor receptor 2- (HER2-) metastatic breast cancer (MBC) is limited by the onset of resistance mutations. For breast cancer, the prognostic value of ctDNA has been validated; however, the clinical utility of serial ctDNA monitoring remains unclear. Results from PADA-1 support the utility of serial ctDNA monitoring to guide an early switch in frontline therapy based on molecular progression (MP) in patients (pts) with HR+HER2- MBC. This strategy led to improvements in median progression free survival (mPFS) of 7 months and time to second progression (PFS2) of 15.4 months.
This is a randomized phase 2 study conducted in pts receiving an AI or ful and a CDK4/6i as first-line therapy for HR+HER2- MBC evaluating the efficacy of switching a frontline regimen upon detection of a molecular signal, rise in ctDNA, suggesting MP prior to clinical progression (CP). Compared to PADA-1, this study is utilizing a more comprehensive biomarker, ctDNA ratio>1. The calculated ctDNA ratio (ctDNA value at time of assessment

over baseline value) corresponds to mutant allele abundance (mutant copies/ml of plasma) at a given timepoint relative to baseline quantification. Use of this ratio as a dynamic marker of response has been validated by O'Leary et al and Darrigues et al. This study design also includes pts with de novo HR+HER2- MBC, a prior history of breast cancer and those who progressed on adjuvant endocrine therapy. In Step 1, enrolled pts will receive frontline therapy with a CDK4/6i and AI or ful. ctDNA will be serially monitored. When a ctDNA ratio >1 is detected and there is no synchronous CP, pts will be randomized in Step 2 to continue the same therapy until CP, which is the current standard of care (SOC), or switch to an alternative second-line regimen. The primary endpoint is PFS in Step 2. Secondary endpoints include the number and percentage of pts with events of MP and no CP and the median time from enrollment to this event, overall response rate, clinical benefit rate, PFS2, overall survival and patient-reported outcomes.
Study feasibility will be evaluated based on the number of pts with MP events (ctDNA ratio>1 and no synchronous CP). Trial accrual will be suspended if <15% of the first 25-30 pts have MP without synchronous CP. To date, 24 pts have enrolled, with follow up < 12 months in all pts. Seventeen pts are in Step 1 and two are in Step 2. Two had CP prior to MP and are off study. Two had MP without CP and proceeded to Step 2 and were randomized to an early switch in therapy or maintenance of frontline therapy. Another 3 have been censored and are off study not related to progressive disease. Plasma samples (n=43) were ctDNA-positive (detectable somatic events), with actionable findings (BRCA2, PIK3CA, ESR1, PTEN variants) and biologically relevant findings (ARID1A, NOTCH3, MSH3, FANCM, NOTCH1, MAP3K1 variants) detected by the BostonGene Liquid Biopsy test in 7 and 6 samples, respectively. At screening/baseline, 20/29 pts (70%) had detectable levels of ctDNA. As expected for pts in Step 1 responding to frontline therapy, ctDNA abundance and cfDNA yield decreased during treatment, with a median pre-treatment and median on-treatment ctDNA abundance of 17.8 mutant copies/ml and 3.3 mutant copies/ml (P<0.01), respectively, for cases with ctDNA-positive baseline timepoints.
This pilot serves to document the feasibility of serial ctDNA monitoring and the early switch approach to then expand to phase II. The overall aim is to validate the utility of serial ctDNA monitoring as a predictive biomarker, and identify a strategy to extend duration of disease control and prolong survival in pts with MBC.

P4-08-16: Modulating Gut Microbiota to enhance the efficacy of Immunotherapy in Triple Negative Breast Cancer (TNBC)

Samarpan Majumder, Manisha Poudel, Fokhrul Hossain, Giulia Monticone, Kristina Larter, Zhi Huang, Justin Brown, Sonali Ghosh

Background: Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer affecting the African American (AA) population disproportionately and is diagnosed more frequently in younger, premenopausal women. Obesity is a known risk factor for increased TNBC incidence, and the association of gut dysbiosis with obesity is now established. Immune checkpoint blockade anti-PD-1 therapy (pembrolizumab) is currently

the standard of care for early TNBC. Evidence suggests modulating the gut microbiome enhances cancer immunotherapy efficacy in melanoma and lung cancer patients. However, there are no studies on early TNBC.

Overarching Challenge

The number of patients with early TNBC who benefit from anti-PD-1 therapy remains suboptimal.

Objective: Our study aims to enhance the efficacy of anti-PD-1 therapy by modulating the gut microbiota in a mouse model of obesity and TNBC. We hypothesize that a probiotic supplement will enhance the efficacy of anti-PD-1 therapy. We will then test the hypothesis that women with early TNBC who respond to anti-PD-1 therapy have distinct gut microbiota profiles compared to non-responders.

Results: We determined the efficacy and safety of two weeks of probiotic supplementation before anti-PD-1 therapy (pembrolizumab) on objective response rates in an obese mouse model of triple-negative breast cancer. This was evaluated by measuring the objective response rate (tumor burden as a read out) after two weeks of anti-PD-1 treatment. The underlying mechanism of microbiota mediated potentiation of anti-PD-1 therapy was further evaluated by a) 16s and metagenomics sequencing; b) total transcriptomic profiling of tumors from control and experimental groups and c) Short Chain Fatty Acids (SCFAs) and cytokine profiling of corresponding harvested plasmas as secondary endpoint. To test the hypothesis that two week of probiotic supplementation before tumor engraftment and anti-PD-1 therapy would cause an increased objective response rate (reduced tumor burden) when compared to groups that received anti-PD-1 therapy alone or isotype control, we conducted a permutation-based extension of multivariate analysis of variance and a two-sample test of independent means. For this, FVB female mice were used and at five weeks of age, mice were switched to a diet that mimics the “Western diet” (ENVIGO, Cat # TD.88137, Indianapolis, IN, USA). This diet reliably induces obesity. After 16 weeks of obesity induction mice were randomized in a 1:1 ratio to a probiotic dietary supplement or usual care for two weeks. We used a probiotic formulation from Creative Enzyme which is a blend of 13 human probiotic strains (Cat # PRBT-035, NY; ~3.38X1010 (Figure 5). It contains most of the species of Lactobacillus and Bifidobacterium that traditionally constitute commensal bacteria that colonize the gut. Authenticated TNBC C0321 claudin low mouse tumor cells (In Vitro Technologies) were injected into the 4th mammary fat pad of all the mice with a 1:1 ratio with Matrigel. Once tumor was engrafted, mice were divided into three groups- probiotics+ anti-PD1, anti-PD1 only, and serotype control and treatment was subsequtly administered. A two-tailed t-test comparing the anti-PD1 group with the probiotics + anti-PD1 group produced a statistically significant ($P<0.0001$) outcome confirming our prediction that probiotics supplementation positively affect anti-PD1 therapy in FVB obese mouse model. A Kaplan-Meier survival plot based on our record of mouse survival over the course of the treatment demonstrates that only probiotics supplement plus Anti-PD1 therapy and not Anti-PD1 therapy alone could provoke statis tically significant survival benefit over isotype control ($p=0.046$) in our study. Furthermore, 16s sequencing profiling on fecal samples illustrates that anti-PD1 therapy alone could induce a significant increase in Akkermensia at genus level consistent with significant

correlation between clinical responses to anti-PD1 therapy in a few other cancer types. We also identified probiotic treated mice showing abundance of Faecalibaculum at genus level. Faecalibaculum blocks tumor progression in mouse model of colorectal cancer. However, detailed metagenomics profiling is imperative to understand species level characterization to illustrate the difference in gene/pathway modulation pertaining to different cohorts subjected to different treatment conditions. Analysis of cytokine profile from collected blood revealed elevated levels of proinflammatory cytokines viz., IL-1b, IL-6, TNF-α, MCP-1 and VEGF in untreated plasma from mice when compared to plasmas from mice treated with probiotics. For feasibility study in human subjects, we already have our pilot study recruiting volunteers to confirm whether the fecal microbiome differs between patients with and without obesity and early TNBC who achieve a pathologic complete response from preoperative anti-PD-1 therapy (NCT06318507). We are recruiting subjects from the Pennington Cancer Center at Baton Rouge General Hospital and the Mary Bird Perkins Cancer Center at this time.

Impact

TNBC is the most aggressive form of breast cancer, with a high risk of early recurrence and death. This study will provide translational insight into a biological mechanism that explains patient outcome heterogeneity in response to anti-PD-1 therapy. The study provides critical experimental data to demonstrate causality and feasibility to justify larger-scale interrogations to modulate the gut microbiome in women with early TNBC who plan to begin anti-PD-1 therapy.

Innovation

This study is the first to determine how a probiotic supplement can enhance anti-PD-1 efficacy in early TNBC.

P4-08-17: First-in-human Phase 1 study of BTX-9341, a first-in-class, CDK4/6 bifunctional degrader, as a monotherapy and in combination with fulvestrant in patients with advanced and/or metastatic HR+/HER2- breast cancer.

Amita Patnaik, Danette Powell, Aparajita H. Chourasia, Massimo Cristofanilli, Ewelina Morawa, Jeremy Barton, Rachel M. Layman, Matthew Goetz

Background: BTX-9341 is a first-in-class, oral bifunctional degrader of cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6), both clinically validated cell cycle targets in hormone receptor (HR)-positive (+)/human epidermal growth factor receptor 2 (HER2)-negative (-) breast cancer. Preclinical data highlight the superiority of BTX-9341compared to CDK4/6 inhibitors in terms of robust inhibition of retinoblastoma (RB) phosphorylation and CDK2 and Cyclin E transcription, cell cycle arrest, in vivo efficacy in breast cancer xenografts and, ultimately, in the ability to overcome key resistance mechanisms that limit the impact of CDK4/6 inhibitors in second-line (2L) HR+/HER2- breast cancer.

Methods: This first-in-human (FIH), Phase 1 trial of BTX-9341 (BTX-9341-101) will be a

multicenter, nonrandomized, open-label trial to evaluate the safety, tolerability, pharmacokinetics (PK), and preliminary efficacy of BTX-9341 in patients with advanced and/or metastatic HR+/HER2- breast cancer who received prior CDK4/6 inhibitor therapy and have no mutations in RB.
The trial will consist of initial dose escalation using accelerated titration and a Bayesian Optimal Interval (BOIN) design (Part A) of BTX-9341, both as monotherapy and in combination with fulvestrant. The dose expansion (Part B) of BTX-9341 in combination with fulvestrant will use a Bayesian Optimal Phase 2 (BOP2) design. The primary objective of Part A is to determine the maximum tolerated dose (MTD)/maximum evaluable dose (MED) of BTX-9341 monotherapy and/or combination therapy. The primary objectives of Part B are to obtain preliminary evidence of BTX-9341 efficacy and to confirm the recommended phase 2 dose (RP2D) of BTX-9341 combination therapy. Secondary objectives include characterization of the plasma PK profile of BTX-9341 following single and multiple doses of the drug administered as a single agent and/or in combination with fulvestrant and exploratory objectives will assess pharmacodynamic (PD) effects and PK/PD correlations. Approximately 82 patients will be enrolled and non-randomly assigned initially to Part A (dose escalation; ~36 participants) and later to Part B (dose expansion; ~46 participants). BTX-9341 will be evaluated as an oral therapeutic administered once a day (QD) in 28-day treatment cycles.
Note: Dr. Rachel M. Layman and Dr. Matthew Goetz have contributed equally to this abstract.

P4-08-18: M6 metabolite enhances the efficacy of the Rac/Cdc42 inhibitor MBQ-167 in metastatic advanced breast cancer

Nilmary Grafals-Ruiz, Julia Medina, Jessica Colon, Jorge Duconge, Cornelis Vlaar, Jose F. Rodriguez-Orengo, Suranganie Dharmawardhane

MBQ-167 is a Rac/Cdc42 specific inhibitor effective in the nM range. In mouse models of spontaneous and pre-existing metastasis, MBQ-167 significantly inhibits triple-negative breast cancer (TNBC) and HER2+ breast cancer tumor growth and metastasis. MBQ-167 shows no toxicity up to 1000 mg/kg in rodents and dogs, thus received IND status from the USFDA, and is currently in Phase 1 clinical trials. Several MBQ-167 metabolites were detected through a mammalian liver microsome assay, and the compound M6 was identified as the most abundant metabolite. In addition, M6 was detected as the major metabolite from dog plasma following oral dosing with 200 or 400 mg MBQ-167 BID. M6 demonstrated parallel pharmacokinetics as MBQ-167, at 10X less concentration than MBQ-167, in plasma during the first 24 hours. In the ongoing clinical trial, “Open-Label, First-in-Human Trial of Oral MBQ-167, as Single Agent in Subjects with Therapy Resistant Advanced Breast Cancer (NCT06075810)”, 20 mg MBQ-167 BID demonstrated a Tmax of 3-4hrs, for both MBQ-167 and M6 (N=4). M6 paralleled the MBQ-167 pharmacokinetics, where MBQ-167 showed a Cmax of 1.9 microM while M6 had a Cmax of 0.014 microM. The Cmax of both compounds was sustained for up to 24 hours. To determine whether M6 is effective as an

anti-breast cancer compound, pre-clinical studies were performed using human TNBC and HER2+ breast cancer cell lines. Rac/Cdc42 activation assays were conducted using pulldowns for GTP-bound Rac or Cdc42 and western blotting for the active phosphorylation status of the Rac/Cdc42 downstream effector P21-activated kinase (PAK). M6 at a similar concentration as MBQ-167 (250nM) significantly inhibited Rac/Cdc42/PAK activation. Wound healing assays for cell migration, MTT assays for viability, and caspase 3/7 assays for apoptosis demonstrated that M6 (250nM) significantly inhibited breast cancer cell motility without affecting viability or apoptosis. Immunocompromised mice bearing green fluorescent protein (GFP)-tagged human HER2+ tumors were treated with 10 mg/kg BW MBQ-167 or M6 by I.P. 5X a week for ten weeks. Tumor growth and metastasis were quantified by GFP fluorescence through in vivo digital whole-body imaging and ex vivo stereoscopy of excised organs following necropsy. M6 was similarly effective as MBQ-167, with a statistically significant 90% reduction in tumor growth and metastasis compared to the vehicle. Therefore, M6 is expected to significantly contribute to the duration and intensity of MBQ-167 efficacy in metastatic advanced breast cancer. This study was supported by the US Army Breast Cancer Research Program (BCRP) W81XWH2010041 and HT9425-23-1-0166 (to SD) and HT9425-23-1-0381 (to JFRO).

P4-08-19: Phase I Safety and Efficacy Study of Autophagy Inhibition to Augment the Antiproliferative and Biological Effects of Low Dose Palbociclib plus Letrozole in Estrogen Receptor-Positive, HER2-negative Metastatic Breast Cancer (MBC)

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Purpose: Endocrine therapy with a CDK4/6 inhibitor is standard therapy for estrogen receptor-positive, HER2-negative metastatic breast cancer (ER+/HER2- MBC), yet clinical resistance develops. Previously, we showed that low doses of palbociclib activate autophagy, reversing initial G1 cell cycle arrest. High concentrations induced off-target senescence. The autophagy inhibitor hydroxychloroquine (HCQ) induced on-target senescence at lower palbociclib doses in preclinical models.
Patients and Methods: We conducted a phase I trial (NCT03774472) of HCQ (400, 600, 800mg/day) with palbociclib and letrozole, using a 3+3 design. Eligible patients were age ≥ 18 years with a histologically confirmed diagnosis of ER+/HER2- MBC and were candidates for treatment with CDK4/6 inhibitor and endocrine therapy. Primary objective included safety, tolerability, and HCQ recommended phase 2 dose (RP2D). Secondary objectives included tumor response and biomarker analysis.
Results: Fourteen postmenopausal women with ER+/HER2- MBC were evaluable [400mg (n=4), 600mg (n=4), 800mg (n=6)]. Grade 3 adverse events (AEs) included hematological (3 at 800mg), skin rash (2 at 600mg), and anorexia (1 at 400mg), with no serious AEs. Best responses were partial (2), stable (11), and progression (1). Tumor decreases ranged from

11% to 30%, with one 55% increase. Biomarker showed drops in Ki67, Rb, and nuclear cyclin E in responders. The combination therapy demonstrated significant overall survival (OS) benefit, with the median OS not reached, significantly exceeding the typical 53.9 months for palbociclib and letrozole alone.
Conclusions: This phase I study demonstrated acceptable safety for HCQ. The RP2D of HCQ was 800mg/day with continuous palbociclib (75mg/day) and letrozole (2.5mg/day). These findings suggest that adding HCQ can enhance the efficacy of standard therapy, potentially offering significant survival advantages in ER+/HER2- MBC patients.These results support further investigation into HCQ as a valuable addition to CDK4/6 inhibitor regimens, offering a promising strategy to improve long-term outcomes and quality of life for patients with ER+/HER2- MBC. This approach could lead to a paradigm shift in managing resistance and enhancing the therapeutic arsenal against this challenging cancer subtype.

P4-08-20: Trial in progress: A first-in-human phase 1a/b, dose-escalation/expansion study of BG-68501/ETX-197 (CDK2 inhibitor) as monotherapy or in combination with fulvestrant for patients with HR+/HER2- breast cancer and other advanced solid tumors

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Background: Cyclin-dependent kinase (CDK) 2 can regulate the cell cycle through the interaction with cyclin E or cyclin A during the G1/S and S/G2 transitions, respectively. Elevated CDK2 activity is a key resistance mechanism to CDK4/6 inhibition in HR+/HER2− breast cancer (BC). Other genomic alterations, eg, loss of RB1, can cause resistance in additional solid tumors, including high-grade serous ovarian cancer, gastric cancer, small cell lung cancer (SCLC), and endometrial cancers. CCNE1 amplification or cyclin E overexpression may confer sensitivity to CDK2 inhibition. BG-68501/ETX-197 is a potent, selective inhibitor of CDK2, with preclinical evidence showing potent activity in biochemical and cellular assays, marked antitumor activity in cancer xenograft models, and superior selectivity for CDK2 over other CDK family members.
Methods: This study is a first-in-human, phase 1a/b, open-label, multicenter study to evaluate the safety, tolerability, PK, pharmacodynamics, and preliminary antitumor activity of BG-68501/ETX-197 in pts with advanced, nonresectable, or metastatic solid tumors, including HR+/HER2− BC. In the dose-escalation phase (phase 1a), sequential cohorts will receive increasing doses of BG-68501/ETX-197 as monotherapy, or in combination with fulvestrant; additionally, safety expansion cohorts will receive BG-68501/ETX-197 at doses recommended for further evaluation. In the dose-expansion phase (phase 1b), pts with HR+/HER2− BC, platinum-refractory or -resistant serous ovarian, fallopian tube, primary peritoneal cancer (PROC), extensive-stage SCLC (ES-SCLC), or CCNE1-amplified advanced solid tumors will receive BG-68501/ETX-197 orally as monotherapy or combined with fulvestrant. Eligibility criteria include pts ≥18 years with histologically or cytologically confirmed advanced or metastatic solid tumors potentially associated with CDK2

dependency who have received ≥1 line of treatment for locally advanced or metastatic disease and prior endocrine therapy and a CDK4/6 inhibitor in either the adjuvant or locally advanced or metastatic setting for HR+/HER2− BC or prior standard of care for all other advanced solid tumors.
For the dose-escalation phase (phase 1a), the primary objectives are to assess the safety and tolerability of BG-68501/ETX-197 monotherapy or in combination with fulvestrant, and to determine the maximum tolerated dose, maximum administered dose, and recommended dose for expansion (RDFE); secondary objectives are to assess preliminary antitumor activity (ORR, duration of response [DoR], time to response [TTR], disease control rate [DCR] and clinical benefit rate [CBR]) as assessed by investigator per RECIST v1.1, and PK.
For the dose-expansion phase (phase 1b), the primary objectives are to assess the antitumor activity (ORR) of BG-68501/ETX-197 in combination with fulvestrant in pts with HR+/HER2− advanced or metastatic BC, and BG-68501/ETX-197 as monotherapy in pts with PROC, ES-SCLC, and other advanced or metastatic solid tumors with CCNE1 amplification; secondary objectives are to further assess the antitumor activity (DoR, TTR, DCR and CBR) of BG-68501/ETX-197 alone in the previously mentioned advanced solid tumors or in combination with fulvestrant in HR+/HER2− metastatic BC, and to assess the safety and tolerability and PK of BG-68501/ETX-197 at the RDFE (NCT06257264).
The study is currently recruiting pts, with 14 sites open across two countries and 6 pts enrolled. Enrollment is complete for 1 cohort and cohort 2A is currently enrolling pts.

P4-08-21: Chlorogenic acid and cinnamaldehyde in combination demonstrate antimetastatic capabilities and induce apoptosis in MCF7 and MDA-MB-231 breast cancer cell lines via downregulation of the Akt pathway

Yusuff Olayiwola, Lauren S. Gollahon

The majority of reported breast cancer-associated deaths are directly correlated with metastatic disease. Furthermore, the nature of current metastatic cancer treatments detrimentally impacts other organ systems. Thus, there remains the need for more effective and safer strategies to treat metastatic breast cancer at a systemic level. Recently, more attention has been given to Natural Products as candidate anticancer approaches. This study is aimed at investigating the synergistic impact of two such natural compounds, chlorogenic acid and cinnamaldehyde (CGA:CA). We hypothesized that CGA:CA in combination, would decrease the metastatic potential of breast cancer cells by suppressing their invasive and migratory abilities induced through AKT pathway activation. We also hypothesized that apoptotic cell death would occur as a result of downregulation of Akt activation. To test this hypothesis, suppression of migration and invasion after treatment was analyzed by wound healing and Transwell Matrigel assays. FACSsort was performed to determine expression of the migration and invasion associated biomarkers fibronectin, vimentin, and EpCAM. Western blotting was used to determine the effect of the natural

products on expression of phospho-AKT, E- and N-cadherin, as well as the apoptotic marker and caspase 3 and BCL2-α. Annexin V/propidium iodide fluorescence microscopy and growth curves were conducted to analyze apoptosis induction. Results showed decreased phosphorylated Akt expression upon treatment with CGA:CA. the anticancer effect of the compounds on Akt activation resulted in inhibition of metastasis, evidenced by restoration of epithelial characteristics such as increased expression of E-cadherin and EpCAM, downregulation of N-cadherin, fibronectin, vimentin and MMP-9 expression and lack of cell migration. Annexin V and Western results showed that CGA:CA significantly inhibited cancer cell growth and induced apoptosis via increased expression of caspase 3 and downregulation of Bcl2-α. Overall, the present study demonstrated that CGA:CA combination downregulated the Akt pathway, induced apoptosis, and arrested the migration and invasion of both triple-positive and triple-negative breast cancer cell lines.

P4-08-22: Overcoming Olaparib Resistance in BRCA-Mutated Triple-Negative Breast Cancer: Synergistic Potential of DNA Repair Pathway Inhibitors

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Background: Triple-negative breast cancer (TNBC) requires new treatment options due to its aggressive nature, high recurrence rates with standard-of-care treatment, and heterogeneous therapeutic effectiveness of existing treatments for metastatic TNBC. Poly(ADP-ribose) polymerase (PARP) inhibitors are approved treatments for HER2-negative breast cancer with germline BRCA (gBRCA) mutation, and they are of particular interest for patients with gBRCA-mutated TNBC due to their role in exploiting DNA repair deficiencies. Two PARP inhibitors (olaparib and talazoparib) are FDA-approved for patients with metastatic gBRCA mutated breast cancer. Although PARP inhibitor treatment has high response rate and improved progression-free survival, resistance to PARP inhibitors poses an emerging and significant challenge, necessitating the identification of novel therapeutic targets to overcome this resistance and improve patient outcomes with gBRCA mutated TNBC. This study aims to identify novel therapeutic targets whose inhibition could enhance the efficacy of PARP inhibitors in TNBC and olaparib-refractory TNBC.
Methods: We conducted a preclinical experiment to achieve our aim. We generated olaparib-resistant (OPR) cell lines using BRCA-mutated TNBC cell lines (SUM149, HCC1937, and MDA-MB-436). These cell lines were treated with olaparib until they achieved at least a 10-fold higher concentration of IC50 than their parent cell lines. To identify potential synergistic kinase targets, we performed a non-biased high-throughput 709 Kinome-Library RNA interference assay using SUM149-OPR cells. Target candidates were selected using the Sensitivity Index (SI > 0.12) and STRING interactome analysis. We then conducted a series of assays, including a sulforhodamine B viability assay, a soft-agar assay, and a Bliss Synergy Score analysis, to determine the combination effect of selected kinase inhibitors and olaparib using SUM149-OPR, HCC1937-OPR, and MDA-MB-436-OPR cell lines. Finally, we

conducted a soft-agar assay to evaluate in vitro tumorigenicity.
Results: Kinome-Library RNA interference screening revealed that the DNA damage pathway (ATR, CDK12, etc.) and phosphoinositide 3-kinases pathway (PIK3CG, PIK3R3, PIK3R4, etc.) as potential canonical pathways targets for synergizing with olaparib efficacy. PLK4 was selected as non-canonical target. Based on target identification, we selected inhibitors of ATR (elimusertib, gartisertib), ATM (AZD1390), PLK4 (CFI-400945), CDK12 (SR-4835), PI3KCA (copanlisib), and AKT (capivasertib) for proliferation assays. We found that targeting the DNA damage repair pathway was the most effective in enhancing the efficacy of olaparib in both parent and olaparib-resistant TNBC cell lines. Bliss score ranges are 5.509 to 48.763 for elimusertib (ATR inhibitor), 7.872 to 71.406 for gartisertib (ATR inhibitor), and 4.660 to 45.836 for AZD1390 (ATM inhibitor). Further, we observed a significantly enhanced in vitro antitumor effect of the olaparib and elimusertib combination ($P<0.05$).
Conclusions: Our preclinical study identified DNA repair pathways as potential targets to enhance the efficacy of olaparib in both parent and olaparib-refractory gBRCA mutated TNBC cell lines. Future in vivo studies, including patient-derived xenografts and additional mechanistic analyses to further understand dual DNA targeting synergy, are warranted to validate this therapeutic approach for TNBC with gBRCA mutation.

P4-08-23: Preclinical characterization of DF1001, a first-in-class dual NK and T cell engager targeting HER2-high or HER2-low tumors

Laurens Lambert, Gregory Chang, Mitchell Bigelow, Tanmay Dichwalkar, Daniel Fallon, Patrick Kirby, Stuart Hicks, Nicolai Wagtmann, Ann Cheung

Background: DF1001 is a first-in-class TriNKET® immune engager, designed to activate both innate and adaptive immune effector cells, including NK, CD8+ T, NKT, and gd T cells, through engagement of the activation receptors CD16a and/or NKG2D. DF1001 targets immune cell effectors against tumor cells by anchoring to HER2, a clinically validated target that is widely expressed across breast, gastric, esophageal, lung, bladder, and other oncologic indications. DF1001 is uniquely differentiated from HER2-targeted therapeutics due to its distinct immune-engaging mechanism of action (MOA) which is active against HER2-low expressing cancers, where HER2 mAbs such as trastuzumab are clinically ineffective. In addition to DF1001's ability to target a range of HER2 expressing tumors, its favorable safety profile enables therapeutic combination with standard of care agents, including the TROP2-targeted ADC sacituzumab govitecan-hziy (Trodelvy®) for treatment of HR-positive metastatic breast cancer (MBC). Sacituzumab govitecan-hziy has been reported to enhance NK cell lysis and deplete immunosuppressive cells in the tumor microenvironment, suggesting potential synergy with DF1001 to drive a more potent therapeutic response against tumor cells expressing HER2 and/or TROP2. Here we present the preclinical characterization of the mechanism of action of DF1001 and the safety profile of DF1001 in non-human primates as well as the potential of combining with sacituzumab govitecan-hziy.

Methods: DF1001 affinity measurements were conducted with recombinant protein reagents using surface plasmon resonance. DF1001 cell binding and HER2 signal inhibition assays were performed in HER2-high and HER2-low cell lines. DF1001 cytolysis, cytokine release, and combination with sacituzumab govitecan-hziy was assessed in primary immune effector: tumor cell co-culture assays. The safety profile of DF1001 was assessed in a repeat-dose 28-Day GLP intravenous study in cynomolgus monkeys and a human in vitro cytokine release assay.
Results: DF1001 bound HER2 with high affinity and NKG2D with low affinity, while retaining native IgG1-like binding to CD16a through the Fc domain. On cell lines with a range of HER2 levels, DF1001 consistently exhibited higher maximal binding compared to trastuzumab. DF1001 led to HER2 signal inhibition as measured by reduced AKT phosphorylation and arrested the growth of HER2-high cells. DF1001 elicited robust CD8+ T cell and NK cell-mediated lysis of HER2-expressing cell lines, including cells expressing HER2 at low levels. In addition to direct tumor cell killing, DF1001 triggered potent NK cell activation when bound to HER2 and elevated secretion of proinflammatory cytokines and chemokines, including IFN-gamma, TNF-alpha, CCL5/RANTES and CXCL10, a known T cell chemoattractant. In long-term immune: tumor co-culture assays with HER2- and TROP2-expressing cell lines, sacituzumab govitecan-hziy enhanced DF1001 NK cell-mediated tumor lysis, while preserving immune cell effector viability. DF1001 was well tolerated in a repeat-dose GLP intravenous NHP study, and the top dose of 120 mg/kg was the no observed adverse effect level (NOAEL). There was no evidence of cytokine release syndrome (CRS) in monkeys or the human in vitro cytokine release assay.
Conclusions: The DF1001 TriNKET® targets robust immune-mediated activities, which enhances cytolytic responses directed at both HER2-high and HER2-low tumors and elevates local release of inflammatory cytokines/chemokines without causing CRS. DF1001 TriNKET's favorable drug safety profile and distinct immune-engaging MOA positions it to complement and synergize with SOC agents such as sacituzumab govitecan-hziy in the HER2-low solid tumor setting. DF1001 is currently under evaluation in patients with locally advanced or metastatic solid tumors in PhI/II trials (NCT04143711).

P4-08-24: AKTive-001: A Phase 1/1b Multiple Cohort Trial of ALTA2618 in Patients with Advanced Solid Tumors with AKT1 E17K Mutation (Trial in Progress)

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Background: AKT1 E17K is an activating driver mutation with a prevalence of 4-8% in breast cancer, with the vast majority found in HR+/HER2-negative breast cancer. Approximately 90% of AKT driver mutations are AKT1 E17K, and half of AKT1 E17K-mutant breast and gynecologic cancers are homozygous for the AKT1 E17K oncogene with

concurrent loss of the wildtype (WT) AKT1 allele. These genomic features suggest AKT1 E17K exhibits uniquely potent oncogene addiction in these tumor types.

Non mutant-selective AKT inhibitors have demonstrated clinical activity in patients with AKT1 E17K-mutant cancers, however their clinical benefit is hindered by significant on-target WT AKT-related toxicity, including diarrhea, fatigue, hyperglycemia and rash. Selective inhibition of the AKT1 E17K variant is predicted to limit or avoid on-target WT AKT toxicities, including hyperglycemia mediated by AKT2 inhibition. Furthermore, potent and sustained suppression of AKT1 E17K enabled by a mutant-selective approach is anticipated to enhance ORR and DOR relative to WT AKT inhibitors.

ALTA2618 is an oral allosteric AKT1 E17K mutation-selective inhibitor that selectively, covalently, and potently inhibits the AKT1 E17K variant. ALTA2618 exhibits approximately 24-fold and at least 236-fold greater selectivity for AKT1 E17K over AKT1 (with WT E17) and AKT2 E17K, respectively. ALTA2618 has demonstrated potent, dose-dependent, and prolonged anti-tumor activity, including complete regressions, in AKT1 E17K-mutant HR+/HER2-negative breast, triple-negative breast, and uterine endometrial patient-derived xenograft models implanted in mice at dose levels that did not induce body weight loss or hyperglycemia.

The AKTive-001 Phase 1/1b study is evaluating ALTA2618 in patients with previously treated, locally advanced, unresectable or metastatic solid tumors with AKT1 E17K mutation.

Trial Design: ALTA2618 is a Phase 1/1b first-in-human (FIH) clinical trial of ALTA2618 in patients with advanced solid tumor malignancies with AKT1 E17K mutation. Patients will be administered ALTA2618 orally once daily (QD). Patients will be enrolled in 2 segments: a Phase 1 dose escalation segment and a Phase 1b dose expansion segment. The Phase 1 dose escalation segment will use the modified Toxicity Probability Interval methodology (mTPI-2). Three cohorts will be evaluated during the Phase 1b segment, including patients with HR+/HER2-negative breast cancer (Cohort A), gynecological cancers (Cohort B), and all other solid tumor types (Cohort C). Disease assessments per RECIST 1.1 are every 6 weeks for the first 48 weeks, then every 12 weeks.

Patients must be ≥18 years old; have an ECOG PS of ≤1; have adequate organ function; and have had no prior PI3K/mTOR inhibitors. A limited number of patients may have received prior AKT inhibitors during dose escalation. Patients should have received, are ineligible for, or declined treatment with a known overall survival benefit for their specific cancer type. For eligibility of Phase 1b Cohort A (HR+/HER2-negative breast cancer), tumors must express ER with or without co-expression of PR; HER2-negative is defined as 0/1+ on IHC, or 2+ on IHC and ISH-negative. Patients with non-HR+/HER2-negative breast cancer subtypes will be enrolled into Cohort C. Patients will be treated with ALTA2618 orally, QD, until disease progression, unacceptable adverse events, patient refusal, or death.

The main study objectives are to characterize the safety and tolerability, to evaluate the pharmacokinetics and clinical activity, and to identify the recommended doses of ALTA2618 for dose expansion.

Sites will be opened in the US, EU, Asia and Australia. Enrollment will begin in September 2024.

P4-08-25: Overall survival in patients with HR+/HER2- advanced breast cancer treated in a phase 1b trial evaluating gedatolisib in combination with palbociclib and endocrine therapy

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Background: Gedatolisib is a pan PI3K/mTOR inhibitor and was evaluated in a multicenter, open-label, phase 1b dose-escalation and dose-expansion trial in combination with palbociclib and endocrine therapy in patients with hormone receptor-positive/HER-2 negative (HR+/HER2-) breast cancer with varying categories of prior therapy (ClinicalTrials.gov NCT02684032). The primary objective of the dose-escalation cohorts was safety while the primary endpoint of the dose-expansion cohorts was investigator-assessed objective response. Median progression-free survival at 12 months was 72.1%, 54.5%, 23.6%, and 53.2% for Arms A, B, C, and D, respectively, as defined in the methods. In the dose-expansion cohorts gedatolisib triplet therapy achieved overall response rates (ORR) of 85%, 77%, 36% and 63% in Arms A, B, C and D respectively. Based on the promising ORR of the phase 1b trial, further follow-up was warranted to analyze survival outcomes. Here we report survival results of patients who received gedatolisib combined with palbociclib plus letrozole or fulvestrant.

Methods: Female patients aged at least 18 years from 17 sites across the USA with HR+/HER2- advanced breast cancer and an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1 were enrolled in the phase 1b trial. There were 2 dose-escalation arms that assessed safety of gedatolisib 180 mg/week plus standard doses of palbociclib with endocrine therapy of letrozole or fulvestrant. The dose expansion portion had 4 patient cohorts based on prior treatment history, with Arm A comprising patients with no prior systemic treatment for breast cancer, Arm B with patients with 1-2 prior endocrine therapies but no history of CDK4/6 inhibitor, and Arms C and D with patients with one or more prior lines of therapy, including a CDK4/6 inhibitor. Gedatolisib 180 mg was administered intravenously weekly in 28-day treatment cycles for Arms A-C, and on days 1, 8, and 15 for Arm D. Endocrine therapy with letrozole or fulvestrant and CDK4/6 inhibition with palbociclib were administered at standard dose. Participating sites were asked to collect patient outcome for survival analysis.

Results: As of the phase 1b study data lock on June 29, 2022, the median overall survival was not reached in any group. The sponsor has initiated overall survival follow up for all patients that will be presented at the conference which provides at least 2 years of additional follow-up time.

Conclusions: Gedatolisib in combination with palbociclib and letrozole demonstrated promising overall response rates and progression-free survival in patients with HR+/HER2-advanced breast cancer that warrants further evaluation of overall survival outcomes.\

P4-08-26:Trial in progress: First-in-human phase 1a/1b, dose-escalation/expansion study of BGB-43395 (CDK4 selective inhibitor) as monotherapy or combination therapy in Chinese patients with metastatic HR+/HER2- breast cancer & other advanced solid tumors

Jian Zhang, Zhanmin Zhang, Yuping Sun, Tao Qin, Gilbert Y. Wong, Ping Zhou, Yiran He, Zhimin Shao

Background: Dysregulation of cyclin-dependent kinase (CDK) 4 is observed in various solid tumors. CDK4/6 inhibitors provide substantial clinical benefit; however, pts with advanced or metastatic HR+/HER2− breast cancer (BC) eventually develop resistance and may experience toxicities associated with current treatments. BGB-43395 is a potent, selective, orally bioavailable CDK4 inhibitor, with preclinical evidence showing antitumor activity and substantial selectivity for CDK4 over CDK6, thus minimizing off-target toxicity and potentially toxicity-related dose reduction/discontinuations, and therefore supporting further evaluation in pts.

Methods: This phase 1a/1b, open-label, multicenter study is evaluating the safety, tolerability, PK, pharmacodynamics (PD), and preliminary antitumor activity of BGB-43395 given orally as monotherapy or in combination with fulvestrant, letrozole, or other combination partners in Chinese pts with advanced or metastatic solid tumors, including HR+/HER2− BC (NCT06253195). In the dose-escalation phase (phase 1a), sequential cohorts of pts with advanced solid tumors will receive increasing dose levels of BGB-43395 given orally as monotherapy (Part A) and cohorts of pts with 2L+ HR+/HER2− BC will receive increasing dose levels of BGB-43395 in combination with either fulvestrant (Part B) or letrozole (Part C). In the dose-expansion phase (phase 1b), pts with advanced or metastatic HR+/HER2− BC will receive the recommended dose for expansion (RDFE) of BGB-43395 in combination with fulvestrant.

Eligible pts are ≥18 years of age with histologically or cytologically confirmed locally advanced or metastatic solid tumors associated with CDK4 dependency who have received prior standard-of-care therapy, including ≥1 line of treatment for locally advanced or metastatic disease and prior endocrine therapy and a CDK4/6 inhibitor in either the adjuvant or locally advanced or metastatic setting for HR+/HER2− BC, or ≥2 lines of HER2-targeted therapy for pts with HR+/HER2+ BC (phase 1a); locally advanced or metastatic HR+/HER2− BC in pts who have received ≥1 line of treatment for locally advanced or metastatic disease and prior endocrine therapy and a CDK4/6 inhibitor (phase 1b); and ECOG PS ≤1.

For phase 1a, the primary objectives are to assess the safety and tolerability of BGB-43395 monotherapy or as part of combination therapy, and to determine the maximum tolerated dose, maximum administered dose and RDFE; secondary objectives are to assess preliminary antitumor activity (ORR, duration of response [DoR] and time to response [TTR] as assessed by the investigator per RECIST v1.1) and the PK of BGB-43395. Exploratory endpoints are PFS as assessed per investigator, disease control rate (DCR) and clinical benefit rate (CBR) per RECIST v1.1, PD, and assessment of biomarkers associated

with response. For phase 1b, the primary objective is to assess the antitumor activity of BGB-43395 (ORR assessed by the investigator); secondary objectives are to further assess the antitumor activity (DoR, TTR, DCR, CBR, and PFS assessed by the investigator), safety and tolerability, and PK of BGB-43395. Exploratory endpoints are overall survival and potential biomarkers associated with response. As of May 28, 2024, the study is currently enrolling pts, with five pts currently dosed in the dose-escalation phase across 12 sites in China.

P4-08-27: PMD-026, a First-in-Class RSK Inhibitor, Overcomes Acquired Resistance to CDK4/6 Inhibitors or Aromatase Inhibitors in HR+/HER2- Breast Cancer Preclinical Models

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Background: RSK2 (90 kDa ribosomal S6 kinase 2) is a kinase implicated in developing hormone receptor positive (HR+) breast cancer. Additionally, RSK1, RSK3, and RSK4 promote breast cancer metastases and resistance. Upon activation, RSKs translocate from the cytoplasm to the nucleus, directly binding to the estrogen receptor (ER) and Y-box binding protein 1 (YB-1), subsequently promoting tumor growth. Specifically, nuclear RSK2 leads to the development of ER+ breast cancer in mice. RSK2 binding occurs outside the common ER mutational hotspots on the activation function 2 (AF-2) ligand binding domain, triggering a conformation change in ER to promote transcription uniquely. Thus, we hypothesize that targeting RSK2 alongside endocrine therapy improves patient outcomes independent of ER mutations. Its emerging role in resistance is underscored by a study showing that RSK2 is the most highly induced protein in the MAPK pathway following the development of acquired resistance to the aromatase inhibitor letrozole. Invariably, patients will progress on CDK4/6 inhibitors (CDK4/6i) combined with aromatase inhibitors in the first-line setting. In the second-line setting, fulvestrant as a single agent only delivers a median progression-free survival (PFS) of 2.35 months and an overall response rate of 5.9% in patients. Notably, PMD-026, our first-in-class, oral small molecule pan-RSK inhibitor, provided 4.8 months of PFS as a single agent in RSK2+ patients who received five prior lines of therapy, including CDK4/6i and endocrine therapy, in our exploratory Phase 1/1b clinical trial. This study addresses the potential of co-targeting RSK and ER as a novel strategy to overcome resistance to CDK4/6i and/or aromatase inhibitors.
Results: By querying the METABRIC database, we show RSK2 and YB-1 are strongly correlated in primary HR+ ($p < 0.0001$, $n = 1356$ patients), as well as triple negative ($p = 0.03$, $n = 253$ patients) breast cancers. Given that RSK/YB-1 regulates the G2/M phase of the cancer cell cycle, PMD-026 could potentially overcome CDK4/6i- resistance mechanisms that mount at G1/S. In patient-derived xenografts (PDx) that progressed on CDK4/6i or endocrine therapies, RSK2 was highly expressed in 69% (11/16) of the models. Therefore, we evaluated PMD-026 in the CDK4/6 resistant cell lines, T47D-IBR and MCF-7-IBR, both of which are ESR1 WT. PMD-026 blocked RSK signaling and suppressed pYB-1S102 in

parental T47D and T47D-IBR cells, leading to tumor cell death. In the MCF-7-IBR model, PMD-026 combined with fulvestrant or oral selective estrogen receptor degraders (SERDs) consistently demonstrated a high degree of synergy, indicating a class effect. These models had elevated pERK and Cyclin E expression, commonly elevated in CDK4/6i resistance, suggesting dysregulation of the G1/S cell cycle checkpoint can be blocked by PMD-026. Next, we addressed the opportunity to overcome aromatase resistance using PMD-026 in PDx models. Gene expression analysis of primary breast cancer PDx models indicated high expression of RSK2 mRNA in 63% (65/104) of the models screened. The high RSK2 expressing HR+/HER2- BR9469 PDx model was selected from a patient who had progressed on an aromatase inhibitor. This model, which has ESR1 (Y537S), p53, BRCA1/2, and PIK3CA mutations, was inhibited by PMD-026 in a dose-dependent manner.
Conclusion: Inhibiting the RSK pathway with PMD-026 in combination with endocrine therapies shows promising potential in overcoming resistance to CDK4/6 inhibitors. The synergistic effect observed with PMD-026 and fulvestrant supports Dauntless-1, our newly launched Phase 2 clinical trial (NCT04115306) for second-line patients with RSK2+ tumors. This trial is for patients who have progressed on one prior line of CDK4/6i and aromatase inhibitors.

P4-08-28: Results of the Dose-Escalation Cohort of a Phase 1 Trial of Intratumoral HER2- and HER3-Primed Dendritic Cells Injections for the Treatment of Early-Stage TNBC and HR Low Positive Breast Cancer. DecipHER trial

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Background: Patients (pts) with breast cancer (BC) harboring low expression of hormone receptors (HR) and human epidermal growth factor receptor-2 (HER2) have poorer outcomes compored to other subsets of BC. Results from the KEYNOTE-522 trial showed that activation of the immune system using a PD1/PD-L1 approach leads clinically meaningful improvement in the outcomes of patients with these high-risk tumors. Dendritic cells (DCs) are antigen presenting cells which are pivotal for robust cytotoxic responses via broader activation of the adaptive immune system.
Methods: DecipHER is a dose-escalation, dose-expansion phase 1 trial designed to assess the safety and preliminary effiacy of autologous HER2- and HER3-primed DCs in combination with KEYNOTE-522 regimen in a maximum of 30 pts. Pts with clinical stage cT1-cN1/2 or cT2-4cN0/2, HR < 20, HER2-negative BCs are eligible. Pts with inflammatory BC and uncontrolled immune-mediated diseases are excluded. After collection through apheresis, autologous DCs are primed against 6 HER2 and 8 HER3 immunogenic peptides. Pts receive alternating US-guided intratumoral HER2 and HER3 DCs injections administered twice a week for 8 doses starting 2 weeks prior to neoadjuvant KEYNOTE-522 regimen. The

dose-escalation phase of the study had a classic 3+3 design (ie DL1-3 [10-20, 30-50, 80-100 million], maximum n=18). Additionally, 12 pts will be treated at the maximum tolerated dose (MTD) in the dose-expansion cohort. Dose-limiting toxicities (DLT) were defined as grade 3 or higher non-hematologic or hematologic adserve events (AEs) thought to be at least possibly related to DCs; any grade 4 nausea, vomiting or diarrhea [or grade 3 if duration > 3 days]) during the 5 weeks following treatment initiation. Secondary endpoints include absolute risk of AEs, pathological complete response (PCR) and recurrence-free survival. Tumor tissue, blood and stool samples are being collected for correlative analyses.
Results: A total of 12 pts (6 on DL3) were enrolled between 08/2022 and 01/2024. The median age was 51.5 (35-71) and 25% of pts were black; 91% had grade 3, 41.7% had T3 and 75% had N1 BCs. One patient received only 2 HER2- and 4 HER3-primed DCs due to low cell yeild. Grade 1 and 2 AEs that were at least possibiliy related to DCs (>10%) were headache (66.7%) chills (50%), fatigue (33.3%), flue-like symptoms (33.5%), fever (25%), nausea (16.7%) and pain (16.7%). No DC-associated grade 3 or higher AEs were observed. None of the toxicities met the definition of DLT. For DL1, 2 out of 3 pts had an SAE (i. cholelithiasis complicated by sepsis and ii. pneumonitis and syncope; 5 and 14 weeks after last DC injection; respectively). No SAEs were observed on dose levels 2 or 3. Immune-related AEs were pneumonitis (1pt), hypothyrodism and adrenal insuficiency (1 pt), hyperthyroidism (1pt). pCR was observed in 5 out of 10 evaluation pts. Base on thees findings, DL3 (80-100 million) was selected for the dose-expansion cohort.
Conclusion: Intratumoral DCs in combination with standard neoadjuvant chemotherapy and pembrolizumab were well tolerated in pts with high-risk HR <20, HER2-negative BCs. The dose-expansion cohort portion of this trial is ongoing; correlative analyses will follow. The study is open at H. Lee Moffitt Cancer Center. Clinical trial information NCT05504707.
Funding: Shulas' foundation.

P4-08-29: Efficacy and safety of larotrectinib in patients with TRK fusion breast cancer: an updated analysis

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Background: Larotrectinib is the first-in-class, highly selective, central nervous system (CNS)-active TRK inhibitor approved for tumor-agnostic use in patients with TRK fusion cancer; approval was based on a robust and durable objective response rate in both adult and pediatric patients with various cancers. Here, we report long-term efficacy and safety data in patients with TRK fusion breast cancer (BC).
Methods: Patients with TRK fusion BC treated with larotrectinib were identified from 2 clinical trials (NCT02576431 [NAVIGATE] and NCT02637687 [SCOUT]). Most patients received larotrectinib 100 mg twice daily. Responses were assessed by an independent review committee (IRC) per Response Evaluation Criteria in Solid Tumours v1.1. The data cutoff date was July 20, 2023.

Results: At data cutoff, 16 patients were identified, 15 of whom were eligible for IRC assessment. Median age was 54 years (range 10–70). At enrollment, 2 (13%) patients had locally advanced disease and 14 (88%) had metastatic disease. Six (38%) patients had secretory carcinoma, 2 of whom were male; 10 (63%) patients had non-secretory carcinoma. NTRK gene fusions were identified by next-generation sequencing (NGS), fluorescence in situ hybridization, and an unknown method in 13 (81%), 2 (13%), and 1 (6%) patients, respectively. Of the 6 patients with secretory carcinoma, 4 had triple-negative BC (TNBC) and 2 had estrogen receptor-positive (ER+) / human epidermal growth factor receptor 2 (HER2) negative tumors. Of the 10 patients with non-secretory carcinoma, 4 had TNBC, 2 had ER+/HER2-equivocal (eq) BC, 2 had ER+/HER2 negative BC, 1 had ER–/HER2eq BC and 1 had ER+/HER2+ BC.
Three patients with non-secretory carcinoma had known CNS metastases at baseline. Overall, 4, 2, and 10 patients had 0, 1, or ≥2 lines of prior systemic therapy, respectively; 3 of the patients with secretory carcinoma were treatment-naïve.
The overall response rate (ORR) for the IRC-eligible patients (n=15) was 53% (95% confidence interval [CI] 27–79): 4 (27%) patients had complete responses (CRs; including 1 pathological CR [pCR]), 4 (27%) had partial responses (PRs), 3 (20%) had stable disease (SD), and 3 (20%) had progressive disease (PD); response in 1 patient (7%) was not evaluable. ORR for patients with secretory carcinoma (n=6) was 83% (95% CI 36–100): 3 (50%) patients had CRs (including 1 pCR), 2 (33%) had PRs, and 1 (17%) had SD. ORR for patients with non-secretory carcinoma (n=9) was 33% (95% CI 7–70): 1 (11%) patient had a CR, 2 (22%) had PRs, 2 (22%) had SD, and 3 (33%) had PD; response in 1 patient (11%) was not evaluable. In all patients, median time to response was 1.7 months (range 0.9–5.6). Median duration of response (DoR) was not reached (95% CI 7–not estimable [NE]); median follow-up was 43 months. Median progression-free survival (PFS) was 9 months (95% CI 2–NE); median follow-up was 44 months. Median overall survival (OS) was 21 months (95% CI 8–NE); median follow-up was 47 months. The 4-year rates for DoR, PFS, and OS were 73% (95% CI 41–100), 38% (95% CI 13–63), and 38% (95% CI 11–65), respectively. Treatment duration was 0–60+ months. Treatment-related adverse events (TRAEs) were mostly Grade 1/2. Grade 3/4 TRAEs occurred in 3 (19%) patients: hepatic cytolysis and hepatitis, both in 1 patient; paresthesia and thrombocytopenia in 1 patient each. There were no treatment discontinuations due to TRAEs.
Conclusions: Larotrectinib demonstrates rapid and durable responses and a favorable safety profile in patients with TRK fusion secretory or non-secretory BC. These results support the wider adoption of NGS panels that include NTRK gene fusions to identify patients who might benefit from TRK inhibitor therapy.

P4-08-30: Preclinical Characterization and Safety of First-in-Class, brain penetrant ALC1 Inhibitor EIS-12656 in HRD cancers

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EIS-12656, a first-in-class ALC1 inhibitor, has shown promising antitumor activity in preclinical studies, aimed at effective cancer management. Our inhibitor specifically targets ALC1, a crucial chromatin remodeler activated by PARP within the DNA damage response (DDR) context. This study focuses on the comprehensive preclinical characterization and safety evaluation of EIS-12656, currently in a Phase I/II clinical trial. Biochemically, EIS-12656 is an allosteric, activation dependent inhibitor of ALC1. Its inhibition results in the trapping of ALC1 alongside PARP1 and PARP2 at sites of DNA damage.
EIS-12656 demonstrated selective cytotoxicity against multiple human cancer cell lines deficient in homologous recombination, with IC50 values in the nanomolar range. This selective targeting of PARP1, PARP2, and ALC1 at sites of DNA damage resulted in G2/M arrest. In vivo studies indicated that EIS-12656 has monotherapy efficacy outcompeting best in class PARP inhibitors. EIS-12656 is well-tolerated at doses up to 1.000mg/kg in both rodent and non-rodent species and displays no significant adverse effects on body weight, food consumption, or clinical observations. Hematological and clinical chemistry analyses revealed no deviations from normal ranges. Histopathological examinations showed no drug-related toxicities. EIS-12656 is well absorbed, distributed, brain penetrant and accumulates in the tumor. Pharmacokinetic analysis indicated dose-proportional exposure and a half-life suitable for once-daily oral administration, providing a strong rationale for the currently recruiting Phase I/II clinical trial to further assess EIS-12656's safety and efficacy in biomarker selected oncology patients.

P4-09-01: Contrast-enhanced ultrasound traced secondary sentinel lymph nodes in early-stage breast cancer to streamline sentinel lymph node biopsy----a preliminary study

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Background: Sentinel lymph node biopsy(SLNB) has replaced axillary lymph node dissection (ALND) as the standard method for axillary staging in clinically node-negative patients. It avoids the complications associated with ALND and allows assessment of nodal status in patients with clinically node-negative breast cancer. Dual agent tracing method of SLNs using methylene blue dye and radioactive sulphur colloid showed excellent detection rates, however the SLNs picked out were not only the first stop, they were included both the first stop(SLNs) and stops thereafter(secondary SLNs, SSLNs). In this study, we conducted contrast-enhanced ultrasound (CEUS) sentinel lymphnode mapping method, to detect a more accurate and visuallizable method of tracing the very first stop of lymphnode. SLNs and SSLNs were labeled preoperatively with a hook wire and skin surface marking, aiming to streamline sentinel lymph node biopsy (SLNB).
Methods: From December 2019 to June 2024, we prospectively collected data of 66 patients with early breast cancer scheduled for surgery in our hospital. Dual tracing method was conducted to detect SLNs on the all patients. In addition, we also conducted CEUS preoperatively, to trace SLNs, SSLNs, and lymphatic draining vessels. All tracing data were

recorded by sketches and marked on patient's skin. Selected patients were labeled with a novel hook wire for SLN. CEUS mapping for SLNs/SSLNs and SLNB surgery were performed by a fixed ultrasound and surgical team. All lymph nodes marked were picked out and recorded with clinical data, such as blue dye result, radioactive counting, et al. Pathological examination on all lymph nodes were made to determine metastatic state. Statistical analysis were made using SPSS 26.0. Non-parametric paired sample wilcoxon test was performed for SLN during and before operation, and the count data rate (%) was indicated by $\chi 2$ test. $P < 0.05$ was considered statistically significant.

Results: 66 patients underwent dual tracing SLNB identified a total of 290 SLNs, among which, 15 SLNs were found positive by pathological biopsy. CEUS method detected a total of 87 SLNs, which was fewer than the numbers detected by the dual tracing method ($Z=-3.076$, $P<0.05$). The detection rate of positive SLNs was 13.80%, higher than that of the dual tracing method (positive SLN rate as 5.17%) ($\chi 2=7.480$, $P<0.05$). The concordance rate between the two methods was 98.48%, on the first lymph node detected, which were the actual SLN. Following the SLNs, all SSLNs displayed by CEUS were found within the markings of dual tracing method, with a concordance rate of 100%. CEUS cumulatively detected 110 SSLNs, with a detection rate of 90.90%, and a positive SSLN detection rate of 2.73%. The concordance rate between all lymph nodes marked by CEUS and those marked by the combined method was 99.49%, and in all cases, there were no instances of positive SSLNs identified by CEUS while the SLNs were negative, indicating by our study population, no skip metastasis occurred.

Conclusions: Preoperative CEUS mapping of SLNs shows a high concordance with SLNs traced by dual tracing method using methylene blue dye and radioactive sulphur colloid. It can differentiate SSLNs from SLNs, and provide a possibility of deescalating axillary SLNB by avoiding SSLN dissection.

P4-09-02: Multiparametric MRI and Transfer Learning for Predicting Positive Margins in Breast-Conserving Surgery: A Multi-Center Study

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Background: Breast-conserving surgery (BCS) maintains high appearance satisfaction while minimizing postoperative complications. However, positive surgical margin is the most important risk factor to cause local recurrence or recurrence in breast and increase the reoperation rate, even following by additional treatments such as radiotherapy during BCS. Preoperative predictive tools are urgently needed to optimize surgical decisions in BCS. In this study, we developed a series of Base- and Transfer-MMRMs based on multiparameter magnetic resonance imaging (mpMRI) and Radiomics for the preoperative prediction of positive surgical margins in BCS across multiple centers.

Methods: This retrospective study enrolled 444 patients who received BCS in three hospitals from January 2019 to April 2024 in China. The patients were divided into four

cohorts: SUMCCH-1 (from Cancer Hospital of Shantou University Medical College, n = 158), SUMCCH-2 (from Cancer Hospital of Shantou University Medical College, n = 60), XAH (from Xiang'an Hospital of Xiamen University, n = 52), and YNCH (from Yunnan Cancer Hospital, n = 174). All patients had preoperative breast MRI scans before BCS, and surgical margin status was determined through pathological examination. During the model construction stage, clinicopathological and Radiomics features of breast MRI (including DCE, T2WI, and DWI sequences) were extracted and selected via t-test and least absolute shrinkage and selection operator (LASSO) logistic regression. Eight machine learning classifiers were used for model development, and transfer learning (TL) was performed to improve the model's generalization ability. The performance of the Base- and Transfer-MMRMs were assessed using metrics such as area under the receiver operating characteristic curve (AUC), accuracy, sensitivity, and specificity.
RESULTS: The rates of positive surgical margins in BCS varied significantly: 26.6% in SUMCCH-1, 20.0% in SUMCCH-2, 34.6% in XAH, and 9.8% in YNCH. The Base-MMRM achieved an AUC of 0.889 in the internal test cohort and 0.771 in the validation cohort after being trained on SUMCCH-1 and validated on SUMCCH-2 but performed poorly in external centers. TL approach significantly improved AUC in both XAH (from 0.533 to 0.902) and YNCH (from 0.359 to 0.855) cohorts compared with the Base-MMRM. Furthermore, the Shapley additive explanation (SHAP) method, along with subgroup analysis, enhanced the interpretability of this model.
Conclusion: This multi-center study developed a precise preoperative model for predicting the risk of positive surgical margins in BCS by utilizing MRI and clinicopathological data. The TL could enhance the predictive accuracy across external centers, making it easier to implement the model in different hospitals.

P4-09-03: Reoperation Rates Following Breast-Conserving Surgery in a Brazilian Cohort

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Introduction: In early breast cancer, Breast-conserving surgery (BCS) should be performed as the first option whenever is suitable, as it provides the same overall survival than mastectomy with favorable aesthetics results. However, the risks of a positive margin will demand a further surgery, that may affect the cosmesis and add financial and emotional distress. These risks underscore the importance of effective strategies to reduce the rate of reoperation. There is a knowledge gap about the reoperation rate after breast-conserving surgery and adjuvant radiotherapy in Brazil.Objective: Analyze the reoperation rate after BCS in Brazil, aiming to identify predictive factors.Methods: This retrospective multicenter cohort study included breast cancer patients from public and private hospitals in Brazil. The

study focused on patients who underwent breast-conserving surgery and adjuvant radiotherapy between January 2016 and December 2022, with cancer stages ranging from I to III. Patients aged under 65 who underwent conservative surgery without radiotherapy were excluded from the study. Statistical analyses were performed using the R application for Windows, version 4.4.1. The association between independent variables and the outcome was assessed using logistic regression to evaluate the relationship between each potential risk factor (independent variable) and the outcome "Reoperation." Odds ratios (OR) and respective 95% Confidence Intervals (CI) for each variable were presented, along with the p-value obtained from the Wald test. Given the small number of reoperations, multiple logistic regression was not performed due to low statistical power. Values of $p<0.05$ were considered statistically significant. This study was approved by the Research Ethics Committee (n°.6,907,736) in accordance with the ethical principles established by the National Health Council.Results: Of the 705 patients included in the study, 40 (5.7%) underwent reoperation. This included 10 out of 72 patients (13.9%) from public hospitals and 30 out of 631 patients (4.8%) from the private sector. Among these patients, 557 (79.6%) underwent Wide Local excision (WLE) and 141 (20.4%) underwent therapeutic mammoplasty (oncoplastic surgery). Of the 40 patients who required reoperation, 32 (80%) had positive margins, 26 (65%) underwent frozen section and/or margin shaving, while 12 (30%) did not undergo frozen section or margin shaving. Additionally, 25 (62.5%) showed the presence of tumor cells in the surgical specimen. Regarding the type of reoperation performed, there were 24 re-excision, 10 nipple-sparing mastectomies, 2 skin-sparing mastectomies, and 1 modified radical mastectomy. In the univariate analysis, the risk of reoperation was higher in patients with positive margins (OR 23.53; 95% CI 9.79-69.94; $p<0.001$), those treated in public hospitals (OR 3.23; 95% CI 1.44-6.72; $p<0.003$), and those with multifocal tumors (OR 2.92; 95% CI 1.11-6.79; $p<0.019$).Conclusion: This contemporary Brazilian study demonstrated low reoperation rates after breast-conserving surgery (BCS) at 5.7%, regardless of the use of strategies such as frozen section, margin shaving, or therapeutic mammoplasty. Further studies with larger cohorts are needed to better understand this scenario.

P4-09-04: Vacuum-assisted biopsy (VAB) versus standard breast surgery among patients with pCR after neoadjuvant therapy.

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Introduction. Nowadays, in patients with breast cancer (BC) and complete pathological response (pCR) after neoadjuvant systemic therapy (NST) role of surgery is uncertain. Vacuum-assisted biopsy (VAB) of the tumor bed in breast cancer has shown promising results as a minimally invasive method for diagnosing pCR. In a NCT04293796 clinical trial we are investigating refusion of standard breast cancer surgery among patients with pCR,

which was confirmed using VAB. This paper presents a retrospective analysis of treatment of patients with triple-negative (TNBC) and HER2-positive breast cancer who achieved pCR after NST, depending on different types of invasive breast surgery, including data from patients who underwent VAB alone without subsequent breast surgery.
Materials and Methods. We retrospectively analyzed 3247 patients who underwent surgery in the department of breast tumors at the National Medical Research Center of Oncology named after. N.N. Petrov from January 01, 2021 until July 01, 2023. Patients were included if they had unifocal invasive HER2+ and TNBC (cT1–2N0–1M0), who had histologically confirmed pathological complete response (ypT0N0) after NST. Patients with TNBC received 4 cycles of AC q21d followed by 12 cycles of weekly paclitaxel and carboplatin AUC 2.0. HER2+ patients received dual anti-HER2 blockade with chemotherapy (TCHP or AC-DHP regimen). Patients were not included in the analysis if an intraductal component was identified by core-biopsy, as well as if they carried germline mutations in the BRCA1/2 genes. All patients were divided into three groups: breast-conserving surgery (BCS) group, the mastectomy (ME) group, and the VAB group. Primary endpoint was 2-year ipsilateral breast tumor recurrence-free survival (IBTR-FS). Secondary endpoint was 2-year disease-free survival (DFS).
Results. Of the 3247 patients analyzed, 81 patients who met the inclusion criteria were included in the final analysis. There were 39 patients in the BCS group, 19 patients in the ME group, and 23 patients in the VAB group. Median follow-up was 24 months. Median survival was not reached for any endpoint. 2-year IBTR-FS was 100% in the BCS group vs. 100% in the ME group vs. 91.3% in the VAB group [p=.159]. 2-year disease-free survival (DFS) was 97.4% in the BCS group vs 94.7% in the ME group vs 87.0% in the VAB group [p=.396]. The hazard ratio (HR) for recurrence did not differ depending on the surgical procedure performed. When measuring HR adjusted for lymph node status (cN) and disease stage, there was a statistically significantly higher risk in the VAB group [HR adjusted for cN: 12.236, (95% CI, 1.163 - 128.773), p = 0.037; HR adjusted for stage: 17.071, (95% CI, 1.255 – 232.173), p=0.033].
Conclusion. We need further observational studies and large randomized prospective trials to determine the safety of VAB as a method for de-escalation of breast cancer surgery.

P4-09-05: Trends in mastectomy for non-metastatic breast cancer patients in an institution with limited resources

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Introduction: Breast-conserving surgery (BCS) is the preferred surgical treatment for early-stage breast cancer. In recent years, however, an increase in mastectomies has been observed in developed countries. The advent of genetic knowledge, the possibility of immediate reconstruction, and the false impression of greater safety have been associated with the increase in mastectomies. This trend, however, has not yet been adequately

evaluated among breast healthcare in low- and middle-income countries.
Method: This is a retrospective study of patients undergoing surgery for non-metastatic breast cancer between 2012 and 2019 at the General Hospital of Fortaleza (HGF), an institution that exclusively treats patients from the public health system in Brazil (SUS). In Brazil, 80% of the population's healthcare is provided through the SUS. The main objective of the study was to evaluate the rates of mastectomy during the period, with immediate reconstruction or without reconstruction, as well as BCS. The chi-square test, with Bonferroni adjustment, was applied to the relative frequency of procedures performed to test the statistical significance in the evolution of surgery frequencies over the years. The research was initiated after approval by the Research Ethics Committee of the institution (CAAE 29325720.7.0000.5040).
Results: After applying the study's inclusion criteria, 805 patients undergoing surgical treatment for non-metastatic breast cancer were included for analysis, with an average of 100 surgeries per year (range 85-118) during the study period. Mastectomy was performed in 552 cases (68.57%), while 253 patients underwent BCS (31.42%). Among patients undergoing mastectomy, 181 (32.78%) had immediate reconstruction, with the highest proportion using implants (92.26%). Evaluating the proportions of each type of surgery during the study period, a statistically significant change was found only for breast-conserving surgery (p=0.001; Bonferroni adjustment: p=0.003), compared to mastectomy without reconstruction (p=0.623; Bonferroni adjustment: p=0.299) and mastectomy with reconstruction (p=0.591; Bonferroni adjustment: p=0.663).
Conclusion: There was no increase in mastectomy rates, with and without immediate reconstruction, over the years, but a trend of increasing BCS. More studies are needed for a better understanding of this trend in locations with limited access to healthcare.

P4-09-06: Oncologic Outcomes of Breast-Conserving Surgery versus Mastectomy after Neoadjuvant Chemotherapy: Results from a Contemporary Multicenter Cohort

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Introduction: Previous studies have shown a higher rate of local recurrence (LR) in breast-conserving surgery (BCS) after neoadjuvant chemotherapy (NAC) without impacting survival. However, currently, the use of more effective systemic treatment, understanding of disease biology, and improved imaging methods, with lesion marking before NAC, may impact local control.
Material and Methods: The main objective of this study was to evaluate oncologic outcomes: local recurrence (LR), distant recurrence (DR), and death in patients with non-metastatic breast cancer, undergoing BCS compared to mastectomy after NAC, through a contemporary

multicenter retrospective cohort study in Brazil involving public institutions in two different Brazilian regions with limited access to resources. Between 2013 and 2023, at the General Hospital of Fortaleza (Northeast region, HDI 0.754) and the Pontifical Catholic University of Rio Grande do Sul (South region, HDI 0.867) were evaluated. Disease-free survival (DFS) curves, DFS by tumor stage (T1, T2, T3, T4), distant recurrence-free survival (DRFS), as well as overall survival (OS), were developed using the Kaplan-Meier method and Cox proportional hazards model. Finally, multivariate logistic regression was performed to assess the association of clinical-demographic and treatment characteristics with LR. The significance level adopted was 5%.
Results: Patients undergoing NAC (n=365), between 2013 and 2023, at the General Hospital of Fortaleza and the Pontifical Catholic University of Rio Grande do Sul (PUC/RS) were evaluated, with 165 women undergoing mastectomy and 200 undergoing BCS, constituting the analysis groups. In the mastectomy group, there were more cases aged over 70 years (12.7% versus 7%; p=0.02) and T4b tumors (16.4% versus 4.5%; p=0.0003), while in the BCS group, there were more patients with negative axilla (42% versus 31.5%; p=0.02). After a mean follow-up of 65 months (4-124), 18 LR were observed, with 8 cases (4.8%) in the mastectomy group and 10 events in the BCS group (5.0%), with no significant difference (p=0.95), as there were no differences in DR (mastectomy 10.9% [18/165] versus BCS 9% [18/200]; p=0.58). On the other hand, more deaths were observed in the mastectomy group (8.5% [14/165] versus 3% [6/200]; p=0.03). DRFS showed no significant differences between the groups (91% BCS versus 89% HR: 1.25, 0.65-2.42; p=0.4). However, when OS was evaluated, patients undergoing BCS had better outcomes compared to mastectomy (97% versus 91.5%; HR: 2.62; 1.06-6.69; p=0.03). The estimated 10-year DFS stratified by tumor stage showed no significant difference, except in T4 disease which showed a higher risk for the mastectomy group (81.8% versus 94.5% HR: 2.86, 1.54-5.30; p=0.0008). In multivariate analysis, T3/T4 staging (OR: 4.37, 1.03-21.91; p= 0.04) and axillary dissection (OR: 5.11, 1.14-35.52; p= 0.04) were associated with LR in BCS.
Conclusion: In this contemporary cohort of patients undergoing NAC, that received treatment in public institutions with limited access to resources, BCS did not present worse oncologic outcomes compared to mastectomy. Further studies are needed to confirm these findings.

P4-09-07: Clinical Outcomes of Reverse-Sequence Endoscopic Nipple-Sparing Mastectomy with Direct-to-Implant Breast Reconstruction in Breast Cancer Patients with Large or Severe Ptotic Breast: A Single-center Prospective Cohort Study

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Background: Nipple Sparing Mastectomy (NSM) with direct-to-implant breast reconstruction (DIBR) is not recommended or even considered contraindicated in breast cancer patients with large or severe ptotic breasts (LSPB). The commonly used NSM

methods for the patients are often based on the Wise-pattern skin-reducing technique, which have some shortcomings in surgical safety and breast aesthetics. The reverse-sequence endoscopic NSM (R-E-NSM) with DIBR has shown promising clinical outcomes for patients with LSPB, and aesthetic outcomes can be enhanced by the combination of the air inflation adjustment technique (AIAT). This study aimed to evaluate the safety and aesthetic outcomes of the R-E-NSM with DIBR and AIAT in breast cancer patients with LSPB and to investigate the feasibility of NSM with DIBR in the patients.
Methods: The prospective study enrolled breast cancer patients who underwent R-E-NSM with DIBR from September 2020 to August 2024 at a single institution. According to whether or not the suprasternal notch-to-nipple distance was over 25 cm, all patients were categorized into the LSPB group and the non-LSPB (NLSBP) group. The propensity score matching (PSM) was used to balance the baseline characteristics between the two groups. Then, the surgical safety, aesthetic outcomes, and oncologic safety were compared between the two groups.
Results: A total of 562 breast cancer patients were eligible for the study, including 98 patients in the LSPB group and 464 in the NLSPB group. After PSM, 343 patients were retained, with 87 patients in the LSPB group (mean [SD] age: 42.2[9.3] years, median [IQR] follow-up time: 20.9 [13.6, 32.9] months) and 256 in the NLSPB group (mean [SD] age: 42.4[8.9] years, median [IQR] follow-up time: 22.3 [10.3, 31.9] months). The mastectomy weight of patients in the LSPB group was significantly higher than that in the NLSPB group (573.1±146.2g vs. 337.3±105.5g, $P < 0.001$). After PSM, there were no significant differences in any complications (27.6% vs. 21.9%, P = 0.276), major complications (4.6% vs. 2.7%, P = 0.617), minor complications (24.1% vs. 20.3%, P = 0.451) and implant complications (20.7% vs. 25.0%, P=0.415) between the LSPB and NLSPB groups. The Ueda scores and Breast-Q were insignificantly different between the two groups after PSM(all $P > 0.05$). Without AIAT, patients in the LSPB group had relatively inferior Ueda scores to those in the NLSPB group (proportions of “excellent” 37.9% vs. 56.1%), while with AIAT, the difference decreased (proportions of “excellent” 72.4% vs. 78.1%), through both of them have no significant difference. In the LSPB group, the Ueda scores in patients undergoing AIAT were significantly better than those without AIAT (proportions of “excellent” 72.4% vs. 37.9%, P=0.002). The local recurrence-free survival (P = 0.205), distant metastasis-free survival (P = 0.903), and disease-free survival (P = 0.532) were not significantly different between the two groups.
Conclusions: R-E-NSM with DIBR can bring favorable surgical safety, aesthetic outcomes, and oncologic safety for patients with LSPB, similar to patients with NLSPB. AIAT helps improve breast aesthetics for patients with LSPB. We suggest that LSPB is not a contraindication to endoscopic breast reconstruction, which is worthy of clinical promotion and offers a new or even superior option for breast cancer patients with LSPB beyond the NSM with Wise-pattern skin-reducing technique.
Keywords: Breast cancer, Large breast, Severe Breast Ptosis, Reverse-sequence endoscopic nipple-sparing mastectomy, Direct-to-implant breast reconstruction， air inflation adjustment technique.

P4-09-08: Outcomes after Autologous Breast Reconstruction for Triple-Negative Breast Cancer among Black and White Patients

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Introduction: Triple-negative breast cancer (TNBC) is an aggressive subtype disproportionately diagnosed among Black women as compared to White women and for which multidisciplinary treatment and coordination among surgeons, medical oncologists, and radiation oncologists is especially important. Autologous breast reconstruction after mastectomy, an increasingly sought surgical option for patients with TNBC, is associated with significant aesthetic and psychological benefits. However, autologous breast reconstruction is technically challenging and while rare, postoperative complications can potentially confer significant morbidity, delay next steps in treatment, and even result in death. It is unclear, however, whether receipt of autologous reconstruction is associated with adverse oncologic outcomes for patients with TNBC and whether previously observed racial disparities in receipt of and outcomes after post-mastectomy reconstruction are also observed among patients with TNBC. We sought to examine and compare overall survival and recurrence rates after autologous breast reconstruction among Black and White patients with TNBC.
Methods: We identified females ≥18 yo diagnosed with invasive triple-negative carcinoma at our institution between 2007 and 2017 and who underwent autologous reconstruction. We collected data on demographics, medical and surgical history, breast tumor data, systemic and radiation treatment composition and sequence, intraoperative details, reconstruction complication and revision information, recurrence, and mortality. Cox proportional models were used to estimate hazard ratios and 95% confidence intervals of the potential recurrence and mortality differences by race. Survival times were measured from the date of surgery to date of death or date of last contact.
Results: There were 135 patients included in the analysis: 34 Black, 89 White, 4 Asian/Pacific Islander, and 8 with unknown race/ethnicity. A majority of patients had Stage II disease: clinical stages 1 (42, 32%), 2 (65, 51%), 3 (15, 11%), and 4 (3, 2%). There were 47 (35%) patients with nodal involvement. More patients received neoadjuvant therapy first (59, 56%) compared to those who received surgery first (46, 44%). Median time to cancer recurrence and mortality was 5.97 and 6.45 years, respectively. Mean age at diagnosis was 48.53 years while mean body mass index (BMI) was 29.78 kg/m2. There were 87 (64%) patients who never smoked, 6 (4%) who currently smoked and 42 (31%) who smoked prior to surgery. Transverse rectus abdominis myocutaneous flap (TRAM) (61% for left breast and 58% for right breast) and deep inferior epigastric perforator (DIEP) flaps (21% for left breast and 26% for right breast) were the most common autologous reconstruction procedures. Of the 135 patients, 38 (28%) patients had their breast cancer recur and 34 (25%) patients died. There were no differences in mortality (p=0.29) or recurrence (p=0.47) by race/ethnicity, and neither smoking nor obesity status significantly affected risk of mortality or recurrence.
Conclusion: There were no significant differences in recurrence or mortality by race after

mastectomy and autologous reconstruction for TNBC patients. Further investigation with larger patient samples could identify risk factors that better predict adverse outcomes post mastectomy and reconstruction.

P4-09-09: Prepectoral Implant-Based Breast Reconstruction: A New Standard of Care. Experience of a Chilean Breast Oncoplastic Surgery Group

Jaime Letzkus, José Manuel Lagos, María José Del Rio, Jorge Gamboa, Daniela Hidalgo, Guillermo Belmar

Introduction: Prepectoral breast reconstruction (PPBR) has gained popularity among breast surgeons due to its preservation of chest wall anatomy, maintenance of normal pectoral muscle function, minimal morbidity, reduction of breast pain, and avoidance of animated breast deformity. Implant-based breast reconstruction (IBR) remains the most widely utilized technique globally, facilitated by advancements in modern implants and synthetic adjunctive materials, alongside significant improvements in mastectomy and reconstruction methods. Historically, IBR was primarily performed using a sub-pectoral two-stage technique, often in conjunction with post-mastectomy radiotherapy (PMRT). However, IBR can be associated with complications such as capsular contracture, secondary breast elevation, breast pain, and animated deformity.
The aim of this study is to report preliminary outcomes of patients undergoing IBR using the prepectoral technique, delineating specific indications and complication rates.
Method: This is a retrospective analysis from a prospective database of reconstructive patients who underwent PPBR from July 2019 to June 2024 at both public university hospitals and private practice centers in Santiago, Chile. Oncological and immediate reconstructive procedures were performed by a team of breast surgeons with Level 3 oncoplastic and reconstructive training. Patients were preoperatively assessed by a multidisciplinary oncological committee. Surgical feasibility for conservative mastectomy and prepectoral reconstruction was evaluated on an individual basis, with specific informed consent obtained. The study analyzed general demographic data, comorbidities, genetic mutation status, histopathological information, adjuvant therapies (chemotherapy and radiotherapy), and technical details of the mastectomy and reconstruction procedures, including complications.
Results: A total of 119 patients (132 PPBR procedures) were included. The average patient age was 47 years (range 27-70), with an average BMI of 27.2. Seventeen patients had pathogenic mutations, and 18 underwent bilateral reconstruction. Definitive implants were used in 116 (88%) patients, with 95 cases being nipple-sparing mastectomies (NSM). Inframammary fold incision was used in 73 cases, and mesh was utilized in 33 cases. PMRT was administered to 43 patients. Mild to moderate complications occurred in 51 patients and there was an implant loss rate of 6% (8 out of 132 implants).
Conclusion
PPBR demonstrated a low complication rate and minimal implant loss. In well-selected

patients, it is a safe and efficient reconstructive technique offering excellent aesthetic outcomes, often without the need of mesh. PPBR avoids animated breast deformity and is associated with reduced chronic breast pain, making it suitable for both therapeutic and risk-reduction purposes.

P4-09-10: Evaluation of Axillary Treatment in Patients with Early Breast Cancer According to Study Z0011 in a Public Tertiary Hospital in the Federal District, Brazil

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Introduction: The radical mastectomy technique described by Halsted in 1894 was based on the concept that more extensive surgical resection increased the likelihood of patient cure. Over more than 120 years, medical knowledge about breast cancer has expanded, enabling less mutilating treatments with equal or better survival rates, especially following studies on tumor biology. Axillary surgical treatment has been a significant milestone, changing medical management and reducing the rates of axillary lymph node dissection after the publication of the American College of Surgeons Oncology Group Z0011 clinical trial, that demonstrated that patients with initial cT1-T2 tumors without palpable axillary adenopathy, undergoing breast-conserving surgery, systemic adjuvant treatment, and radiotherapy, maintained overall survival and distant recurrence-free survival rates, even with 1 or 2 metastatic axillary lymph nodes, compared to those who underwent axillary dissection.
Objectives: To evaluate the applicability of the Z0011 protocol in the axillary treatment of early invasive breast cancer and its impact on reducing axillary lymph node dissection by omitting this surgical treatment.
Method: This is an observational, descriptive, retrospective study based on data collected from the medical records of patients who underwent surgical treatment at a public hospital, Hospital de Base, in the Federal District, Brazil.
Results: A total of 119 patients from the mastology service at Hospital de Base met the Z0011 eligibility criteria. Nine patients were excluded due to failure in patent blue migration, resulting in 110 patients included in the study. The average age of patients was 58 years (ranging from 35 to 83 years). The predominant immunohistochemical profile was luminal B (55%), followed by luminal A (30%), HER2 positive (9%), and triple-negative (6%). Among the HER2 positive patients, 80% also had positive hormone receptors (HR), while 20% were HR negative. Among the 110 patients, 27% had sentinel lymph node (SLN) metastasis. Specifically, 19% had metastasis in 1 SLN, 3% had metastasis in 2 SLNs, and 5% had metastasis in 3 or more SLNs. Consequently, 83% of patients with 1 or 2 metastatic SLNs did not undergo lymphadenectomy, representing a significant omission of axillary surgical treatment.
Conclusion: The successful replication of the Z0011 protocol in our study demonstrates the significant potential for reducing axillary lymphadenectomy in patients treated at our

hospital, with an observed 83% reduction in axillary dissection among patients with axillary lymph node metastasis. These findings are particularly important as they underscore the feasibility of implementing the Z0011 protocol in a public hospital setting, providing evidence that even in resource-constrained environments, it is possible to achieve substantial improvements in patient care.

P4-09-11: The use of indocyanine green instead of blue dye for dual-tracer sentinel lymph node biopsy in breast cancer

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Introduction: Dual-tracer sentinel lymph node biopsy (SLNB) with technetium-99m sulfur colloid (Tc99) and blue dye has been the standard method utilized in breast cancer surgery. The use of blue dye can be associated with skin tattooing, skin necrosis (methylene blue), and allergic reactions (isosulfan blue). The aim of this study was to determine if indocyanine green (ICG) is a suitable replacement for blue dye for dual-tracer SLNB.
Methods: We performed a single-center retrospective study of female breast cancer patients age ≥18 years who underwent SLNB with Tc99 and ICG from November 2022 to April 2024. ICG mapping was performed by injecting a total of 0.1 ml of ICG intradermally into the areolar skin of the right breast at the 12 and 9 o'clock positions or of the left breast at 12 and 3 o'clock positions preoperatively. Tc99 mapping was performed by injecting Tc99 into the retroareolar breast preoperatively. Intraoperatively, the SPY Portable Handheld Imager was utilized to visualize fluorescent SLNs and a gamma detector was utilized to identify SLNs with Tc99 uptake. Operative reports were reviewed to determine sentinel lymph node (SLN) identification rate with ICG (fluorescent) and Tc99 (radioactive). Pathology reports were reviewed to determine the pathology of excised SLN.
Results: One hundred and nineteen SLNBs were performed on 117 patients. The median age at time of surgery was 59.0 years and the median BMI was 26.9. Most patients had invasive ductal carcinoma (76%) and a primary tumor located in the upper outer quadrant (50%). The proportions of patients receiving mastectomy (47%) and breast-conserving surgery (53%) were similar. Thirty-two patients (27%) received neoadjuvant chemotherapy. The mean number of SLNs retrieved per SLNB was 1.6 (fluorescent, 1.50; radioactive, 1.48). Overall, at least one radioactive or fluorescent SLN was identified in 93.2% of all patients. Of all excised SLNs, 89.4% were fluorescent, 88.4% were radioactive, and 81.9% were both fluorescent and radioactive. Overall, 26 patients (22.2%) had SLN metastases; of SLNs identified with metastases on pathologic examination, 87.2% were fluorescent and 74.6% were radioactive. Three patients (2.6%) experienced skin flap necrosis and one patient (0.85%) experienced prolonged skin discoloration. No patients experienced allergic reactions.
Conclusion: This study demonstrates that SLN identification rates using ICG and Tc99 are comparable to those using blue dye and Tc99. Thus, ICG is a suitable alternative for blue dye in times of tracer shortage and may avoid complications specific to blue dye. Future work

should assess if ICG is a suitable tracer for SLNB in low-resource settings where Tc99 is not available.

P4-09-12: Factors influencing axillary node clearance in early breast cancer care in the UK in 2023

Stuart A McIntosh, Helen Flint, Julie Douglas, Philipp A. Dietrich, Victoria Bush, Richard Simcock

Early breast cancer (EBC) treatment is multimodal; as more effective systemic therapies become available, decision-making and personalised treatment selection become more complex, although factors such as tumour size, grade and nodal status remain important. Advances in treatment are driving an evolution in care. This study sought to understand the UK breast cancer management landscape and challenges facing healthcare professionals (HCPs) in relation to decision-making around axillary surgery in patients with ER+ HER2- disease.
HCPs involved in EBC patient management (n=70; 29 medical oncologists, 13 clinical oncologists, 10 surgeons, 13 pharmacist prescribers and 5 nurses) were surveyed August to December 2023.
NICE guidance states that patients with radiologically detected node-positive (N+) disease should be offered axillary node clearance (ANC). However, only 21% of HCPs stated their multidisciplinary teams would perform ANC with 1 involved node identified pre-operatively and 30% where 2 nodes were seen. Approximately 40% stated the decision to carry out ANC would depend on other factors such as tumour size, comorbidities and age. However, of those citing other factors, 56% said this decision would be to inform further treatment. Similarly, following a positive sentinel lymph node biopsy (SLNB), only 11% would perform ANC for a single positive node, and 30% for 2 involved nodes. Again, almost 40% said the ANC decision would depend on other factors, but here 81% of those said that the decision for ANC would be to inform further treatment. For greater burdens of nodal disease, ANC was much more likely in both radiologically detected and SLNB detected nodal disease.
These findings show that for patients with a low burden of radiologically detected axillary nodal disease, only a small proportion routinely undergoes ANC, in contrast to NICE recommendations. However, this proportion is higher in those with a positive SLNB. It appears that some patients may undergo ANC to inform further treatment decisions. Given that more extensive axillary surgery does not improve outcomes, but is associated with significant morbidity, it is clear that improved methods to inform decisions about further adjuvant treatment are required.

P4-09-13: Intraoperative assessment of margins in breast conserving therapy: Comparison of intraoperative specimen mammography and intraoperative pathologic evaluation

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Background: Breast-conserving therapy is a long-standing standard surgical procedure for treating breast cancer. The aim is the complete resection of the tumor with free margins to avoid additional surgery and possible relapse. The optimal way to document tumor-free margins intraoperatively is still unclear.
Methods: In a retrospective study, we analyzed patients who underwent breast-conserving surgery for the treatment of early breast cancer in our institution. Patients with ductal carcinoma in situ (DCIS), locoregional relapse and those after neoadjuvant chemotherapy were excluded. We investigated the value of intraoperative pathologic evaluation compared with intraoperative specimen mammography for the assessment of tumor-free margins in breast conserving surgery.
Results: Between 2009-2022, 465 patients underwent breast conserving surgery and received both intraoperative pathologic evaluation by frozen section and specimen mammography at our institution. The median age of the patients was 62 years. We found 59.1 % of the patients to have an invasive tumor not otherwise specified (NOS), 14.2% to present with an invasive lobular cancer and 18.1% with a mixture of both types, while 8.6% of our patients had another type of tumor, e.g. medullar. Most of the patients, 76.3 %, were node negative and had a tumor stage pT1c. The highest accordance between the results for frozen section and specimen mammography was seen in patients older than 70 years (77.7%), having an invasive breast cancer of no special type (73.8%) and a pT2 tumor stage according to the TNM classification (73.1%). Patients with a hormone-receptor negative tumor had higher accordance rates as well as those with a HER2 positive breast cancer. The difference between the accordance of the two methods was statistically significant only for patients younger than 50 years and those having a G3 tumor. To evaluate the value of the two methods for determining the margin during surgery we calculated the sensitivity, the specificity and the positive predictive value for the specimen radiography as compared to the frozen section resulting in 52.6%, 76.7% and 31.3%, respectively.
Conclusion: The intraoperative specimen mammography is not a reliable method to assure tumor free margin for patients undergoing breast conserving surgery. The pathological evaluation of tumor specimen in addition to the specimen mammography resulted in a very low re-excision rate. Only 2.4% of the patients in our cohort had positive margins after complete pathological evaluation postoperatively. While the intraoperative frozen section seems to be the best way to avoid a second surgery, this method is costly and requires the presence of a pathologist. Thus, an improvement of the specimen mammography and the development of new imaging methods are needed.

P4-09-14: Results of combined breast cancer treatment depending on various types of reconstruction after mastectomy and radiation therapy

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Background. Implant-based breast reconstruction, in contrast to autologous reconstruction, often requires revision surgery and ultimately leads to reconstructive failure, especially in the context of adjuvant radiation therapy. Reconstructive failure and capsular contracture (CC) remain the most common complications leading to unsatisfactory aesthetic results. To date, there is no therapeutic target for the direct treatment of CC, which emphasizes the importance of its prevention. Also the consensus is lacking regarding the optimal reconstruction plan for patients requiring adjuvant radiation therapy.
Objective. To determine the impact of risk factors on the development complications of breast reconstruction and the incidence of capsular contracture and reconstructive failure in the era of multidisciplinary breast cancer treatment using modern surgical and therapeutic approaches.
Materials and methods. This retrospective study included 466 patients diagnosed with breast cancer who received combined treatment from 2016 to 2021 at the FSBI “N.N. Petrov NMRC of Oncology” of the Russian Ministry of Health. The patients were divided into 3 groups: immediate reconstruction (N=158), immediate-delayed (N=210) and delayed (N=98). Features of the surgical intervention, neoadjuvant and/or adjuvant systemic therapy were analyzed to determine the association with the risk of developing capsular contracture.
Results. Every fourth patient (24.0%, 112/466) may encounter a complication after mastectomy with reconstruction and adjuvant radiation therapy. There was no correlation between potential risk factors depending on the general status of patients (age, obesity, diabetes mellitus, hypertension, history of smoking) and the occurrence of complications after combination treatment. Capsular contracture during alloplastic reconstructive plastic surgery with adjuvant radiation therapy, according to the literature, is a common complication with a complex and multifactorial pathogenesis. Despite the low incidence of CC III-IV Baker scale in the present study (6.9%, 32/466), the majority of patients required revision surgery (90.6%, 29/32). There was no correlation between risk of CC depending on the systemic treatment of patients ($z < 1,96$).
Conclusion. The analysis of complications and reconstructive failures showed that immediate breast reconstruction at the FSBI "N.N. Petrov NMRC of Oncology" of the Ministry of Health of Russia is the safest method of reconstruction in the combined treatment of breast cancer with the lowest incidence of severe complications (9.5%, 15/158, $z>1.96$), which plays a key role in the development of reconstructive failures (Pearson Correlation=0.861, $p<0.001$). However, these surgeries are associated with a

significant number of capsular contractures (15.2%, 24/158, z>1.96, Pearson Correlation=0.230, p<0.001) and statistically depend on the subpectoral location of the endoprosthesis (22.1%, 21/95; z = 2.79), the choice of textured implants (20.8%, 22/106; z = 2.02) and periareolar access during surgery (31.4%, 11/35; z > 1,96). That's why these complications require finding ways to minimize risks to optimize treatment in each individual case in short and long term.
Key words: breast cancer, mastectomy, adjuvant radiation therapy, reconstructive plastic surgery, complications, capsular contracture, reconstructive failure, risk factors.

P4-09-15: The Role of Axillary Reverse Mapping during Sentinel Lymph Node Biopsy in Breast Cancer Patients

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Introduction. The incidence of lymphedema after axillary lymphadenectomy varies widely, ranging from 14.1% to 33.4%, with the highest rates seen in patients receiving adjuvant radiation therapy. The routine use of sentinel lymph node biopsy (SLNB) instead of lymphatic axillary dissection (LAD) resulted in a lower incidence of lymphedema, ranging from 3.5% to 11%. Therefore, even when we perform an SLNB procedure, we cannot always offset the risk of complications. This is because the lymph nodes responsible for lymph drainage in the upper limb may be among the removed sentinel lymph nodes.
Aim. To evaluate the relationship between sentinel lymph nodes and lymph nodes responsible for the lymph drainage from the upper limb in BC patients when performing SLNB by radioactive isotope (RI) method and with visualization of the lymph collector of the upper limb using indocyanine green (ICG), an axillary reverse mapping (ARM) technique.
Materials and Methods. The retrospective analysis was carried out in the FSBI N.N. Petrov NMRC of Oncology. Thirty-five patients diagnosed with breast cancer during the surgical stage of treatment, including SLNB and the method of ARM using ICG was applied to visualize lymph nodes and vessels responsible for lymph drainage of upper limb tissues.
Results. In 6 (17.14%) cases, the sentinel lymph node turned out to be the lymph node responsible for the lymphatic collector of the upper limb. Standard histology showed tumor cells in only 1 in 6 cases. In the remaining 29 cases (82.86 %) it was possible to preserve the lymphatic collector (lymph nodes and lymphatic vessels) from the tissues of the upper limb.
Conclusion. Performed analysis showed that the marking of lymphatic vessels in the upper limb allows the differentiation of the zones responsible for lymphatic drainage, which in turn determines a selective approach to lymph node removal. Also, if the lymph node responsible for draining lymph from the upper limb is removed, preventive measures can be taken to prevent lymphedema.

P4-09-16: Progesterone Receptor Expression Significantly Correlates with Recurrence Score Regardless of Menopausal Status in HR+/HER2- BC Patients

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Background: Clinical and pathological (CP) features have been historically considered the most reliable parameters to define breast cancer (BC) prognosis and select adjuvant therapy accordingly. In the last decade, the OncotypeDX genetic test has entered clinical practice as it refines risk stratification and anticipates chemotherapy benefit for HR+/HER2- BC after surgery. To date, several retrospective series have correlated classical histopathological parameters with the recurrence score (RS) with heterogenous results. Moreover, most of these reports did not take into account patient menopausal status.
Material and Methods: We retrospectively collected data on consecutive patients with HR+/HER2- early BC who underwent OncotypeDX testing between September 2021 and March 2024, followed at a single Institution. CP characteristics were retrieved. Continuous variables were reported as median and interquartile ranges (IQR), while binomial and categorical variables were classified as rates. Linear regression analysis was performed considering RS as a continuous outcome, while logistic regression analysis was performed separately for pre- and post-menopausal patients, dichotomizing the RS with 16 and 25 as high-risk thresholds for each group. Statistical analyses were carried out with the STATA v18.0 software.
Results: Of the 187 identified patients, 180 had complete CP data and were included in the analysis. 63 (35.0%) were pre-menopausal and 117 (65.0%) were post-menopausal. Median age in our patient cohort was 57 years (IQR 49-67.5). Most patients had pT1 pN0 (111, 61.7%), moderately differentiated (G2=119, 66.1%) disease with high ER expression (median 95%, IQR 90-95%) and a median Ki67 proliferation index of 25% (IQR 15.30%). Median PgR expression was 80% (40-90%) in the overall population, 90% (IQR 55-90%) in pre-menopausal women and 70% (IQR 30-90%) in post-menopausal patients. Median recurrence scores were 16 (IQR 12-22), 15 (IQR 11-18) and 17 (IQR 12-22) in the overall, pre- and post-menopausal cohorts.
The RS displayed a direct correlation with G3 disease (p 0.01), HER2 2+ status (p 0.02) and Ki67% (p<0.0001), and an inverse correlation with ER% (p 0.03) and PgR% (p<0.0001). Among pre-menopausal patients, only Ki67% (0.02), ER% (0.01) and PgR% (<0.0001) retained statically significant correlations with the RS, while in the post-menopausal population significance persisted for G3 (p 0.03), HER2 2+ status (p 0.003), Ki67% (p 0.001) and PgR% (p <0.0001).
Using logistic regression analysis, in pre-menopausal patients PgR% was the only variable predicting an RS>16, with an odds ratio (OR) of 0.95 [95% confidence interval (CI) 0.93-0.98, p 0.001]. The area under the ROC curve for PgR% to predict RS was 0.75. In post-menopausal patients, HER2 2+ status (OR 5.53; 95% CI, 1.29-23.68; p 0.021), Ki67% (OR

1.08; 95% CI, 1.01-1.16, p 0.031) and PgR% (OR 0.95; 95% CI 0.93-0.98, p<0.0001) significantly correlated with an RS>25. In this subset of patients, the area under the ROC curve for PgR% to predict RS was 0.87.
Conclusion: In our cohort of HR+/HER2- early BC patients, patient menopausal status influenced the correlation between the RS and classical histopathological features. The rate of PgR expression was the only variable consistently correlating with the RS, regardless of menopausal status. Hence, the prognostic and predictive role of this, often overlooked, parameter deserves further investigation in larger and multicentric cohorts, along with a prospective validation.

P4-09-17: Oncotype DX® breast cancer assay in older patients: a real-life cohort

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Introduction: The 21-gene Oncotype DX® Breast Recurrence Score test was designed for HR+, HER2- early-stage breast cancer (eBC) to aid in the decision-making process regarding adjuvant chemotherapy. Its validity and utility have been demonstrated prospectively across multiple studies, though data on older patients remain limited.Methods: This retrospective cohort study included all consecutive patients over 70 with eBC treated between January 2018 and December 2023 at Tenon Hospital, Paris, France.Results: Of the 365 patients included, the mean age was 77.6 years (range 70-96). Among these, 84,4% had HR+/HER2- eBC. Most patients had invasive ductal carcinomas (75%), N0/Nmic node involvement (75%), grade 2 tumors (60%), and tumor sizes <5 cm (65%). Axillary lymph node dissection was performed in 20% of the patients, while 66% underwent sentinel lymph node biopsy. Oncotype DX® testing was requested for 86 patients, 27,9% of HR+/HER2- eBCs. A Recurrence Score (RS) >25 was found in 13 patients (15%), including those with N+ (15%), grade 3 (69%) and Ki67 >20% (62%). Three patients had a RS >25 with T2, grade 2 breast cancer. Chemotherapy was not initiated in 6 out of the 13 patients with a high RS: 2 were deemed unfit according to geriatric evaluation, 2 refused chemotherapy, and 2 for unknown reasons.Conclusion: This observational study provides valuable insights into the management of HR+ eBC in older patients, emphasizing the importance of considering Oncotype DX® to optimize treatment strategies in these patients.

P4-09-18: Adjuvant abemaciclib use and outcomes in patients with high-risk hormone receptor-positive, HER2-negative breast cancer: a real-world analysis.

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Background: More than 90% of patients with breast cancer are diagnosed with early-stage disease, of whom approximately 70% have tumors that are hormone receptor-positive (HR+) and human epidermal growth factor receptor 2 negative (HER2 -). Standard treatment varies depending on the risk of recurrence but includes combinations of surgery, radiotherapy, adjuvant/neoadjuvant chemotherapy, and endocrine therapy (ET). Patients with HR+/HER2-, node-positive early breast cancer (EBC) are at high risk of recurrence (up to 30% at five years) and need intensification of treatment. The MONARChE study demonstrated the efficacy of abemaciclib combined with endocrine therapy as an adjuvant treatment for patients with high-risk early breast cancer. This study evaluated the real-world applicability of these findings in a cohort of Brazilian patients, highlighting patient characteristics, treatment regimens, adverse events, and outcomes.
Methods: We collected data from patients diagnosed with high-risk (MONARChE criteria) RH+/HER2-, early breast cancer, who received and treated at A. C. Camargo Cancer Center, Brazil, from January 2021 to May 2024. Data collected included patient demographics, menopausal status, body mass index (BMI), germline BRCA1/2 mutation status, receptor status, chemotherapy and surgery details, histological grade, KI67 levels, pathological staging, and treatment outcomes.
Results: We 89 HR+/HER2-, high-risk early breast cancer patients who received adjuvant abemaciclib following the MONARChE study protocol. The median follow-up was 33 months. Among the 89 patients, 87 (98%) were female, with a median age of 50 years (range 26-85). Of these, 54 patients (62%) were pre-menopausal, and 32 (37%) were post-menopausal. Sixteen percent were older than 65, and 84% were 65 or younger. The median BMI was 27, with 58% having a BMI >25. Approximately 5.62% had a germline BRCA1/2 mutation. Most patients had tumors with histological grade (HG) 2 (55%) and ≥ 20% (N=71, 81.6%). Estrogen receptor (ER) > 20% was observed in 86 tumors (97%), progesterone receptor (PR)> 20% in 72 patients (81%), and HER2-low in 79% of patients. Thirty-six patients (40%) received neoadjuvant chemotherapy, 46 (51%) received adjuvant chemotherapy, and 7 (7.87%) did not receive chemotherapy. Chemotherapy regimens included AC-T in 70 patients (79%), TC x 4 cycles in 2 patients (2%), and TC x 6 cycles in 9 patients (10%). Regarding surgery, 39 patients (43%) underwent segmental resection, and 50 patients (56%) underwent mastectomy. T stage was T1 in 37%, T2 in 42%, T3 in 17%, and T4 in 2.27%, while N stage was N1 in 43%, N2 in 25% , and N3 in 22% . Grouped staging was II in 54% and III in 46%. The response rate among the 36 patients who received neoadjuvant chemotherapy was RCB-0 in 4.6%, RCB-1 in 1%, RCB-2 in 12%, and RCB-3 in 18% . Ninety-two percent of patients received adjuvant radiotherapy. Adjuvant endocrine therapy consisted of tamoxifen in 6% of patients, goserelin + tamoxifen in 2%, aromatase inhibitors (AI) in 39%, and AI + goserelin in 31%. Programed duration of endocrine therapy was for seven years in 13% of patients, ten years in 29%, and five years in 26%. The most commonly reported adverse events were diarrhea in 49% of patients, fatigue in 12%, neutropenia in 9%, elevated transaminases in 13%, nausea in 5%, and no toxicity in 14%. Dose adjustments were necessary in 26 patients (29%) (20% reduced to 200mg/day

and 9% to 100mg/day). Suspension of treatment with abemaciclib occurred in 16 (16%) patients due to limiting toxicity. During the follow-up, only one patient experienced disease recurrence during adjuvant therapy.
Conclusion: This cohort was composed of younger premenopausal patients with a high prevalence of overweight and low frequency of germline BRCA1/2 mutations. Tumors were predominantly HG2, T2, N, and stage II. Most received adjuvant chemotherapy with anthracyclines and taxanes, adjuvant radiotherapy, and double hormonal blockade with AI. Diarrhea was the most common toxicity and the leading cause of dose reduction or discontinuation of therapy. Until the last follow-up, most were still using adjuvant abemaciclib without evidence of disease.

P4-09-19: Exploring cellular heterogeneity of localised breast cancers

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Breast cancer exhibits heterogeneity at various levels, from subtype differences between patients (inter-patient heterogeneity) to diverse malignant cell compositions, variability in hormone receptor (HR) expression, and cellular makeup within single breast cancer samples (intra-tumour heterogeneity). Despite the availability of numerous effective therapies, many patients still experience incomplete treatment responses and subsequent relapse. These adverse outcomes are often due to the critical but frequently overlooked factor of cellular heterogeneity.
To gain deeper insights into intra-tumour heterogeneity, we applied single-cell technologies to a cohort of 250 primary, untreated breast cancers. We optimized tissue cryopreservation methods, eliminating the need for fresh sample processing, and developed multiplex tissue profiling techniques. These cost-efficient processes improve handling of small tissue sizes, such as biopsies, and reduce batch effects. To ensure accurate and reliable data processing, we created a scalable computational workflow that includes benchmarked methods for sample SNP-demultiplexing, doublet detection, high-resolution cell annotation, and cellular integration. Additionally, we extended our existing methods to study the cellular heterogeneity of breast cancers.
Our method, scSubtyper, examines the phenotypic differences between malignant cells within tumours by comparing each single cell to features associated with different molecular subtypes and assigning each cell to one of these subtypes. Our previous study and preliminary results from this project revealed that over 90% of samples exhibit a mix of malignant cells of different subtypes, and 50% of samples contain cells with characteristics of all subtypes, demonstrating that cellular heterogeneity exists not only between malignant cells but also within malignant cells of a tumour. Our second approach, known as ecotyping, assesses patterns of cell type frequencies across samples and groups them based on the similarity of these co-occurrences. Preliminary results have revealed the existence of five ecotypes that lack significant associations with clinical subtypes. Application of the same approach exclusively within the HR-positive samples identified four ecotypes characterized by distinct abundances of immune and stromal cells. This analysis revealed that ecotypes

are not simple surrogates for clinical and molecular subtypes, but their presence could influence different responses to treatment.
Together, our high-throughput tissue processing and computational approaches to study intra-tumour heterogeneity are now being applied to our large, well-annotated clinical cohort. Supported by preliminary results, we hypothesize that this study will play a vital role in optimizing breast cancer patient stratification to improve treatment management and outcomes.

P4-09-20: Extended Endocrine Adjuvant therapy for Early HR+ Breast Cancer, Comparisons Between Molecular Expression Profiles

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Molecular gene expression profiles are commonly used now in early stage HR+ breast cancer to determine adjuvant systemic therapy and can prognosticate recurrence risk in years 0-5, although predictive and prognostic use for prevention of late relapse in years 5-10 with extended hormonal therapy remains controversial and direct comparisons of the available assays are few. Breast Cancer Index (BCI) is a genomic assay that analyzes 11 genes using qRT-PCR expression profiling to interrogate multiple cell-signalling pathways associated with breast cancer recurrence risk. These genes do not overlap with the other commonly used early breast cancer genomic assays.
We reviewed retrospective data from 75 patients in Florida and North Carolina in both a private practice and an academic practice who had early stage HR+ breast cancer and had a commonly used 30 gene or 70 gene genomic assay sent, as well as a later BCI assay. We sought to determine if patients classified as low or intermediate risk on the standard early assays had a higher risk BCI profile or a potential benefit from longer term hormonal therapy, and also if high risk patients might show a potential lack of benefit from longer term hormonal therapy and therefore avoid possible adverse effects such as bone loss and menopausal symptoms.
Results- We did find that a significant number of patients with low or intermediate risk on 30 or 70 gene assays had a higher than expected late relapse risk on the BCI assay, approximately 25-30%. Data will be presented in the poster, along with outcomes. In addition we found that some patients with high risk 30 or 70 gene assays had a low risk of late relapse on the BCI assay, suggesting lack of benefit from extended hormonal adjuvant therapy.
Conclusions- We were able to confirm in this contemporary group the comparison findings of the 2016 TransATAC analysis that there can be a significant disparity between early recurrence risk as determined by the standard 30 or 70 gene expression profiles, and the BCI risk of late recurrence, as well as the potential benefit of longer term hormonal therapy in these patients. This suggests that the 11 gene BCI assay may be able to help patients decrease risk of late recurrences and avoid also unnecessary treatment related toxicity.

P4-09-21: Metastatic disease in patients with early breast cancer and a low Oncotype DX® Recurrence Score: A review of clinicopathological features

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Background: Prognostication and prediction in the adjuvant setting in early breast cancer has been evolving since the introdcution of gene expression profiling, with a move away from traditional clinical parameters such as tumour size, histological grade and lymph node status toward mutligene panel testing. However, it has become increasingly evident that both approaches are needed to provide personalised risk prediction. Although these tools have helped inform adjuvant therapy decisions and prognostication in early breast cancer, the aspiration of personalised prediction is still awaited as these tests may not reflect tumour heterogeneity. A retrospective analysis exploring the clinicopathological features of patients with genomic scores who developed metastatic disease within ten years of diagnosis was undertaken to investigate potential risk factors for recurrence.
Methods: A retrospective review was conducted to identify patients with node negative early breast cancer who had undergone Oncotype DX RS testing between 2007 and 2018. Exclusions included preeinvasive disease, and patients with no follow up data or outcomes measures. Clinicopathological details of patients with recurrent disease and low genomic risk, defined as a Recurrence Score less than 26, were evaluated. NPI was calculated. Chemotherapy regimes, use and duration of endocrine therapy was noted. The date of recurrence, metastatic site, and the date of death was recorded.
Results: The cohort consisted of 121 caucasian female patients with a mean age of 68.36 years (range 35 to 84). Histological subtypes included 79.34% (96/121) ductal cancers, 16.53% (20/121) lobular cancers, 2.48% (3/121) mucinous carcinomas, 0.83% (1/121) micropapillary cancers, and 0.83% (1/121) mixed ductal lobular cancers. mean tumour size of whole cohort was 23.75mm. Percentage of recurrence scores <11 was 27.27% (33/121), 11-17 was 46.28% (56/121) and 18-25 was 26.45% (32/121). Mean recurrence score of cohort was 13.80. Mean NPI 3.4. Of the 121 patients, seven developed recurrence (5.79%), and five patients (4.13%) developed a new/second breast primary. The cases with metastatic disease were all grade 2 ductal cancers. Most recurrences were diagnosed greater than 5 years following diagnosis (range 2 to 10 years). Metatastic sites were lung 100% (7/7), liver 28.57% (2/7), bone 28.57% (2/7) and local recurrence noted in 28.57% (2/7). The overall mortality rate was 6.61% (8/121 - two non-cancer deaths). Of the patients who developed metastatic disease (n=7), all tuours were PR positive, and 6 of the 7 were ER8, PR8 suggesting luminal A subtype.The mean NPI of the metastatic group was 3.54. The percentage of patients with metastatic disease with a recurrence score of 0-10, 11-15, 16-20 and 21-25 was 42.86% (3/7), 42.86% (3/7), 0%, and 14.28% (1/7), respectively. 57.14% (4/7) received adjuvant Letrozole for a minimum of 5 years. The patient with a recurrence score of 24 received adjuvant chemotherapy and developed metastatic 4 years post diagnosis while on Tamoxifen.
Discussion: The mortality rate of the cohort is consistent with the literature, with a 5 to 10

year disease free survival of 94.21%. There was no statistically significant difference in the recurrence scores, NPI and clinicopathological findings in the two groups. The incidence of pulmonary metastases in this cohort warrants further investigation. Primary breast tumour site was in the inner quadrant in two patients and one patient had a positive intramammary nodes with negative sentinle nodes. The cohort investigated is from a homogeneous population. Records suggest that compliance with adjuvant endocrine therapy was good, although this is difficult to verify. Studies suggest that noncompliance can be a factor in recurrence in these cohorts.
Conclusion: The quest for personalised prediction and prognostication in early breast cancer is ongoing. Genomic tests have resulted in a significant reduction in the number of patients receiving adjuvant chemotherapy. However, a more multifacted approach may be required to tailor treatment decisions incorporating genomic testing, clinicopathological features and individual characteristics with the aim of improving outcomes for patients with hormone receptor positive early breast cancer.

P4-09-22: Distant disease-free survival (DDFS) across key subgroups from the phase 3 NATALEE trial of ribociclib (RIB) plus a nonsteroidal aromatase inhibitor (NSAI) in patients with HR+/HER2− early breast cancer (EBC)

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Background: In the NATALEE trial, RIB + NSAI has demonstrated invasive disease-free survival and DDFS benefits in patients with stage II and III HR+/HER2− EBC. In an analysis with all patients off RIB, RIB + NSAI reduced the risk of distant disease recurrence vs NSAI alone. Understanding the impact of adjuvant treatments on distant disease recurrences across patient subgroups is critical for clinical decision-making. We present DDFS data from the 4-y landmark analysis of NATALEE across clinically relevant subgroups.
Methods: In NATALEE, patients were randomized 1:1 to receive either RIB (400 mg/d, 3 wk on/1 wk off for 3 y) + NSAI (anastrozole 1 mg/d or letrozole 2.5 mg/d for 5 y) or NSAI alone, with men and premenopausal women also receiving goserelin. NATALEE included patients with anatomic stage IIA (either node-negative [N0] with additional risk factors or N1 [1-3 axillary lymph nodes]), IIB, or III disease per AJCC (8th edition). DDFS was a secondary endpoint and was defined as the time from randomization to the first event of distant recurrence, second primary non-breast invasive cancer (except for basal and squamous cell skin carcinomas), or death from any cause. DDFS was assessed across patient subgroups, including anatomic stage and nodal status, using Kaplan–Meier analysis and a Cox proportional hazards model.
Results: At the data cutoff of April 29, 2024, with a median duration of follow-up for DDFS of 44.2 months and all patients off RIB, RIB + NSAI demonstrated both a DDFS benefit (HR, 0.715 [95% CI, 0.604-0.847]; nominal P value <.0001) and a distant recurrence-free survival

benefit (HR, 0.705 [95% CI, 0.589-0.844]; nominal P value <.0001) in the intent-to-treat population. The DDFS benefit was consistent regardless of anatomic stage (stage IIA [n=1001]: HR, 0.396 [95% CI, 0.218-0.720]; stage IIB [n=1045]: HR, 0.806 [95% CI, 0.524-1.238]; stage IIIA [n=1832]: HR, 0.697 [95% CI, 0.524-0.926]; stage IIIB [n=317]: HR, 0.569 [95% CI, 0.326-0.994]; stage IIIC [n=890]: HR, 0.878 [95% CI, 0.649-1.188]). The absolute DDFS benefit with RIB + NSAI vs NSAI alone increased from 3 y to 4 y for all stage subgroups (stage IIA, 2.3% to 5.1%; IIB, 0.8% to 2.6%; IIIA, 3.1% to 4.6%; IIIB, 6.8% to 11.4%; IIIC, 2.4% to 4.5%). Similarly, a consistent improvement in DDFS was observed regardless of nodal status (N0 [n=613]: HR, 0.696 [95% CI, 0.403-1.204]; node-positive [N+] [n=4480]: HR, 0.726 [95% CI, 0.608-0.867]), and the absolute DDFS benefit increased from 3 y to 4 y across nodal subgroups (N0, 2.7% to 4.2%; N+, 2.4% to 4.6%). The DDFS benefit with RIB + NSAI vs NSAI alone, with increasing benefit up to 4 y, was consistent across other clinically relevant subgroups, including those based on menopausal status and Ki-67 status. Conclusions: With all patients off RIB, RIB + NSAI consistently reduced the risk of distant recurrence across clinically relevant subgroups, including in patients with N0 disease. The DDFS benefit was sustained after the 3-y RIB treatment duration, with increasing absolute benefit up to 4 y. These findings further support adding RIB to adjuvant NSAI in a broad population of patients with HR+/HER2− EBC.

P4-09-23: Challenges and opportunities for CDK4/6i therapy in adjuvant early breast cancer

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CDK4/6 inhibitors (CDK4/6i) are a class of medicines that have been studied and introduced in early Breast Cancer (EBC) in the UK since June 2022. Currently, abemaciclib is the only licensed and reimbursed CDK4/6i for use in hormone receptor positive, human epidermal growth factor receptor 2 negative (HR+/HER2-) EBC in the UK. Prognosis in HR+/HER2- EBC has improved significantly over recent decades. However, despite these advances, the risk of recurrence remains high for many patients. To reduce the risk, the arrival of CDK4/6i therapy in combination with endocrine therapy in EBC occurs at time when there are multiple other therapeutic advances in the neoadjuvant, adjuvant and metastatic Breast Cancer treatment landscape, which can pose challenges for adoption. This study sought to understand the UK Breast Cancer treatment landscape and challenges facing Healthcare Professionals (HCPs), focusing on the impact of the introduction of current and future CDK4/6is.
HCPs involved in EBC patient management who were not necessarily prescribers were surveyed between August to December 2023 (n=70, oncologists, surgeons, pharmacists and nurses). The aim of the survey was to better understand the early breast cancer patient pathway in the UK from diagnosis, through risk stratification and finally treatment decisions and to explore key challenges faced by clinicians and how these differ around the country. 72% of respondents had treated between 5-30 patients with a CDK4/6i in this

setting. The majority of HCPs (89%) have experience of adjuvant CDK4/6i prescribing in routine clinical practice, while 23% of HCPs have used adjuvant CDK4/6i in clinical trials. Staff shortages and clinic capacity are the most significant challenges for breast cancer services when delivering adjuvant therapy. Most HCPs (91%) cite the introduction of CDK4/6is to have impacted on time spent educating patients. In line with this, digital patient education, patient apps and psychological support were common suggestions from survey respondents to help manage EBC patients on new therapies.
Survey respondents reported that on average 10% patients decline adjuvant CDK4/6i, mainly due to additional hospital visits, toxicity from previous treatment and side effect concerns. Poor performance status and prohibitive comorbidities were the most likely factors to prevent CDK4/6i therapy initiation in EBC.
Patient reviews were reported to be typically monthly with a medical oncologist for up to 3 months after initiation of adjuvant CDK4/6i treatment. After this period, follow-up was commonly quarterly and predominantly maintained by Medical Oncologists, however there was no consistent standard of follow-up.
If permitted through commissioning, 70% of HCPs would consider rechallenging a patient with a CDK4/6i if their disease progressed. 78% of respondents highlighting they would use a different CDK4/6i and different endocrine therapy backbone following a 12-month treatment-free interval.
The introduction of CDK4/6is in EBC offers improved outcome for patients, but comes with additional strain on service delivery, particularly staff shortages, capacity constraints and additional patient follow up by HCPs. These findings suggest further need to streamline EBC care. UK service providers may need to evaluate and adjust healthcare infrastructure in order to deliver optimal care for EBC patients.

P4-09-24: Revealing the connection: Clinicopathological characteristics and Oncotype DX Scores in Invasive Lobular Breast Cancer

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Background: Oncotype DX™ (ODX) score estimates prognosis of cancer recurrence and predicts benefit of chemotherapy. It also individualizes the patient's adjuvant chemotherapy prescription for BC. Invasive lobular carcinoma (ILC) ranked second as the most common type of breast carcinoma, accounting for approximately 15% of tumors of breast origin. In this analysis, we aimed to explore the association between OncotypeDX Recurrence Score (RS) and clinical characteristics of ILC patients.
METHODS: We retrospectively reviewed clinical data from 55 ILC patients diagnosed at Instituto Nacional de Enfermedades Neoplásicas (INEN, Lima-Peru) and Oncosalud with available ODX reports. We evaluated the linear relationship between the Recurrence Score (RS) and continuous variables like age, tumor size, ER, PR, and Ki 67 using scatter plots with confidence intervals and Pearson correlation coefficients. Boxplots were also generated to

compare RS across categorical variables, using statistical tests such as Kruskal-Wallis and Mann-Whitney U. P-values less than 0.05 were considered significant.
RESULTS: In our cohort, 96.4% (n=53) were classified as low-risk (RS=0-25), and only two cases with high-risk (RS=26-100). Progesterone Receptor (PR) status was negative in 18.2% (n=10), and 31.6% had Ki67 levels <20%. We found that only Ki67 expression was positively correlated with RS (p=0.0089). Patients stratified according to Ki67%<=14% (p=0.03) or Ki67%<=20% (p=0.02) also showed significant differences in RS. Regarding the histologic grade, G3 seems to have a higher mean RS (u=19.40) compared to G1/G3 (u=14.36), the significance of this variable remains unclear in our cohort (p=0.07).
CONCLUSIONS: Stratifying patients according to Ki67 levels (% <= 14% and % <= 20%) revealed significant differences in RS (p=0.03 and p=0.02, respectively). This underscores the importance of Ki67 as a potential biomarker for predicting recurrence scores and, consequently, the risk of recurrence in ILC.

P4-09-25: Estrogen Receptor Expression and Clinical Outcomes in Breast Cancer: Insights from a Single-Center Study

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Breast cancer presents a diverse clinical spectrum characterized by distinct subtypes that significantly influence treatment strategies and prognostic outcomes. Hormone-sensitive breast cancers, defined by estrogen receptor (ER) and/or progesterone receptor (PR) expression, play a crucial role in guiding therapeutic decisions. Understanding the complexities of ER-positive tumors is paramount due to their diverse biological behaviors and varied responses to treatment.
This retrospective, single-center study conducted at Chihuahua's State Cancer Center reviewed the medical records of 1224 breast cancer patients diagnosed with positive estrogen receptor status, aged ≥ 18 years, between January 2012 and June 2023. The study specifically focused on categorizing patients into two groups based on their ER levels: those with levels greater than 10% (high ER) and those with 10% or less (low ER). Clinicopathologic characteristics and recurrence rates were meticulously evaluated using Pearson's chi-square test to establish comparisons and determine statistical significance. Among the estrogen receptor-positive patients, 420 (34.31%) exhibited low ER expression, while 804 (65.69%) had high ER expression. Significant differences were observed in tumor size (T) between the groups (p=0.00). In the low ER group, 56.54% had T1 and T2 tumors, whereas 43.46% had T3 and T4 tumors. Conversely, the high ER group predominantly also presented with T1 and T2 tumors (71.32%) but compared to T3 and T4 tumors (28.68%). Regarding lymph node involvement, 67.41% of the low ER group had positive lymph nodes, compared to 60.36% in the high ER group, showing a statistically significant difference (p=0.010). Tumor grade analysis revealed that 59.52% of low ER patients were in grade 3,

while only 30.05% of high ER patients were in grade 3 (p=0.00). Finally, concerning recurrence rates, the high ER group exhibited an 11.44% recurrence rate, whereas the low ER group exhibited nearly double the rate at 21.43% (p=0.00).
In conclusion, our findings highlight that low estrogen receptor expression is significantly associated with more aggressive tumor biology, characterized by larger tumor sizes, higher grades, and a substantially increased recurrence rate. These results underscore the critical importance of integrating estrogen receptor levels into treatment planning and management strategies for breast cancer patients, advocating for tailored therapeutic approaches particularly in cases of low ER expression.

P4-09-26: Impact of NCCN/ASCO Guidelines and Epic Software Enhancement on Utilization of the Breast Cancer Index Test and its Role in the Quality of Cancer Care at Community Centers

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Background: The Breast Cancer Index® or BCI™ test is a gene-based test that analyzes two key aspects of early-stage, hormone receptor-positive breast cancer. In a first aspect, the HOXB13:IL17BR ratio (H/I) assesses how well the cancer is likely to respond to continued hormone therapy. In a second aspect, the molecular grade index (MGI) predicts the likelihood of recurrence. This combination of features helps inform the appropriate duration of endocrine therapy beyond traditional tests. Importantly, the BCI test is effective for both lymph node-negative and lymph node-positive patients. The BCI test was incorporated into the NCCN Clinical Practice Guidelines in Oncology (NCCN® Guidelines) in January 2021 and the ASCO® Clinical Practice Guideline (ASCO Guideline) in April 2022. Additionally, Epic® medical record software was recently updated to allow users to order the BCI test. We examined the frequency of BCI test orders as a quality improvement metric, both at the main campus of the City of Hope Cancer Center and our community network.
Methods: Data was obtained from Biotheranostics, Inc., for the period January 2020 to mid-2024. We analyzed the data for testing rates with the BCI test over four time periods: pre-NCCN Guidelines (Jan 2020-Jan 2021), post-NCCN Guidelines (Jan 2021- April 2022), post-ASCO Guideline (April 2022-May 2024) and post-Epic medical record software integration (May 2024 - current).
Results: A total of 822 women with early-stage breast cancer underwent testing with the BCI test during the study period. Following the NCCN Guidelines update in January 2021, there was a notable increase in adoption of testing with the BCI test, with utilization increasing by 83% (p=0.026). Following endorsement of the BCI test by the ASCO Guideline update in April 2022, there was a further increase in test ordering by 30% (p=0.22). Increased utilization of the BCI test was comparable at the main campus and our community network. The test was ordered comparably for all age groups, LN- and LN+ disease, HER2- and HER2+ patients, and pre- and post-menopausal status. The Epic

software enhancement has been in place for one month with early evidence of increased test ordering.
Conclusion: The updates to the NCCN and ASCO Guidelines led to a significant increase in utilization of the BCI test at all City of Hope sites of practice. We anticipate that the Epic software enhancement will lead to further utilization of the BCI test.

P4-09-27: The clinical benefits of CDK 4/6 inhibitors and biomarker interactions on HR+/HER2- advanced breast cancer patients: an updated pairwise meta-analysis of randomized controlled trials and network meta-analysis

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Background: The combination of cyclin-dependent kinase (CDK) 4/6 inhibitors with endocrine therapy has significantly improved the outcome of advanced hormone receptor-positive (HR+) and human epidermal growth factor receptor-2 negative (HER2-) breast cancer. However, challenges persist in identifying biomarkers of response or resistance to CDK4/6 inhibitors in the context of primary or secondary endocrine resistance and in determining their impact on overall survival (OS). A comprehensive pooled investigation and the identification of biomarker interactions may lead to a better understanding of the clinical benefits of CDK4/6 inhibitors.
Methods: To address these issues, we conducted a systematic review and a pairwise meta-analysis of randomized controlled trials of oral CDK4/6 inhibitors, including palbociclib, ribociclib, abemaciclib, dalpiciclib, and lerociclib. We examined the clinical benefits and biomarker (such as PIK3CA, TP53, and ESR1) interactions of CDK4/6 inhibitors in HR+ and HER2- advanced breast cancer. The analysis involved calculating the hazard ratio (HR) and exploring the efficacy of different CDK4/6 inhibitors in specific patient populations using network meta-analysis. The P-score was utilized to rank the effectiveness of various treatments by calculating their probabilities, facilitating comparisons in the network meta-analysis.
Results: The findings indicated that CDK4/6 inhibitors consistently confer clinical advantages to patients with HR+/HER2- advanced breast cancer, showing significant improvements in progression-free survival (PFS, HR 0.55, 95% CI 0.52-0.59) and OS (HR 0.80, 95% CI 0.74-0.86). Sensitivity analysis confirmed the robustness of these results. Subgroup and meta-regression analyses demonstrated that the clinical benefits of CDK4/6 inhibitors are consistent across various subgroups (lines of therapy and category of endocrine therapy), covariates (follow-up duration and patient characteristics), and biomarkers, with the exception of the PIK3CA mutation status (p=0.03). Specifically, we found that the interaction of PIK3CA mutation status might be essential to endocrine-therapy naive patients (First-line treatment: PIK3CA wild-type vs. mutation, HR 0.41 vs. 0.58, p for interaction = 0.03; second-line or subsequent treatments: PIK3CA wild-type vs.

mutation, HR 0.49 vs. 0.54, p for interaction = 0.68). Furthermore, the network meta-analysis revealed that all approved CDK4/6 inhibitors are equally effective for HR+/HER2- advanced breast cancer patients, providing confidence in the current treatment options. However, the clinical benefits and ranking probabilities varied for different outcomes in specific individual populations.
Conclusions: In conclusion, the PIK3CA mutation status emerges as a promising biomarker for the use of CDK4/6 inhibitors in HR+ and HER2-advanced breast cancer patients with endocrine therapy-naive breast cancer. This finding suggests the potential for future targeted and effective treatments. Moreover, the similar efficacy of currently approved CDK4/6 inhibitors for patients with HR+/HER2- advanced breast tumors provides a strong foundation for further research and development in this field.

P4-09-28: Perioperative treatment regimens and efficacy outcome measures in early-stage HR+/HER2- breast cancer: A systematic literature review of randomized controlled trials

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Background: Hormone receptor positive and human epidermal growth factor receptor 2 negative (HR+/HER2-) breast cancer (BC) accounts for ~70% of early-stage cases. A systematic literature review was conducted to summarize the perioperative (neoadjuvant therapy and/or adjuvant therapy) treatment regimens and the efficacy outcomes in this setting.
Methods: Embase, Medline and Cochrane were searched from database inception to May 19th 2023; relevant conferences were also searched from 2021 to 2023 (inclusive). English language publications of randomized control trials (RCTs) including adult patients with early-stage HR+/HER2- BC receiving neoadjuvant therapy and/or adjuvant therapy (excluding endocrine [ET] only neoadjuvant therapy) were included.
Results: Of 3,490 records identified, 71 RCTs (window of opportunity: n=2 [3%], Phase II: n=39 [55%], Phase III: n=27 [38%], not reported: n=3 [4%]) were included. The cumulative sample size was 78,457, which ranged from 21 to 9,719 across the trials. The HR status among the included populations of these trials were reported as follows: HER2-/HR+ (n=57 trials) and HER2-/ER+ (n=14). Mean/median age of the patients varied from 46.9 to 65.6/46 to 76 years, respectively across the 26 trials where age was reported. Anatomical stage varied across studies (Stage I [Range]: 0.1% to 59%, Stage II: 25.3% to 100%, Stage III: 5% to 74.1%). Among studies which reported histological grade, the proportion of patients with grade 3 disease ranged from 3% to 55%. Where reported, nodal status varied among patient cohorts (N0: 5.1% to 98.1%, N1: 33.1% to 67.4%, N2: 2% to 37.0%, N3: 1.6% to 13.6%). Treatment interventions varied across the RCTs. Chemotherapy (CT) with or without ET was included in 33 studies overall; as neoadjuvant treatment in 21 RCTs (30%), adjuvant treatment in 9 RCTs (13%) and both neoadjuvant and adjuvant in 3 RCTs (4%). Targeted therapies (including CDK4/6, PARP, PI3K and AKT inhibitors) with or

without CT or ET was included as neoadjuvant treatment in 19 RCTs (27%) and as adjuvant treatment in 6 RCTs (8%). Immunotherapies with or without CT or targeted therapies were included as neoadjuvant treatment in 12 RCTs (17%). Antibody drug conjugates (SGN-LIV1A) were included in 1 RCT (1%). Primary endpoints included pathologic complete response (pCR: n=32; 45%), invasive disease-free survival (IDFS: n=8; 11%), disease free survival (DFS: n=5; 7%), objective response rates (ORR: n=5; 7%) and change in Ki67 expression (n=3; 4%). Event free survival (EFS) was not reported as a primary endpoint in any included trials. Median duration of follow-up ranged between 0.5 to 14.4 years across the included trials.
Conclusions: Heterogeneity was observed in terms of patient characteristics, treatment regimens, and outcome measures among clinical trials in perioperative setting. EFS, a relevant efficacy outcome measure for perioperative interventions, was not reported as a primary endpoint in the existing literature; to date clinical trials evaluating perioperative neoadjuvant plus adjuvant regimens in HR+/HER2- BC have been limited. An emerging trend for clinical trials investigating immunotherapy-based perioperative neoadjuvant plus adjuvant interventions was observed, reflective of the unmet need for innovative therapies among patients with early-stage high-risk HR+/HER2- BC.

P4-09-29: PR status cannot predict Oncotype DX Recurrence Score®: a study in a Greek cohort of N0M0/ER+/PR-/HER2- breast cancer patients tested with Oncotype DX

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Background: The Oncotype DX assay is a widely utilized genomic test that helps predicting the risk of breast cancer recurrence and the potential benefit of chemotherapy in early-stage, hormone receptor-positive (HR+) breast cancer patients. Low/absent expression of PR in ER-positive breast tumors is associated with more proliferative and aggressive disease, poorer prognosis and recurrence. However, the predictive value of progesterone receptor (PR) status in determining chemotherapy benefit remains unclear. This study focuses exclusively on PR-negative breast cancer patients to assess whether PR status can predict chemotherapy benefit using Oncotype DX scores.
Methods: We retrospectively analyzed data from a Greek cohort of pT1-3N0, cM0/ER+/PR-/HER2- breast cancer patients who underwent Oncotype DX testing between 2008 and 2020. Patients were categorized based on their Oncotype DX recurrence scores (RS) into

low (0-25) and high (>25) risk groups.
Results: A total of 268 PR-negative, ER+/HER2- patients were included in the analysis. The mean RS was 20.3. (Mean RS reported in TailorX study regardless of PR status was 18.2). RS 0-25 was reported in 130 (48,5%) of them, while 138 (51,5%) had a RS >25.
Conclusion: In PR-negative, pT1-3N0, cM0/ER+/HER2- breast cancer patients, PR status alone does not predict the RS and hence, the benefit of chemotherapy. The Oncotype DX assay effectively stratifies these patients, highlighting those with high RS (>25) who will benefit from chemotherapy. These findings underscore the importance of using Oncotype DX scores rather than PR status alone to guide chemotherapy decisions in PR-negative breast cancer patients.

P4-09-30: The Impact Of Online Education On Clinician Knowledge, Competence, And Confidence In Understanding the Latest Clinical Trial Data for HR+/HER2- Early Breast Cancer With a High Risk of Recurrence

Urvi Patel, Nabil Dorkham, Victoria Phoenix

Background: The treatment landscape in HR-positive, HER2-negative early breast cancer with a high risk of recurrence is constantly evolving with new data being regularly released at different congresses. Clinicians need to be updated regarding this data and its implications for clinical practice. Two global educational activities were developed that consisted of a 30-minute panel discussion between 2 experts and a 30-minute online enduring activity of a live symposium. We assessed the educational impact of the online educational activities.

Methods: A repeated-pairs pre-/post-assessment study design of knowledge, competence and confidence was used to assess impact, in which everyone served as their own control. A paired samples t-test assessed mean differences in the average number of correct responses to the matched pre-/post-assessment questions, and a McNemar's test assessed significance of improvement in single questions (and learning objectives) from pre- to post-assessment. P values <.05 are statistically significant. The first activity launched in January 2023 and the second activity in December 2023 and data was collected till January 2024.

Results: The analysis included 266 oncologists from the US and outside of the US who answered all the pre-/post-assessment questions. Each assessment question was mapped to an activity learning objective., which were grouped into three core topics. All core topics showed a significant improvement in education. The core topic knowledge of the latest data group had an n of 266, there was a relative change of 35% from pre-correct/confidence of 51% to post-correct/confidence of 69% ($p < 0.001$). For the core topic competence in applying the latest clinical trial data, the group had an n of 55, there was a relative change of 15% from pre-correct/confidence of 48% to 55% post-correct/confidence ($p < 0.05$). Lastly, the core topic confidence in interpreting the clinical implications of the latest data group had an n of 266, there was a relative change of 44% from pre-correct/confidence of

32% to post-correct/confidence of 46% ($p < 0.001$)
Conclusions: There was a significant improvement of oncologists' knowledge, competence and confidence related to clinical trial data. As new data is continuously being released at different congresses, regular educational activities to update clinicians are important to apply this knowledge in clinical practice.

P4-10-01: Testosterone & Tamoxifen (T&T) trial: adding Testosterone to Tamoxifen in male breast cancer patients

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Background: Male breast cancer (MBC) is very rare and has worse outcome compared to BC in women. MBC specific interventions are lacking. Non-adherence to mainstay tamoxifen treatment is common, due to feminizing side effects and reduced quality of life, and related to worse survival. Thus, well-tolerated endocrine treatment is an unmet need in MBC. MBC expresses both estrogen- and androgen receptors (ER and AR) in 97% of cases. As the AR functions as tumor suppressor in ER+ BC, adding testosterone to tamoxifen may improve both tumor response and tolerability in MBC. Therefore, we assessed the safety profile of tamoxifen plus testosterone in patients with metastatic MBC.
Methods: In this single-arm pilot study, patients received tamoxifen 20mg plus testosterone 25mg once a day, increased to 50mg after 3 weeks in case of good tolerance. Primary endpoint was safety (assessed as grade 3-4 adverse events (AEs) according to Common Terminology Criteria of Adverse Events (CTCAE) v5.0). Secondary endpoint was tumor response and duration (according to RECIST1.1) assessed after 8 weeks, thereafter every 12 weeks.
Results: Five MBC patients were enrolled with median age of 63 years, and mean two (1-3) previous endocrine treatment lines for metastatic MBC. All patients had received prior tamoxifen monotherapy, in either adjuvant or metastatic setting. No grade 3-4 AEs were observed. Two out of 19 grade 1-2 AEs were possibly related to the treatment: grade 1 anxiety and nausea, both spontaneously resolved. Other low grade AEs were probably related to (progressive) disease (n=7) or from another cause not likely related to the treatment (n=10). Regarding responses, 3/5 patients showed clinical benefit with in one patient a partial response duration of 10 months and in two patients ongoing stable disease at the time of analysis (resp. 12 and 15 months), 2/5 patients had progressive disease at 8 weeks. All patients spontaneously reported improved well-being.
Conclusions: In this unique MBC intervention trial, tamoxifen plus testosterone showed good safety, tolerability as well as long-term responses in the majority of patients. Therefore, this could be a promising new endocrine approach in this rare MBC population.

P4-10-02: RYZ101 (225Ac-DOTATATE) ± pembrolizumab in estrogen receptor-positive, human epidermal growth factor receptor 2-negative, locally advanced and unresectable or metastatic breast cancer progressing after prior therapy: the phase 1b/2 TRACY-1 study

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Background: RYZ101 (actinium-225 [225Ac]-DOTATATE) is a radiolabeled somatostatin analog (SSA) for the treatment of patients with solid tumors expressing somatostatin receptor-type 2 (SSTR2). RYZ101 is composed of the alpha-emitting radioisotope 225Ac, the chemical chelator DOTA (tetraxetan), and SSA octreotate (TATE). RYZ101 binds with high affinity to SSTR2 on the cell surface and is internalized, where the alpha-particle emission of 225Ac results in lethal double-strand DNA breaks. Although SSTR-directed therapy is widely used in well-differentiated gastroenteropancreatic neuroendocrine tumors (GEP-NETs), its use in non-GEP-NET SSTR-expressing neoplasms is still emerging. Clinical positron emission tomography (PET) imaging has reported SSTR expression in breast cancers and a correlation between tracer uptake and estrogen receptor (ER) expression. Available data support investigating the efficacy of RYZ101 in patients with ER+, HER2-negative, locally advanced and unresectable or metastatic breast cancer who are endocrine-refractory and have received prior chemotherapy and/or antibody–drug conjugates (ADCs). As preclinical evidence suggests that alpha-particle emitter radiopharmaceutical therapy (RPT) has immunostimulatory effects that may enhance anti-tumor activity of immunotherapy, the combination of RYZ101 and pembrolizumab will also be investigated.
Methods: TRACY-1 (IND#170007) is a global, multicenter, open-label, 3-part (dose escalation, randomization, expansion) phase 1b/2 study. Key inclusion criteria are: age ≥18 years; histologically confirmed, ER+, HER2-negative locally advanced and unresectable or metastatic breast cancer not amenable to treatment with curative intent; endocrine-refractory disease; documented progression (per RECIST v1.1) after ≥2 and ≤4 prior lines of chemotherapy or ADC (≥1 must be ADC); ≥1 RECIST-measurable SSTR–PET-positive lesion with ≥80% of RECIST-measurable lesions being SSTR–PET-positive on screening scan. Key exclusion criteria are: prior RPT, anti-PD-1/L1/L2 therapy or a prior agent directed to another stimulatory or co-inhibitory T-cell receptor; prior anticancer therapy in past 4 weeks or external beam radiotherapy in past 6 weeks. Primary objectives are to determine the recommended phase 2 dose (R2PD) of RYZ101 (dose escalation; anticipated n=6–18), the optimal treatment regimen of RYZ101 alone or in combination with pembrolizumab (randomization; n=30 per arm), and the efficacy of RYZ101 at the optimal treatment dose and regimen (expansion; n=100). During dose escalation, patients will receive RYZ101 by IV infusion every 6 weeks for up to 6 cycles at a starting dose of 6.5 MBq (Dose Level [DL] 1), with escalation to DL 2 (8.3 MBq) and DL 3 (10.2 MBq), or dose de-escalation to 4.6 MBq if DL 1 is not tolerated, based on dose-limiting toxicity rates. In randomization, RYZ101 will

be administered at the RP2D every 6 weeks for a total of 6 infusions; patients receiving combination therapy will also receive pembrolizumab 400 mg IV every 6 weeks for up to 2 years or until progression. In expansion, patients will receive RYZ101 alone or in combination with pembrolizumab as determined in randomization step. Concomitant amino acid IV infusions (containing L-arginine and L-lysine) will be co-infused with RYZ101 for renal protection. The study is expected to begin enrolment in July 2024.

P4-10-03: Potential risk factors for health-related quality of life (HRQoL) in palbociclib (PAL) plus endocrine therapy (ET) and ET alone patients with HR+/HER2- advanced breast cancer (ABC): exploratory analysis from 6-month longitudinal study (JBCRG-26)

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Background: PAL is a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) for the treatment of HR+/HER2- ABC. In phase 3 clinical trials, HRQoL was generally maintained and/or improved in patients (pts) treated with PAL+ET compared with placebo+ET. Previously, we reported that PAL+ET and ET monotherapy did not impact on HRQoL or physical activity (SABCS2023, PO1-05-06). Here, we explored potential risk factors related to HRQoL changes in the pts treated with PAL+ET or ET alone evaluated with a mobile application.
Methods: This prospective, observational, multicenter study was conducted in Japanese women with HR+/HER2- ABC initiating PAL+ET (Group 1) or ET alone (Group 2) in 1L/2L setting. HRQoL was assessed using EORTC-QLQ-C30 at baseline and Day 15 of each cycle via a smartphone-based app for 6 cycles (24 weeks). Patient data, including baseline characteristics, treatment, and adverse events, were collected via electronic case report forms. A multivariate mixed effects model was conducted to explore risk factors for HRQoL changes, including treatment group, cycle, baseline HRQoL, line of therapy, education, employment, menopausal status, ECOG-PS, TMN stage, BMI, visceral metastases, and dose reduction as fixed effects. This was exploratory analysis and a trend of GHS change and related risk factors were evaluated. Positive score of estimated mean change represents improvement of global health status (GHS), and negative score represents deterioration of GHS.
Results: Ninety-nine pts were enrolled (Group 1: 78, Group 2: 21). Pts had a median age of 56/52 years (Group 1/2); 51%/33% had visceral metastases; 59%/43% were stage IV at initial diagnosis; 36%/33% were fully employed. In each group, 95% (78 pts and 20 pts, respectively) completed the 6-cycle observation period. In the overall population for each group, GHS was maintained across the observation period. Based on the analysis, treatment cycle demonstrated an association with GHS change from baseline score in early cycles (Cycle 2: mean change=4.79, 95%CI 0.93-8.65,; Cycle 3: mean change=6.53, 95%CI 2.75-10.31, p=0.001). Later cycles 4-6 continued to show a trend toward improvement in QOL;

however these were less prominent. Additional trends included that CDK4/6i combination treatment (mean change=3.02, 95%CI -4.04- 10.07), first-line treatment (f mean change=2.87, 95%CI -4.18-9.93), and part-time employment (mean change=3.82, 95%CI -3.68- 11.32), which all showed improvement towards GHS from baseline; whereas, stage IV/other (mean change=-2.45, 95%CI -8.58- 3.65) and dose modification (mean change=-2.06, 95%CI -6.02-1.90) trended towards GHS decrease from baseline. Notably, by cycle 4, 91 pts remained on study (Group 1: 73, Group 2: 18).
Conclusion: In this Japanese cohort, a gradual improvement in HRQoL was observed in the initial cycles of PAL+ET and ET alone. Although this is a small cohort of pts, our exploratory data provide an initial hypothesis around which pts may experience a benefit/detriment in HRQoL. Further investigation is warranted. (NCT04736576)

P4-10-04: Utility of genomic testing and targeted treatment for patients with hormone receptor-positive metastatic breast cancer

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Background: Optimal sequencing of subsequent treatments after progression on initial hormone therapy plus CDK4/6 inhibitor (HT + CDK4/6i) in patients with hormone receptor-positive (HR+), HER2-negative metastatic breast cancer (MBC) has not been well established; however, we prefer to continue hormone-based therapy for as long as possible before switching to chemotherapy, due to its favorable toxicity profile. Next-generation sequencing (NGS) of tumor tissue or circulating tumor DNA (ctDNA) can reveal actionable alterations for targeted therapy. Several targeted agents have been approved for genes commonly mutated in MBC, including ESR1, PIK3CA, AKT, PTEN, ERBB2, and BRCA1/2, and many newer agents are under investigation in clinical trials. Consequently, we aimed to evaluate the use of genomic testing and the adoption of targeted therapies in patients with HR+, HER2-negative MBC and hereby present updated results.
Methods: Our retrospective cohort included patients with HR+, HER2-negative MBC who experienced progression after initial treatment with HT + CDK4/6i and received treatment at the Koontz Center for Advanced Breast Cancer (KCABC) at St. Luke's Cancer Institute. KCABC is a specialized clinic dedicated to the care of MBC patients. MBC patients underwent genomic testing using commercially available NGS assays. Clinically actionable alterations were defined as those with targeted treatment available as standard of care or as part of a clinical trial: PIK3CA, PTEN, AKT, ESR1, ERBB2, BRCA1, BRCA2, PALB2 mutations, and high TMB (≥10). A subgroup of patients received alteration-targeted therapy (ATT), and a comparison was made with those who did not. Baseline clinical characteristics and genomic testing results were described using summary statistics. Time to initiation of chemotherapy and overall survival (OS) were compared between groups using Kaplan-Meier analysis and the log-rank test.
Results: In the study, 136 HR+, HER2 negative MBC patients received treatment following initial HT + CDK4/6i treatment. Of these, 110 patients (80.8%) underwent genomic testing,

with 54 (39.7%) being tested before starting the second line of treatment. Of those tested, 95 (86.8%) were found to have a targetable alteration. Alteration-targeted therapy (ATT) was administered to 39 patients, and 17 patients received ATT as second-line therapy after HT + CDK4/6i. The most common targetable alterations identified were ESR1 (39.0%) and PIK3CA mutations (33.8%), followed by PTEN alterations (11.8%), BRCA1/2 (7.4%) and ERBB2 (5.1%) mutations. The most utilized ATT was alpelisib in 21 patients (53.8%), followed by neratinib in 6 (15.4%), elacestrant in 5 (12.8%), talazoparib in 3 (7.7%), olaparib in 2 (5.1%) and capivasertib in 2 (5.1%) patients. No significant difference was observed in time to initiation of chemotherapy or OS for patients who received ATT compared to those who did not.
Conclusions: A significant proportion of patients with HR+, HER2-negative MBC in our cohort underwent genomic testing after progression on initial treatment with HT + CDK4/6i, and the vast majority (86.6%) of these individuals were discovered to have at least one alteration that could be acted upon. We found no significant improvement in OS or time to initiation of chemotherapy for those who received ATT compared to those who did not. However, it is worth noting that the proportion of participants who received ATT was small. Only 39 patients (28.7%) received ATT, with alpelisib being the most common treatment. Since the approval of alpelisib in 2019, many new targeted agents have been in development. This includes two new agents approved in the past two years. These new treatments are poised to have better tolerability and potentially greater efficacy, which could lead to broader acceptance and more significant benefits of ATT in HR+ HER2-negative MBC.

P4-10-05: LITESPARK-029: A Phase 2, Randomized, Open-label Study of Belzutifan Plus Fulvestrant in Patients W/ Estrogen Receptor Pos, HER2-Negative Unresectable Locally Advanced or Metastatic Breast Cancer After Progression on Previous Endocrine Therapy

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Background: Endocrine-based therapy (ET), either alone or in combination with cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, prolongs progression-free survival (PFS) and overall survival (OS) in patients with metastatic hormone receptor positive (HR+) and human epidermal growth factor receptor 2 negative (HER2–) breast cancer. After progression on first-line therapy, next line therapy options provide limited gains in PFS owing in part to resistance mechanisms (e.g., hyperactive FOXA1). The transcription factor hypoxia-inducible factor 2α (HIF-2α), a major target of FOXA1, regulates key components of angiogenesis and subsequent development of metastasis. Preclinical studies show the ability of a HIF-2α antagonist to significantly suppress tumor growth, particularly when combined with fulvestrant. Belzutifan, a HIF-2α inhibitor, is approved for the treatment of patients with advanced renal cell carcinoma following a PD-(L)1 inhibitor and vascular endothelial growth factor tyrosine kinase inhibitor. LITESPARK-029 (NCT06428396) evaluates the efficacy and safety of belzutifan plus fulvestrant versus everolimus plus

fulvestrant or exemestane in patients with estrogen receptor positive (ER+)/HER2– unresectable locally advanced or metastatic breast cancer.
Methods: This phase 2, randomized, active-controlled, open-label, multicenter study is enrolling patients aged ≥18 years with centrally confirmed ER+/HER2– unresectable, locally advanced or metastatic disease. Eligible patients must have had radiographic disease progression on ≥12 months of ET plus CDK4/6 inhibitor therapy in the noncurative setting or received ≥2 lines of ET in the noncurative setting including CDK4/6 inhibitor where the CDK4/6 inhibitor was discontinued due to intolerance (not due to progression). Patients must also be eligible for additional ET with everolimus in combination with either fulvestrant or exemestane per local investigator assessment, have an Eastern Cooperative Oncology Group performance status of 0 or 1, have adequate organ function, and provide a new or recent core biopsy for central determination of ER and HER2 status. Previous treatment with chemotherapy, antibody-drug conjugates, or PARP inhibitors in the noncurative setting is prohibited. Patients are randomized 1:1 to receive belzutifan 120 mg orally once daily plus fulvestrant 500 mg on days 1 and 15 of cycle 1 and on day 1 of all subsequent 28-day cycles or everolimus 10 mg orally once daily plus fulvestrant (as above) or exemestane 25 mg orally once daily until disease progression or unacceptable toxicity. Randomization is stratified based on treatment with previous ET plus CDK4/6 inhibitor therapy (<18 months duration prior to progression vs ≥18 months duration prior to progression or no progression). Tumor imaging is performed at screening, every 8 weeks from randomization through week 56, and every 12 weeks thereafter. The primary endpoint is PFS per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 by blinded independent central review (BICR). Secondary endpoints include PFS rate per RECIST version 1.1 by BICR at 6 and 12 months, OS, objective response (complete response [CR] or partial response [PR]) per RECIST version 1.1 by BICR, clinical benefit (CR, PR, or stable disease for ≥24 weeks), and safety. The study start date was July 2024.

P4-10-06: Preclinical characterization of BGB-43395, a potential best-in-class CDK4 selective inhibitor with potent pharmacodynamic and anti-tumor activity in HR+HER2- breast cancer models

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Cyclin-dependent kinase (CDK) 4/6 inhibitors (palbociclib, ribociclib and abemaciclib) in combination with endocrine therapies have become the standard of care for patients with metastatic hormone receptor-positive, HER2-negative breast cancer (HR+HER2- BC). However, HR+HER2- BC is primarily dependent on CDK4, while CDK6 inhibition by dual CDK4/6 inhibitors often leads to dose-limiting neutropenia, which requires treatment holidays or dose reductions, thus limiting sustained CDK4 inhibition. Therefore, BGB-43395, a CDK4 selective inhibitor, was developed to reduce neutropenia by sparing CDK6,

thereby maximizing CDK4 inhibition to further improve clinical benefit.
BGB-43395 is a highly potent CDK4 kinase inhibitor with high selectivity over CDK6 and other CDK family kinases at biochemical level. In addition, BGB-43395 also demonstrated great selectivity against a panel of other kinases. These properties translated into a desirable toxicity profile in nonclinical toxicity studies, where BGB-43395 was well tolerated without concerning of neutropenia and gastrointestinal toxicity issues.
In the biochemical assay, BGB-43395 exhibits superior kinase inhibition against CDK4 compared to approved CDK4/(6) inhibitors (palbociclib, ribociclib and abemaciclib) and investigational CDK4 inhibitor PF-07220060. The potency of BGB-43395 was further determined by RB1 phosphorylation inhibition in human breast cancer cell lines. Compared to PF-07220060 and approved CDK4/6 inhibitors, BGB-43395 demonstrated more potent inhibition of RB1 phosphorylation (pRB1-S780) in CDK4-dependent HR+HER2- BC cell lines. As a result, BGB-43395 showed greater anti-proliferative activity in HR+HER2- BC cell lines as well as other cancer cell lines including prostate, ovarian, endometrial and lung cancer.
The in-vivo pharmacodynamic and anti-tumor activity of BGB-43395 were further evaluated in CDK4-dependent tumor models. BGB-43395 monotherapy demonstrated significant inhibition of RB1 phosphorylation in a dose-dependent manner in MCL Jeko1 and HR+HER2- MCF7 mouse xenograft tumors. BGB-43395 monotherapy treatment resulted in a greater tumor growth inhibition than palbociclib at clinically relevant dose in Jeko1 xenograft models. BGB-43395 in combination with fulvestrant also demonstrated a greater tumor growth inhibition compared to palbociclib in combination with fulvestrant in HR+HER2- MCF7 xenograft models.
In summary, BGB-43395 is a potential best-in-class CDK4 inhibitor with high potency and selectivity over CDK6 and other kinases, providing an opportunity to achieve high exposure and thus maximum on-target CDK4 inhibition for the treatment of HR+HER2- breast cancer and other CDK4 dependent cancers. BGB-43395 is currently undergoing clinical investigation as monotherapy or in combination with endocrine therapies in patients with metastatic HR+HER2- BC and other advanced solid tumors (NCT06120283).

P4-10-07: Evaluation of pharmacokinetics and safety of imlunestrant in participants with hepatic impairment

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Background: Imlunestrant is a next-generation, oral selective estrogen receptor (ER) degrader designed to deliver continuous ER-target inhibition. Imlunestrant is under study for the treatment of ER+ advanced breast and endometrial cancers. Hepatic impairment (HI) is a common condition, particularly in cancer patients, and it can alter the pharmacokinetics (PK) of anticancer drugs, impacting their safety. Given that the intended patient population for imlunestrant may include cancer patients with HI, it is crucial to determine whether HI can impact the imlunestrant PK and safety profile. Here we present

PK and safety data of imlunestrant in postmenopausal females of nonchildbearing potential (FONCBP) with and without HI following a single oral dose in a fasted state.
Methods: This phase 1, open-label, 3-site study was conducted between July 2022 and February 2024 in FONCBP with normal hepatic function or HI. Participants (pts) were assigned to 4 different treatment arms, according to the Child-Pugh (CP) score: Group (G) 1 - normal hepatic function; G2: mild HI; G3: moderate HI, and G4 - severe HI. Pts were screened 28 days prior to enrollment, admitted to a clinical research unit (CRU) on Day -1, where they remained resident for PK and safety assessment following drug administration. In G 1, 2 and 3, pts received a single dose of 400 mg of imlunestrant, whereas pts in G4 received a single dose of 200 mg, while fasted. Plasma samples were collected for PK analyses. Key endpoints included PK parameters (AUC(0-tlast), AUC(0-∞), and Cmax) and safety.
Results: Twenty-seven females (G1: n=9; G2: n=6; G3: n=6; G4: n=6) were included in the study (age: 45-71 years). Compared to pts with normal hepatic function, the AUC(0-tlast) geometric least square mean (GLSM) ratios for pts with mild or moderate HI by the CP scores were 1.2 (90% confidence interval (CI); 0.82, 1.8) and 2.2 (1.5, 3.3), respectively. Compared to pts with normal hepatic function, the dose-normalized (DN)-AUC(0-tlast) in pts with severe HI by the CP scores was 2.9 (90% CI;1.8, 4.7). The AUC (0-∞) GLSM ratios were 1.2 (90% CI; 0.8, 1.8) and 2.2 (90% CI; 1.5, 3.3) for the mild and moderate HI groups, respectively. The AUC(0-∞) normalized ratio of the severe HI group was 3.1 (90% CI; 1.9, 4.9). The imlunestrant Cmax GLSM ratio was similar between pts with normal hepatic function and those with mild (1.3 (90% CI; 0.8, 2.0)), moderate (1.5 (90% CI; 1, 2.4)) and severe (1.6 (90% CI; 1, 2.7)) HI. The median elimination half-life was 33.1 hours in pts with normal hepatic function, 42.8, 46.3 and 67.0 hours in pts with mild, moderate and severe HI, respectively. Additional exploratory analysis of PK parameters based on the National Cancer Institute (NCI) classification of HI, showed there were significant differences in imlunestrant PK when administered to pts with mild and moderate HI compared to pts with normal hepatic function. Only 1 pt was categorized with severe HI by NCI classification as such, this group was excluded from NCI based analyses. The average fraction unbound in plasma was similar across groups. Most TEAEs were mild or moderate in severity. TEAEs were reported by 2 pts with moderate HI and severe HI, respectively. Nausea and headache were the only TEAEs reported by more than 1 pt.
Conclusions:
Imlunestrant administered as a single oral dose in the fasted state was well tolerated in healthy FONCBP, as well as pts with mild, moderate and severe HI determined by the CP classification. There were no significant differences in the exposure profiles of imlunestrant in pts with mild HI in comparison to pts with normal hepatic function. However, in pts with moderate and severe HI, statistically significant increases in imlunestrant AUC (but not Cmax) were observed when compared with normal hepatic function. This data will inform the recommendations for dosing patients with HI under treatment with imlunestrant.

P4-10-08: Impact of an Online Educational Activity on Oncologists' Knowledge and Confidence Regarding the Management of HR-Positive/HER2-Negative Metastatic Breast Cancer

Zhizhi Fiske, Stephen Dunn, Deborah Grainger, Eloise Ballard, Jamie Habib, Peter Schmid

Background: The management of HR+/HER2− metastatic breast cancer (mBC) is evolving with the introduction of novel therapies, particularly in the post-CDK 4/6 inhibitor setting. The objective of this study was to assess if an online continuing medical education (CME) activity could improve oncologists' knowledge of the latest evidence regarding novel and emerging therapies for HR+/HER2− mBC.
Methods: This educational activity consisted of a 30-minute video presentation with synchronized slides, including a whiteboard animation illustrating the mode of action of novel agents. Educational effect was assessed using a repeated-pair design with pre-/post-assessment. 3 multiple choice questions assessed knowledge, and 1 question rated on a Likert-type scale assessed confidence, with each individual serving as their own control. A chi-squared test assessed significance of improvement in the percentage of correct responses to knowledge questions from pre- to post-assessment. P values < .05 are statistically significant. The activity launched on 25th of August 2023, with data collected through 11th December 2023 being reported in the current study.
Results: The analysis set consisted of responses from oncologists (n=59) who answered all assessment questions during the study period. Analysis of pre- vs post-intervention responses demonstrated a significant improvement in oncologists' overall knowledge (P< .01). Overall correct responses increased from 57% pre- to 75% post-CME. Specific areas of improvement include:

- Knowledge of the latest clinical data of emerging therapies for HR+/HER2− mBC (pre 65%, post 80%; P < .01)
- Knowledge of the mode of action of novel agents for HR+/HER2− mBC (pre 41%, post 64%; P < .01)

After education, 44% of oncologists had a measurable increase in confidence in their ability to identify patients suitable for participation in clinical trials investigating novel treatment, and that increase was, on average, 64%.
Conclusions: This study demonstrates the success of an online, 30-minute CME activity on improving the knowledge and confidence of oncologists regarding the evolving treatment landscape of HR+/HER2− mBC, including in areas where many clinicians appeared to have relatively high knowledge levels at baseline. However, as new data continue to emerge, it will be important to provide ongoing education to clinicians so that they feel confident in interpreting the clinical evidence and translating it into practice.

P4-10-09: Genomic Profiles of Early Progressors vs Exceptional Responders on CDK4/6i in ER+ HER2- Advanced Breast Cancer

Tim Kong, Shana Thomas, Andrew Davis, Emily Podany, Jing Xi, Cynthia Ma, Katherine Clifton

Background: CDK4/6 inhibitors (CDK4/6i) paired with endocrine therapy (ET) remain first-line (1L) therapy for patients (pts) with hormone receptor positive (HR+) HER2 negative (HER2-) advanced breast cancer (aBC). A subset of pts will demonstrate primary resistance to CDK4/6i, as characterized by early progression, while other patients will remain on CDK4/6i for an extended duration prior to progression. We sought to examine clinical and genomic differences between a cohort of early progressors and exceptional responders.
Methods: Pts with HR+ HER2- aBC from a phase II trial of an alternative schedule of palbociclib (palbo alt dosing trial NCT 3007979) and from a retrospective CDK4/6i study were included in this analysis. Pts in the retrospective CDK4/6i study included pts receiving CDK4/6i as part of standard of care (SOC) first-line therapy for HR+ HER2- aBC at Washington University in Saint Louis from 2016 to 2024. Clinical information, including treatment start and stop dates, was collected from the electronic medical record. Progression-free survival (PFS) was estimated by the treatment duration on a specified treatment regimen. Early progression (EP) on CDK4/6i was defined as PFS < 6 months (mo) and late progression (LP) was defined as PFS > 48 mo. Overall survival (OS) was defined as time to death from the initiation of CDK4/6i. NGS testing was performed using the Guardant360 or Tempus platforms per SOC at time points per the treating physician's discretion in the retrospective CDK4/6i study patients and Whole Exome Sequencing (WES) of circulating tumor DNA was performed on the palbo alt dosing trial patients at baseline and progression.
Results: Of the 54 pts enrolled on the palbo alt dosing trial, 10 experienced EP and had WES available. The median age of EP pts with WES was 64 years at time of metastatic diagnosis and the majority (6/10) had recurrent [AD1] disease with visceral involvement (8/10). The median PFS of EP pts with WES was 3.1 mo and OS was 14.5 mo. Of retrospective study pts, 20 patients had EP on CDK4/6i, 10 of which had SOC NGS testing during at least one time point. The median age of EP pts with SOC NGS testing was 61 years at time of metastatic diagnosis and the majority (8/10) had recurrent [AD2] disease with visceral involvement (6/10). The majority of these pts received palbo (9/10) paired with letrozole (9/10) and did not have recurrence on adjuvant endocrine therapy (6/10). The median PFS of EP pts with SOC NGS was 3.9 mo and OS was 22.3 mo. Of the 38 pts on the retrospective study with LP on CDK4/6i[EP3], 17 were excluded as they were still receiving therapy. 10 pts with LP had NGS testing available. The median age these pts was 64 at time of metastatic diagnosis and the majority (7/10) of the LP pts had recurrent disease with bone only involvement (6/10). The majority of these pts received palbo (10/10) paired with letrozole (8/10) and did not have recurrence on adjuvant endocrine therapy (8/10). The median PFS was 65.7 mo and most patients (6/10) were alive at data analysis. When analyzing genomic differences between the EP and LP pts, there was no significant difference in rates of PIK3CA, ESR1, TP53, and ATM mutations. More patients in the EP cohort had amplification

(amp) of AR or AR mutations of uncertain significance (4/14 samples), CCND1 amps (2/14) and GATA3 mutations (2/14) at any time point. More patients in the LP cohort had RB1 (3/15 samples), GNAS (3/15), KRAS (2/15), EGFR (2/15) mutations and FGFR1 amps (2/15) at any time point.Frequencies of ESR1 alterations were similar in WES baseline (5/10), SOC EP (5/11), and SOC LP (5/9) samples, but less frequent in WES progression samples (1/10).
Conclusions: Early progression on CDK4/6i is associated with a particularly poor prognosis; however, there are patients with exceptional response to CDK4/6i who may remain on therapy for an extended time. There were variations in the mutation profiles between the two cohorts, though this data set was limited in size. Additional analysis of genomic variants is needed to identify profiles of patients who may significantly benefit from CDK4/6i.

P4-10-10: Changing treatment paradigms and unmet needs in patients with hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2-) metastatic breast cancer (mBC): Results from health records data

Alberto Montero, Di He, Miguel Miranda, Luis Berrocal-Almanza, Timothy Pham, Sam Hillman, Clara Chen

Background: Despite treatment advances for patients with HR+, HER2− mBC, most tumors acquire resistance, leading to disease progression. Outcomes worsen with subsequent treatment, underscoring a need for novel treatments that prolong disease control with earlier lines of therapy. We aim to describe treatment paradigms and real-world clinical outcomes in patients with HR+, HER2− mBC.
Methods: This retrospective cohort study included US adult patients from the Flatiron Health oncology database who were diagnosed with HR+, HER2− mBC between January 2017 and March 2023 and received any systemic oncologic therapy. Eligible patients were grouped into 3 cohorts according to the year of mBC diagnosis: 2017–2018, 2019–2020, or 2021–2023. Patients were followed through September 2023. Baseline characteristics at mBC diagnosis and treatment patterns were summarized using descriptive statistics. The unadjusted Kaplan–Meier method was used to estimate time to treatment discontinuation (TTD) and overall survival (OS). A Cox regression analysis was used to determine hazard ratios for OS. Inverse propensity weighting was used to eliminate confounding factors (age, sex, race, region, practice type, payer type, de novo vs recurrent disease, and ECOG performance score).
Results: Of 6,838 eligible patients (median age, 65 y; 99% female), 38% were initially diagnosed with de novo mBC, and 81% were treated at a community practice. The most prescribed first-line (1L) treatments overall were CDK4/6 inhibitor (CDK4/6i)-containing regimens (57%), single-agent endocrine therapy (ET; 28%), and chemotherapy (CT; 14%). From 2017–2018 to 2021–2023, CDK4/6i-containing regimen use in the 1L increased from 49% to 62% of patients. Median TTD for 1L CDK4/6i plus an aromatase inhibitor was 12.7 mo (95% CI: 12.0–13.4), with 28% and 48% of patients discontinuing therapy by 6 mo and

12 mo, respectively. In the second-line (2L; n=3,841), the main treatments overall were CDK4/6i-containing regimens (49%), CT (25%), and single-agent ET (16%). CDK4/6i-containing regimen use in the 2L increased from 45% in 2017–2018 to 56% in 2021–2023. Of patients who received 2L CDK4/6i-containing regimens, 54% were new to CDK4/6i, and 46% were rechallenged with CDK4/6i (i.e., switched to another CDK4/6i and/or changed ET). In the third-line (3L; n=2,050), treatments overall included CT (35%), CDK4/6i-containing regimens (32%), single-agent ET (16%), mTOR inhibitor–containing regimens (8%), and PI3Kα-specific inhibitor–containing regimens (5%). CDK4/6i-containing regimen use in the 3L increased from 31% in 2017–2018 to 36% in 2021–2023. Of patients who received 3L CDK4/6i-containing regimens, 33% were new to CDK4/6i, and 67% were rechallenged with CDK4/6i. Across treatment lines, median OS was similar in 2017–2018, 2019–2020, and 2021–2023 (41.4 mo, 42.0 mo, and not reached, respectively; p=0.9956). Relative to 2017–2018, mortality risk was comparable in 2019–2020 (HR 1.00; 95% CI: 0.92–1.09) and 2021–2023 (HR 1.01; 95% CI: 0.90–1.14).
ConclusionsCLUSIONS: CDK4/6i-containing regimens were the most used 1L treatment for patients with HR+, HER2– mBC; however, about half of patients discontinued CDK4/6i treatment within 1 year, suggesting the need for novel therapies that extend 1L treatment time and improve outcomes. There was no predominant treatment used in the 2L or beyond; CDK4/6i reuse was consistently observed. CT use increased when patients progressed to subsequent lines, suggesting that the percentage of patients who no longer responded to current standard of care increased. Despite increased use of CDK4/6i beyond the 1L, mortality has not improved in real-world practice since 2017, highlighting the need for novel therapies to improve outcomes.

P4-10-11: The Impact of Live and Online Education on Clinical Knowledge, Competence, and Confidence in the Use of Novel Antibody–Drug Conjugate Therapy for HR-Positive Breast Cancer

Zhizhi Fiske, Stephen Dunn, Deborah Grainger, Jamie Habib, Javier Cortés

Background: Antibody–drug conjugates (ADCs) are rapidly changing the treatment landscape for advanced breast cancer and are now being explored in earlier disease setting. The objective of this study was to assess the effect of an online continuing medical education (CME) activity on clinicians' knowledge in the clinical data on novel ADCs for the treatment of HR-positive breast cancer, competence in managing patients on treatment with an ADC, and confidence in their ability to integrate novel ADC therapy into clinical practice for patients with HR-positive advanced breast cancer.
Methods: This educational activity consisted of a 90-minute live symposium and video enduring activity with a downloadable slide deck. Educational effect was assessed using a repeated-pair design with pre-/post-assessment. 3 multiple choice questions assessed knowledge, and 1 question rated on a Likert-type scale assessed confidence, with each individual serving as their own control. A McNemar's test assessed significance of improvement in the percentage of correct responses to knowledge questions from pre- to

post-assessment. P values < .05 are statistically significant. The live symposium took place on 20th of October, 2023 and the enduring activity launched on 20th of November, 2023, with data collected through 12th April, 2024 being reported in the current study.
Results: 50 oncologists and 20 surgeons who answered all the assessment questions were included in this analysis. Both physician groups demonstrated an improvement in knowledge and competence across all 3 learning themes:

- Knowledge of the latest clinical trial data: correct responses from oncologists increased from 44% pre-CME to 58% post-CME, P < .05; correct responses from surgeons increased from 30% pre-CME to 44% post-CME, P < .05
- Knowledge of ongoing clinical studies: correct responses from oncologists increased from 32% pre-CME to 44% post-CME, P < .05; correct responses from surgeons increased from 16% pre-CME to 40% post-CME, P < .01
- Competence related to managing patients receiving ADC therapy: correct responses from oncologists increased from 26% pre-CME to 55% post-CME, P < .001; correct responses from surgeons increased from 18% pre-CME to 26% post-CME, P < .248

All increases are statistically significant expect Surgeons' competence related to managing patients on ADC therapy.
Additionally, 36% of oncologists and 36% of surgeons reported increased confidence in integrating ADC therapy into clinical practice for patients with HR-positive advanced breast cancer, and that increase was, on average, 89% and 86% among the two physician groups, respectively.
Conclusions: This analysis demonstrates the positive educational impact of a live and subsequent online CME activity on clinicians' knowledge, competence and confidence regarding ADC therapy for breast cancer. As the treatment landscape continues to evolve in this field, it is important to provide ongoing education to clinicians so that they feel confident and competent when applying novel therapies in clinical practice.

P4-10-12: Abemaciclib monotherapy after disease progression on prior CDK4/6 inhibitors in patients with metastatic hormone-receptor positive breast cancer

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Introduction: Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) in conjunction with endocrine therapies have transformed the treatment landscape for patients with metastatic hormone-receptor positive (HR+)/HER2- breast cancer. Studies exploring the clinical utility of CDK4/6i re-introduction after disease progression on prior CDK4/6i-based therapy have yielded mixed results, including the recent phase III postMONARCH trial (which interrogated the combination of fulvestrant and abemaciclib in the second-line metastatic setting). Here, we explore the clinical outcomes of abemaciclib monotherapy after disease progression on prior combined CDK4/6i and endocrine therapy.

Methods: We collected retrospective clinical data at two academic institutions (Massachusetts General Hospital and Barnes-Jewish Hospital) according to site-specific IRB-approved protocols from patients with metastatic HR+/HER2- breast cancer who had received abemaciclib monotherapy after disease progression on another CDK4/6i-based therapy in the metastatic setting. We summarized patient and treatment characteristics and conducted time-to-event analyses.
Results: In this preliminary analysis, a total of 16 patients received abemaciclib monotherapy after disease progression on prior CDK4/6i-based therapy. All 16 received prior palbociclib-based therapies. Eleven of these patients continued abemaciclib until disease progression or death, while five patients stopped therapy due to toxicity (three due to gastrointestinal side effects, one due to fatigue, and one due to atrial fibrillation). Patients received palbociclib-based therapy for a median of 16.2 months and abemaciclib for a median of 3.8 months (95% confidence interval, 2.0-7.5 months). Five patients received abemaciclib for >180 days prior to disease progression/death. Conversely, only two patients discontinued abemaciclib prior to 90 days after initiation due to disease progression. Four out of the 16 patients received abemaciclib as the subsequent line of treatment immediately following progression on palbociclib. The median time from progression on palbociclib-based therapies to starting abemaciclib was 10.7 months, with a median number of two intervening lines of therapy. Efforts are underway to combine this cohort with additional patients receiving abemaciclib monotherapy in this setting at other institutions to enhance clinical sample size. Planned analyses will include retrospective review of radiographic images to estimate overall response rate and exploration of genomic sequencing to identify molecular mediators of response and resistance to abemaciclib monotherapy. These ongoing efforts will be presented at the meeting.
Conclusions: A subset of patients tolerated abemaciclib monotherapy for > 180 days before progression/death despite progression on prior combination palbociclib and endocrine therapies. This is the first effort to interrogate the utility of abemaciclib monotherapy in this setting and will provide additional insights related to serial CDK4/6-directed therapy in this patient population. These results demonstrate clinical promise in continued CDK4/6 inhibition and raise questions about mechanisms of resistance and the identification of patients that would benefit from abemaciclib monotherapy.

P4-10-14: Real-world palbociclib dose adjustment and outcomes in HR+/HER2- metastatic breast cancer: Flatiron database analysis

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Background: A cyclin dependent kinase 4/6 inhibitor (CDK4/6i) in combination with endocrine therapy has become standard of care for HR+/HER2- advanced/metastatic breast cancer (MBC). CDK4/6i dose adjustment is recommended based on individual safety and tolerability during the treatment of MBC. Clinical trial data demonstrated that Palbociclib (PAL) dose adjustment had no significant impact on progression-free survival (PFS). Small

real-world studies have not demonstrated consistent outcomes associated with PAL dose adjustment, including real-world PFS (rwPFS) and overall survival (OS). Large real-world studies with longer follow-up are needed to understand patient characteristics and clinical outcomes associated with CDK4/6i dose adjustment. This study examined PAL dose adjustment and outcomes in HR+/HER2- MBC in routine clinical practice.
Methods: Using Flatiron Health longitudinal database, we conducted a retrospective analysis of HR+/HER2- MBC patients who started PAL plus an aromatase inhibitor (AI) as first-line therapy between February 2015 and March 2020 (index period). Patients were assessed from start of PAL+AI to September 30, 2020 (data cutoff), death, or last medical activity, whichever came first. Dose adjustment was defined as a change of PAL daily dose compared to initial/previous prescription dose. Treatment duration was defined as months from start of PAL+AI to end of the treatment. OS was defined as months from start of PAL+AI to death. rwPFS was defined as months from start of PAL+AI to death or disease progression, evaluated based on clinical assessment or radiographic scan/tissue biopsy. Kaplan-Meier analysis was used to estimate treatment duration, rwPFS, and OS. Multivariable Cox proportional hazard regression models were performed to adjust for baseline characteristics: age, sex, race/ethnicity, healthcare practice type, initial diagnosis, Eastern Cooperative Oncology Group Performance Status, National Cancer Institute-Comorbidity Index, disease free interval from initial breast cancer to MBC diagnosis, bone only disease, lung/liver involvement, and number of metastatic sites.
Results: A total of 1,324 patients received PAL+AI during the index period. Mean age was 67.1 years (SD=9.6), 99.2% were female, and 68.0% were white. Of these patients, 1,110 (83.8%) initiated PAL at 125 mg/day, 144 (10.9%) at 100 mg/day, 48 (3.6%) at 75 mg/day, and 22 (1.7%) did not have information about initial dose. Among 1,302 patients who had initial dose documented, 524 (40.3%) experienced dose adjustment. Comparted with patients without dose adjustment, those with dose adjustment were more likely to be white (71.8% vs 65.8%) and had a higher proportion of lung/liver involvement (35.5% vs 32.4%). Median follow-up was 28.5 and 22.6 months in patients with and without dose adjustment, respectively. Median treatment duration was longer in patients with dose adjustment than in those without dose adjustment (27.4, 95%CI = 24.5–30.6 vs 21.4, 95%CI = 19.7–26.5 months). Patients with and without dose adjustment showed similar median rwPFS (20.5, 95%CI=17.8–25.9 vs 19.6, 95%CI=16.9–21.7 months; unadjusted HR = 0.90, 95%CI = 0.77–1.04, p=0.162; adjusted HR = 0.89, 95%CI = 0.76–1.04, p=0.133). Median OS was significantly prolonged in patients with dose adjustment than in those without dose adjustment (57.8, 95%CI=49.0–NA vs 51.4, 95%CI=45.3-58.7 months; unadjusted HR = 0.72, 95%CI = 0.59–0.88, p=0.001; adjusted HR = 0.73, 95%CI = 0.59–0.89, p=0.002).
Conclusions: Similar to other CDK4/6is, PAL dose adjustment is common in the treatment of HR+/HER2- MBC. PAL dose adjustment did not have significant effect on rwPFS but was associated with prolonged treatment duration and OS. Further research is needed to confirm these findings and understand the reasons for PAL dose adjustment in real-world settings.

P4-10-15: Evaluating the APIS ESR1 Mutations Kit: Performance and LoD Testing in Varied Wildtype Backgrounds

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Introduction: Mutations in the estrogen receptor 1 (ESR1) gene are crucial for understanding and predicting resistance to endocrine therapy in ER-positive breast cancer. The APIS ESR1 Mutations Kit (APIS Assay Technologies, Manchester, UK) offers a cost-effective, rapid, and highly sensitive qPCR assay designed to detect eleven ESR1 mutations (E380Q, S463P, P535H, L536R/Q/H/P, Y537C/S/N, and D538G). The kit is optimized for use with DNA from plasma (cfDNA) or FFPE tissue. This study evaluates the performance of the APIS ESR1 Mutations Kit, particularly its sensitivity in detecting ESR1 mutations in varied wildtype backgrounds.
Methods: DNA fragments encoding all ESR1 mutations detected by the APIS kit, were spiked into wildtype (WT) DNA background. The Limit of Blank (LoB) and Limit of Detection (LoD) for each mutation were determined using approximately 5,000 copies WT background DNA. To evaluate the effect of higher WT backgrounds on LoD and LoB, samples with a 0.5% mutant allele frequency (MAF) were prepared with higher WT DNA backgrounds (approximately 10,000 and 50,000 copies). This approach aimed to highlight the importance of assessing sensitivity based on the copy number rather than %MAF. A dilution series of DNA fragments, ranging from 5 to 10,000 copies per reaction, was analyzed to determine linearity. The kit's performance was further assessed using the SensID ESR1 Reference Set 1% AF cfDNA (SensID GmbH, Rostock, Germany). All PCR runs were conducted using a QuantStudio™5 Dx Real-Time PCR System.
Results: The APIS kit successfully detected all mutations at ≤ 1.0% MAF in WT background of approximately 5,000 copies, detecting the most prevalent mutations, D538G and Y537S, at 0.4% and 0.1% MAF, respectively. In higher WT backgrounds, mutations D538G, Y537S/C/N, L536H/Q and P535H were detected in 0.5% MAF samples with approximately 10,000 copies background. In approximately 50,000 copies background, mutations D538G, Y537S/C/N, E380Q and P535H were detected in 0.5% MAF samples. No false positive calls were observed for any mutations, regardless of the WT background. Linearity was maintained within 90-110% across a range of 50 to 10,000 copies per reaction. When tested with the SensID ESR1 Reference Set, the APIS kit accurately detected all ESR1 mutations, with no false positives calls.
Conclusions: The APIS ESR1 Mutations Kit exhibited high sensitivity and specificity as a qualitative qPCR assay for detecting ESR1 mutations in varying wildtype backgrounds. Additionally, testing with the SensID ESR1 Reference Set 1% AF cfDNA confirmed the kit's performance and LoD at ≤ 1% MAF with external material. The APIS kit is a valuable tool for assessing ESR1 mutations in both clinical and research settings, offering a more accessible alternative to traditional NGS and dPCR assays.

P4-10-16: INX-315, an oral, potent and selective CDK2 inhibitor in patients with CDK4/6 inhibitor resistant ER+/HER2- breast cancer or CCNE1 amplified solid tumors: phase 1 monotherapy dose escalation

Antoinette Tan, Antonio Giordano, Catherine Shannon, Carey Anders, Nashat Gabrail, Kevin Kalinsky, Patrick Roberts, George Au-Yeung

Background: Cyclin-dependent kinases (CDK) are a family of serine/threonine kinases that heterodimerize with regulatory subunits called cyclins to drive cell cycle progression, cell division, and associated biological processes. CDK2 plays a crucial role in promoting G1/S transition and S phase progression. Dysregulated CDK2 activity commonly occurs through amplification of CCNE1 (gene that encodes cyclin E1 protein) and/or overexpression of cyclin E1, and mutations that inactivate CDK2 endogenous inhibitors (e.g., p27), respectively. INX-315 is an oral, potent, and selective small molecule CDK2 inhibitor in early clinical development with best-in-class potential.
Methods: NCT05735080 is a first-in-human study designed to evaluate the safety, tolerability, pharmacokinetics (PK) and preliminary antitumor activity of INX-315 in patients with recurrent advanced/metastatic cancer, including estrogen receptor positive (ER+)/Human Epidermal Growth Factor Receptor 2 Negative (HER2-) breast cancer who progressed on a prior CDK4/6 inhibitor (CDK4/6i) regimen and patients with CCNE1 amplified solid tumors (defined by next generation sequencing), including high grade serous ovarian cancer (HGSOC), who progressed on prior standard of care treatment. This study is evaluating dose levels of INX-315 QD in 28-day cycles in Part A, with dose escalation utilizing a Bayesian Optimal Interval Design (BOIN) to select dose levels for the Expansion Phase. Blood samples for PK and circulating biomarker analyses were collected.
Results: By June 27, 2024, 27 patients were enrolled in 5 dose cohorts with increased dose levels (100 mg to 600 mg) and are included in the safety analysis. Median age was 60 years old (range 29-78), 78% were female, and 78% white. Tumor types studied include ER+/HER2- Breast Cancer (n=8), HGSOC (n=10), and other solid tumors (n=9). Median prior lines of therapy were 5 with a range of 2-10. At the time of data cut, 11 patients were receiving treatment, of which 2 are ER+/HER2- breast cancer, 13 discontinued treatment due to progression of disease (PD), 2 withdrew consent, and 1 physician decision. No discontinuations due to adverse events (AEs) were observed. The most frequent treatment-related adverse events occurring in ≥15% of cases, included decreased platelet count (48%), nausea (37%), decreased neutrophil count (29%), anemia (26%), diarrhea (26%), vomiting (22%) and decreased white blood cells (15%). Treatment-related Grade 3 AEs included fatigue (7%) and decreased white blood cell count (7%), as well as diarrhea, anemia, and decreased neutrophil count (4% each). Two treatment-related grade 4 AEs were decreased neutrophil count and decreased platelet count (4%). At the time of the data cut off, two patients (ER+/HER2- breast cancer and CCNE1 amplified HGSOC) have confirmed partial responses. Additionally, 14 pts (63.6%) had a best response of stable disease, with 4 patients (2 ER+/HER-) achieving at least a 20% tumor reduction. INX-315 plasma concentrations increased with dose and achieved 24-hour trough concentrations ≥ the predicted effective concentrations (Ceff) starting at the first dose level (100 mg QD).

Pharmacodynamic studies are ongoing.
Conclusions: INX-315 monotherapy at increasing QD dose levels was generally well tolerated and demonstrated monotherapy antitumor activity in heavily pretreated patients with ER+/HER2- breast cancer and CCNE1amplified solid tumors. Dose escalation and optimization is ongoing to determine the dose levels to be further evaluated in monotherapy (CCNE1 amplified HGSOC) and combination (ER+/HER2- breast cancer) expansion parts of the study.
Clinical trial information: NCT05735080.

P4-10-17: An Analysis of the Breast Cancer Vaccine Landscape

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Background: Cancer vaccines and the idea of using the immune system to improve cancer treatment has a long history, and until recently, has been met with limited success. However, advances in technology and improved understanding of the immune system have led to promising vaccine approaches to both treating and preventing cancer. This analysis explores the evolution of breast cancer (BC) vaccines.
Methods: We conducted a systematic search of clinicaltrials.gov (CT.gov) for BC vaccine trials registered through Feb 29, 2024, yielding 220 studies that were downloaded into a Microsoft Excel file. Manual searches of published BC vaccine review articles identified 13 additional BC vaccine trials not retrieved in our CT.gov search. A total of 233 studies were screened by two of the authors for relevance. Eligibility criteria included that all studies be clinical studies, including randomized, non-randomized, and cohort trials that evaluated a tumor vaccine in patients with BC. Trials of multiple tumor types were included if BC was among the cancer studied. Non-cancer vaccine trials were excluded, as were studies with a withdrawn status yielding 186 trials for review and classification according to a pre-defined taxonomy that included: BC subtype; cancer stage; primary purpose of the vaccine; vaccine platform; antigen targets; drug combinations; trial phase; trial design; primary and secondary endpoints; key sponsors, collaborators, and funders; trial status; start/completion year, and results. Four BC patient advocates (all graduates of Project LEAD®) systematically reviewed and abstracted each trial according to the taxonomy. Coding was reviewed by two individuals. Disagreements were adjudicated by team review. Following detailed abstraction, 11 additional studies were excluded yielding 175 registered trials for analysis. Additional searches of PubMed and Google Scholar were done to identify published findings for all included clinical trials.
Results: Of the included trials, 101 were Phase 1, 33 Phase 1-2, 37 Phase 2, and four Phase 3. A total of 75 trials did not restrict participation to a specific BC subtype whereas 40, 46, and 14 focused on HER2+, TNBC, and HR+ BC, respectively. Five trials included patients with DCIS. For disease stage, 96, 63, 11, and 7 involved patients with advanced/metastatic stage, early stage, early stage/metastatic-no evidence of disease, and no BC (i.e., DCIS or high risk), respectively. Vaccine trials focused in early-stage disease have increased in recent years whereas vaccines targeting advanced disease have remained relatively stable over time. Peptide-based vaccine platforms were the most common (n=80), followed by 50

cell-based, 26 viral vector-based, and 26 DNA-based vaccines, including numerous examples of mixed platforms. HER2 was the most common target antigen (N=50 studies), followed by CEA (N=29), MUC1 (N=11), hTERT (N=7), and Brachyury (n=7). Most vaccines targeted a single antigen, with an increasing trend toward vaccines with multiple antigen targets. All but 45 trials included some form of combination therapy, including 27 with immune checkpoint inhibitors, 45 chemotherapy, and 21 with HER2 targeted agents. A total of 28 of 108 completed trials had results posted on CT.gov. Twenty-nine had results published elsewhere while 30% had completion dates beyond 2023.
Conclusions: This analysis provides a wealth of information on the evolution of the BC vaccine landscape, including key trends in antigen targets, vaccine platforms, and immune modulatory adjuvants. While most BC vaccines have had therapeutic purposes, largely in the advanced/metastatic setting, there has been an increased focused in recent years on secondary and primary prevention.

P4-10-18: Potential predictive biomarkers for a fibroblast growth factor receptor (FGFR) inhibitor: Phase 1b trial of tasurgratinib (E7090) with endocrine therapies (ET) for ER+, HER2+ recurrent/metastatic breast cancer (BC) resistant to CDK4/6 inhibitors

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Background: CDK4/6 inhibitors (CDK4/6i) combined with ET are standard first-line treatment for people with ER+, HER2− recurrent/metastatic BC. Previous studies suggest that FGFR gene alterations or overexpressions may contribute to resistance to CDK4/6i + ET. Here, we investigated the relationship between gene alterations or expression and sensitivity to the FGFR inhibitor, E7090.
Methods: We evaluated E7090 with or without ET in patients with ER+, HER2− recurrent/metastatic BC after CDK4/6i exposure (NCT04572295). Part 1 included treatment with fulvestrant (FUL) 500 mg + E7090 (105 or 140 mg) or exemestane (EXE) 25 mg + E7090 (105 or 140 mg). In part 2, patients with high FGFR1–2 protein expression by IHC received E7090 140 mg monotherapy. In part 3, patients received the recommended regimen (FUL + E7090 105 mg) to evaluate preliminary efficacy and safety. Tumor samples were collected at baseline (BL) and on cycle 3 day 1 (C3D1). Gene abnormalities were evaluated using FoundationOne®CDx (F1CDx) or whole-exome sequencing (WES), while mRNA expression was assessed using nCounter® PanCancer Pathways Panel. DNA sequencing analyzed FGFR-related gene abnormalities, ESR1 gene mutations, and PI3K/AKT pathway genetic mutations. Leveraging mRNA expression data from this study and the TCGA database, we established 3 gene-signature scores related to FGF/FGFR signaling—Signature Score 1, 2, and 3 (based on the TCGA database, BL gene expression in this study, and expression changes on pre/post samples from this study, respectively)—to

predict the clinical outcome of E7090 in BC. We explored potential correlations between gene alterations or gene-signature scores at BL and clinical outcomes (objective response rate [ORR] and progression-free survival [PFS]).
Results: Fifty-one patients received E7090 (E7090 105 mg + FUL, n=35 [3 from part 1 and 32 from part 3]; E7090 140 mg + FUL, n=3; E7090 105 mg + EXE, n=3; E7090 140 mg + EXE, n=9; E7090 140 mg monotherapy, n=1). Gene alterations in 42 patients were assessed by F1CDx (n=14) or WES (n=28) and gene expression was related to FGF/FGFR signaling assessed in 48 patients (BL only, n=40; paired BL and C3D1, n=8). The most frequently altered genes (> 20%) were: TP53 (38%); PIK3CA (33%); CCND1 (26%); ESR1 (26%); FGF19 (26%); FGF4 (26%); FGF3 (24%); and FGFR1 (24%). In the groups with or without FGF/FGFR pathway gene alterations, the ORRs were 15.0% (n=3/20) and 27.3% (n=6/22), and the PFS medians (mPFS) were 3.5 and 3.7 mos (altered vs unaltered: HR=1.100 for PFS), respectively. Among patients with or without ESR1 mutations, the ORRs were 9.1% (n=1/11) and 25.8% (n=8/31), and the mPFS were 3.5 and 3.7 mos (HR=1.631), respectively. Among patients with or without AKT pathway gene alterations, the ORRs were 15.8% (n=3/19) and 26.1% (n=6/23), and the mPFS were 3.5 and 5.4 mos, respectively (HR=1.513). For the gene-expression biomarker analyses, the Signature Score 1 high-score group had an ORR of 42.1% (n=8/19) and mPFS of 16.6 mos, whereas the low-score group had an ORR of 10.3% (n=3/29) and mPFS of 3.6 mos (high vs low: HR=0.524). For Signature Score 2, ORRs and mPFS in the high-score (n=19) and low-score (n=29) groups were the same as for Signature Score 1 (HR=0.506). For Signature Score 3, the high-score group had an ORR of 39.1% (n=9/23) and mPFS of 16.6 mos, whereas the low-score group had an ORR of 8.0% (n=2/25) and mPFS of 3.6 mos (HR=0.484).
Conclusions: These results emphasize that FGFR pathway activation evaluated with mRNA expression of selected genes at BL—rather than FGFR gene abnormalities—is a key molecular determinant for sensitivity to E7090, an FGFR inhibitor. Future validation of these biomarkers is necessary in a larger population of people with ER+/HER2− BC.

P4-10-20: First-in-human phase 1a, dose-escalation study of BGB-43395 (CDK4-selective inhibitor) as monotherapy and in combination with fulvestrant or letrozole in patients with metastatic HR+/HER2+ breast cancer and other advanced solid tumors

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Background: Despite the approval of cyclin-dependent kinase (CDK) 4/6 inhibitors (CDK4/6i) in HR+/HER2− breast cancer (BC), pts may develop resistance and experience toxicities with current treatments. BGB-43395 is a potent and selective CDK4i, showing preclinical antitumor activity with improved CDK4 coverage and greater selectivity for CDK4 over CDK6, thus minimizing off-target toxicity and potentially toxicity-related dose reduction/discontinuations. Here, we present the preliminary results of an ongoing first-in-

human, phase 1a dose escalation, open-label, multicenter trial of BGB-43395 given orally as monotherapy in pts with advanced solid tumors (Part A) or as part of combination therapy in pts with fulvestrant (Part B) or letrozole (Part C) in pts with 2L+ HR+/HER2− BC (NCT06120283).
Methods: Eligible pts were ≥18 years of age with histologically or cytologically confirmed advanced, metastatic, or unresectable solid tumors associated with CDK4 dependency. Permitted prior therapies included ≥2 lines of treatment including endocrine therapy (ET) and CDK4/6i in either the adjuvant or advanced metastatic setting for pts with HR+/HER2− BC, ≥2 lines of HER2 targeted therapy for pts with HR+/HER2+ BC, and standard of care for pts with other advanced solid tumors. Primary objectives were to assess the safety and tolerability of BGB-43395 as monotherapy or combined with fulvestrant or letrozole, and to determine the maximum tolerated dose or maximum administered dose and the recommended dose for expansion. Secondary endpoints were to evaluate pharmacokinetics and preliminary antitumor activity assessed per RECIST v1.1 by investigator.
Results: As of May 20, 2024, 23 pts (17 in Part A [including 6 with HR+/HER2− BC], 3 in Part B, and 3 in Part C) were enrolled in the ongoing dose escalation portion of the study. In all, there were 7 dose cohorts (5 in Part A, 1 in Part B and 1 in Part C). In Parts A, B and C, respectively, 14/17 (82.4%), 2/3 (66.7%) and 3/3 (100%) pts had metastatic disease. The median (range) number of lines of prior therapy was 3.0 (1–10) in all pts in Part A (3.5 [2–10] in the 6 HR+/HER2− pts in Part A), 4.0 (2–8) in Part B, and 4.0 (1–5) in Part C. All of the 12 HR+/HER2− BC pts in Parts A, B, and C, received prior CDK4/6i, ET, and chemotherapy (CT), except 1 pt in Part C who did not receive CT. TEAEs occurred in 15/17 (88.2%), 1/3 (33.3%) and 0 pts in Parts A, B, and C, respectively, and were primarily grades 1 and 2. For all 23 patients, the most common TEAEs were diarrhea (12/23; 52.2%; 1 pt grade 3), nausea (7/23; 30.4%; all grades 1 and 2), anemia (3/23; 13.0%; 1 pt grade 3), fatigue (3/23; 13.0%; all grades 1 and 2), and vomiting (3/23; 13.0%; all grades 1 and 2). Treatment-related AEs occurred in 14/23 (60.9%) pts (13 in Part A, 1 in Part B and 0 in Part C) and were primarily grade 1 and 2 except for 3 pts with grade 3. There were no DLTs or TEAEs leading to treatment discontinuation or death. Updated clinical data will be presented.
Conclusion: BGB-43395 is a novel CDK4-selective inhibitor and a promising agent for tumors with high CDK4 dependency with the potential to minimize off-target toxicity. To date, BGB-43395 has been safe and tolerable, supporting continued development. The dose-escalation phase is currently ongoing.

P4-10-21: Enhanced anti-tumor activity of zelenectide pevedotin in triple negative breast cancer (TNBC) patients (pts) with NECTIN4 gene amplification (amp)

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Background: Zelenectide pevedotin (zele, formerly BT8009) is a highly selective Bicycle® Toxin Conjugate (BTC) comprised of a bicyclic peptide targeting Nectin-4 conjugated to monomethyl auristatin E. BTC® molecules have lower molecular weight and shorter plasma half-life than antibody-drug conjugates, with distinct pharmacokinetics/dynamics, i.e., potential for rapid tumor penetration and minimal healthy tissue exposure. NECTIN4 amp has been shown to be a predictive biomarker for response to Nectin-4 targeted therapy in metastatic urothelial cancer (Klümper et al., 2024). An analysis of NECTIN4 amp in a large, independent sample of 245 breast cancer (BC) pts indicates that amp is common, seen in 19% (30/161), 14% (5/36) and 23% (11/48) of HR+/HER2-, HER2+ and TNBC, respectively (Klümper et al., unpublished data). This initial post-hoc analysis assesses the utility of NECTIN4 amp as a predictor of zele response in heavily pretreated BC pts.
Methods: Zele is being evaluated in the ongoing Phase 1/2 study BT8009-100/Duravelo-1 (NCT04561362) for safety and efficacy in pts with advanced solid tumors associated with Nectin-4 expression including BC pts. This analysis focuses on TNBC study pts who had baseline tissue sample available, had consented to optional future research, and tested for NECTIN4 amp by fluorescence in-situ hybridization. NECTIN4 amp positivity was defined as a ratio of NECTIN4: centromere 1 (CEN1) of 2.0 or higher. Assessment of anti-tumor activity was per RECIST v1.1 by investigator. Objective response rate (ORR) is based on the efficacy evaluable population.
Results: As of 29 August 2024, 32 heavily pretreated pts with TNBC enrolled in the monotherapy dose escalation and expansion cohorts of BT8009-100. Thirty-one TNBC pts were treated with the zele recommended Phase 2 dose of 5 mg/m2 weekly. At baseline, TNBC pts had a median age of 52 (30-76), median prior lines of therapy of 6 (2-13), and ECOG of 0 or 1 (50.0% each). Of 32 enrolled TNBC pts treated with zele, 30 were efficacy evaluable. Four pts achieved partial response (PR) resulting in an ORR of 13.3% (95% CI: 3.8, 30.7).
Nineteen pts were tested for NECTIN4 amp, 6 of whom were positive (31.6%). Of these, 3 achieved a PR with an ORR of 50.0% (95% CI: 11.8, 88.2), and the remaining 3 had stable disease (SD; disease control rate 100.0%). All 3 NECTIN4 amp responders were previously treated with sacituzumab govitecan. Of the NECTIN4 non-amp pts, 1/13 had a PR: ORR of 7.7% (95% CI: 0.2, 36.0). Of note, the non-amp responder pt harbored a polysomy, with NECTIN4 and CEN1 copy number >6 (NECTIN4:CEN1 ratio <2.0). Four HR+ BC pts were tested for NECTIN4 amp. One responder (1/ 4) was also the only one harboring a NECTIN4 amp.
Safety and tolerability of zele in the TNBC population was similar to a previously reported cohort of bladder cancer pts (Reig et al., 2024). Grade 3 or higher zele-related adverse events (AEs) occurred in 34.4% of all TNBC pts, and Grade 3 or higher zele-related serious adverse events (SAEs) occurred in 12.5% of all TNBC pts. Zele-related AE frequencies were similar in the NECTIN4 amp pts; however, no NECTIN4 amp pts had a zele-related SAE.
Conclusions: NECTIN4 amplification appears to show predictive clinical utility in identifying pretreated TNBC pts with enhanced response to zelenectide pevedotin, with an ORR of 57.1% in NECTIN4 gene amp positive pts including polysomy versus an ORR of 13.3% in biomarker unselected TNBC. Despite the limited sample size, this post-hoc analysis

underscores the promising anti-tumor activity of zelenectide pevedotin in pts with NECTIN4 amp TNBC, who continue to have significant unmet medical need for effective, well-tolerated therapy. These findings support further exploration of zele and NECTIN4 amp stratification strategies in BC pts, particularly TNBC.

P4-10-22: Eflapegrastim, a long-acting GCSF, administered the same day as chemotherapy in patients with early-stage breast cancer: Results from a multicenter, open-label, study

Manuel Modiano, Omkar Marathe, Shanta Chawla, Jeffrey L. Vacirca, Kenneth Crist, Howard Franklin, Lee Schwartzberg

Introduction: Patients (pts) with early-stage breast cancer (ESBC) receiving docetaxel and cyclophosphamide (TC) chemotherapy have a high risk of severe neutropenia (SN) and febrile neutropenia (FN) that can increase the risk of life-threatening infections and lead to chemotherapy dose reductions or treatment delays. The National Comprehensive Cancer Network recommends prophylactic treatment with granulocyte colony stimulating factor (GCSF) to reduce the risk of FN. Eflapegrastim is a novel long-acting GCSF consisting of recombinant human GCSF covalently linked to human IgG4 Fc fragment via a short polyethylene glycol linker. Eflapegrastim, via transcytosis and recycling, demonstrates increased bone marrow residence compared with pegfilgrastim, potentially improving its bioavailability following chemotherapy and allowing same-day dosing. Same day dosing has the potential to improve pt convenience by reducing multiple office visits. In the phase 3 pivotal trials ADVANCE (NCT02643420) and RECOVER (NCT02953340), eflapegrastim was administered ~24 hours post TC administration. Both studies showed non-inferiority of eflapegrastim to pegfilgrastim in reducing the duration and incidence of neutropenia in pts with ESBC receiving TC. In this study, we evaluated the safety of same-day dosing of eflapegrastim in the same disease setting.
Methods: This multicenter, open-label study (NCT04187898) enrolled pts with confirmed ESBC (stage I to IIIA), aged ≥ 18 years who were candidates for neoadjuvant or adjuvant TC. Pts received subcutaneous eflapegrastim (single, fixed dose of 13.2 mg [3.6 mg GCSF]) 0.5 h ± 5 min post TC (intravenous docetaxel 75 mg/m2 + cyclophosphamide 600 mg/m2) The primary endpoint was the time to recovery of absolute neutrophil counts (ANC) from nadir to ≥ 1.5×109/L in C1. Secondary endpoints included incidence of SN (ANC < 0.5×109/L) and FN (ANC < 1.0×109/L and temperature of > 38.3 oC or 2 consecutive readings ≥ 38.0 oC over 2 hours), duration of SN (DSN), and incidence of neutropenic complications, including use of antibiotics and/or hospitalizations. Blood samples for complete blood counts with differential were collected pretreatment and on day 1 and daily on days 4–10 of C1. Safety assessments began with the first dose of TC and lasted until 35 ± 5 days after the last dose of eflapegrastim. Here, we report the treatment-emergent adverse events (TEAEs) of interest. All results reported are descriptive.
Results: A total of 53 pts (mean [SD] age: 62.7 [11.9] years; female: 100%) from 13 sites

across the US, were enrolled (White: 62.3%; Black or African American: 9.4%; others: 28.3%). Pts were relatively healthy (ECOG 0, 52.8% [n = 28] patients; ECOG 1, 47.2% [n = 25] patients). Efficacy in C1 was evaluable in 49 patients. Mean (SD) time to ANC recovery was 1.8 (1.1) days. Incidence of SN was 46.9% (n = 23) and mean (SD) DSN was 0.8 (1.0) days. Incidence of FN was 2% (n = 1). No neutropenic complications were observed during the study. Safety was assessed in all 53 patients who received at least 1 dose of eflapegrastim. Overall, 43 patients (81.1%) experienced any TEAE of musculoskeletal pain. Common musculoskeletal-related TEAEs experienced by ≥ 10% of patients were bone pain (52.8%; n = 28); back pain (26.4%; n = 14), arthralgia or pain in extremity (17.0%; n = 9 for each); and myalgia (13.2%; n = 7). No deaths were reported during the study.
Conclusions: These findings suggest that administration of eflapegrastim on the same day as TC chemotherapy may be advantageous in reducing the time to ANC recovery and related complications in pts with ESBC. The AEs observed in this study were consistent with those generally observed in pts receiving TC and other GCSF products.
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Disclosures: MM and JLV have no conflicts to disclose. OM has received personal fees for consulting and research funding from OnQuality Pharmaceuticals. SC was an employee of Spectrum Pharmaceuticals, Inc. and still holds shares in Assertio Holdings, Inc. as a result of Assertio's acquisition of Spectrum. KC and HF are employees of Assertio Holdings, Inc. LS has received personal fees for consulting from Assertio Holdings, Inc., and Coherus Biosciences.

P4-10-23: Preclinical anti-tumor activity of BB-1701, a HER2-targeting eribulin conjugated ADC, in HER2-low breast cancer PDx models.

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Background: HER2-low breast cancer (BC) is the diagnosis in approximately 60~65% of HER2 "negative" breast cancer patients. Recently, the clinical landscape of HER2-low BC has changed with the approval of a HER2 targeting antibody-drug conjugate (ADC), trastuzumab-deruxtecan (T-DXd). Suitable drug candidates for next-line therapy in HER2-low BC after T-DXd are in need of investigation. BB-1701 is an ADC consisting of a trastuzumab and eribulin payload. When BB-1701 targets HER2-expressing cancer cells and is internalized, free eribulin is cleaved from the ADC by cathepsin B and causes cytotoxicity. Neighboring cells are also affected with bystander cytotoxicity as well as non-cytotoxic effects on the microenvironment. We evaluated the anti-tumor activity of BB-1701 using clinically relevant BC patient-derived xenograft (PDx) models to identify the potential clinical usefulness of BB-1701 in HER2-low BC.
Method: We established 32 human BC PDx models. These models were characterized using gene expression with RNA-seq and by HER2 immunohistochemistry (IHC). We basically grouped these 32 models into 3 classes: 1) HER2-negative (TPM<90), 2) HER2-low (TPM=90~400) and 3) HER2-high (TPM>1000) with HER2 mRNA expression and confirmed with HER2 IHC analysis. We selected 6 HER2-low PDx models, characterized

them by stroma composition, and tested the anti-tumor activity of T-DXd at clinically relevant doses. The anti-tumor activity of BB-1701 was also evaluated in these models at single or double administration doses of 3, 10, and 30 mg/kg (Q3W). Tumor samples were collected before treatment, at the tumor regression phase, and at the tumor re-growth phase. These samples were then evaluated for mode-of-action investigations.
Result: 32 PDx models were classified into 5, 23, and 4 models with HER2 negative, low, and high respectively by HER2 expression levels based on RNA-seq analysis. To investigate the anti-tumor activities of BB-1701 against HER2-low models, we selected 2 groups of models: 3 HER2 lower models (TPM=90-130) and 3 HER2 higher models (TPM=180~400) from 23 HER2-low PDx models. While T-DXd exhibited significant anti-tumor activity in all 6 models, we found one (IM-BRE-029) to be the least sensitive to T-DXd. In this model, tumors progressed approximately 21 days after the administration of 10 mg/kg T-DXd and a shorter duration of tumor regression than was observed in other PDx models. IM-BRE-029 PDx tumors are characterized by a rich stromal component in histopathological analyses. BB-1701 also showed anti-tumor activities with tumor regression in all models in both with the HER2 lower and HER2 higher groups. And in IM-BRE-029 model, BB-1701 exhibited a clear anti-tumor activity at 3 mg/kg and long-term tumor regression was achieved with 2 shot administrations at 10 or 30 mg/kg. In this symposium, we will describe model characterization results of these 6 PDx models, focusing on IM-BRE-029, and present the anti-tumor activities of BB-1701.
Conclusion: These results indicate that BB-1701 has the potential to provide clinical benefit to HER2-low BC patients. A phase 2 clinical trial in patients with HER2 positive or HER2-low unresectable or metastatic BC is ongoing (NCT06188559).

P4-10-24: Extracellular matrix remodelling is a targetable feature of invasive lobular carcinoma (ILC)

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Background: Invasive lobular carcinoma (ILC) has previously been identified as overexpressing collagens and elastin as well as LOXL1, a collagen cross-linking enzyme belonging to the lysly oxidase (LOX) family. Abberant LOX activity can promote breast cancer invasion and metastasis through remodelling of the extracellular matrix (ECM). The clinical stage pan-LOX inhibitor PXS-5505 has demonstrated anti-tumour potential through the disruption of the collagen matrix in models of pancreatic and myeloid cancer, and exhibits an excellent safety profile. Given that ILCs are enriched for ECM remodelling, we propose that inhibition of LOX be investigated as a therapeutic strategy in the treatment of ILC.
Methods: We xenograft ILC cells into the milk ducts of mice (MIND) to faithfully recapitulate ILC disease progression including ECM remodelling processes. We used cell line and PDX MIND models of ILC to examine their response to LOX inhibition (LOXi) alone and

combination with ovariectomy (OVX) to mimic aromatase inhibition (AI).
Results: Treatment with LOXi alone significantly decreased primary tumour growth compared to vehicle in cell line and patient derived xenograft models of ILC. This was accompanied by changes in the “matrix structure” and downmodulation of proliferative markers, and a transcriptional level MYC and ER signalling pathways. The magnitude of response to LOXi differed across ER+ ILC models, either being sufficient to reduce primary tumour growth alone (SUM44, p=0.000410), or in combination with OVX (MM134, p=0.032). In the metastatic ER- IPH-926 model LOXi significantly reduced metastatic dissemination to the lungs (p=0.013). Histologically, combination treatment decreased collagen matrix density and alignment as well as the Ki67 index. Proteomic profiling revealed LOXi alone and in combination with OVX downmodulated matrix-associated proteins, as well as MYC transcription factor targets. Functional pathway and keyword enrichment analysis identified down modulation of cell adhesion, cell cycle and apoptosis-related pathways.
Conclusion: These data demonstrate that ECM remodelling is a targetable feature of ILC and suggest that the LOXi PXS-5505, a well tolerated drug provides additional benefit to standard of care endocrine therapy. Changes in fibrillar collagen and matrix-associated proteins result in decreased MYC activity, which may serve as an endpoint in a prospective window trial.

P4-10-26: PRISM high-throughput screening to elucidate how tumor microenvironments modulate drug responses of breast cancer cells

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Cancer treatment options including small molecules, antibody-drug conjugates (ADCs), CAR T-cells, and combination therapies are rapidly expanding to improve cancer patient survival. However, efficacy of targeted therapies is limited by interpatient and intratumoral heterogeneity in breast cancer, and may be further exacerbated by dysregulated tumor microenvironments. For example, studies suggested that changes in tumor and breast tissue microenvironment in obesity (e.g. inflammation, increased growth factors) could lead to worsened prognosis that could be related to therapy resistance. Systematic characterization of the cellular effects of emerging drug candidates, particularly across different tumor microenvironments, is challenging, as it needs to be surveyed in diverse cancer contexts beyond just a handful of cancer cell lines.
To overcome this challenge, we created PRISM (Profiling Relative Inhibition Simultaneously in Mixtures), an effective drug discovery screening platform with over 900 genetically distinct cancer cell lines of diverse lineages, to rapidly test therapeutics in a high-throughput manner. These cell lines, including 33 different breast cancer cell lines covering diverse cell types and subgroups, carry a specific 24 nucleotide “barcode” tag and have rich baseline genomic and functional characterization. They are first pooled into 20-25 cell pools of different lineages with similar growth rates, then subsequently cultured together with or

without drugs. The relative barcode quantity read out by NextGen sequencing determines cell line responses to drugs. This pooled approach significantly reduces the time and the resources required for drug screening. Importantly, utilizing the rich cell line characterization to interpret viability profiles enables the identification of drivers of differential sensitivity and potential biomarkers of compound response. Furthermore, we created a breast cancer cell specific pool to investigate how media conditions mirroring different tumor microenvironments [e.g. media filtration, addition of insulin (0.01 mg/mL) or EGF (0.01 ug/mL)] can impact drug effects on breast cancer cells.
In the PRISM 900+ cell panel treated with ADCs conjugated to different payloads that target HER2, there was a clear association between cell line sensitivity and ERBB2 (encoding HER2) gene expression, with the strongest effects on viability observed in a subset of cell lines overexpressing ERBB2. Upon further comparison of biomarker profiles across different ADCs, we established relationships between payload resistance and ABCB1 efflux pumps emerged for a subset of ADC payloads.
Using the breast cancer cell specific pool, our data showed that estrogen receptor (ER) degraders fulvestrant and vepdegestrant (ARV-471) had significantly greater effects in reducing viability of ER+ versus ER- breast cancer cell lines. Media filtering reduced the drug effects of fulvestrant but not ARV-471, suggesting that ARV-471 efficacy may be less sensitive to the tumor microenvironment than fulvestrant. Lapatinib (HER2/neu and EGF receptor inhibitor) was more effective in killing HER2+ than HER2- negative breast cancer cells, with cells cultured in EGF-spiked media being more sensitive to lapatinib, whereas media filtration diminished its effects. We did not observe breast cancer subgroup specific killing by alpelisib (PI3K inhibitor) or olaparib (PARP inhibitor), but breast cancer cells became less sensitive to alpelisib when cultured in either insulin- or EGF-spiked media, and less sensitive to olaparib in EGF-spiked media.
Overall, our study highlights how the PRISM 900+ cell panel can provide insights into drug specificity, cancer subtype selectivity, and to uncover clinically relevant targets. The breast cancer specific pool can further increase the throughput of understanding how drug responses may be altered in different tumor microenvironments in breast cancer.

P4-10-27: Epigenetic Drug Profiling for Breast Cancer Therapy: A Focus on Receptor Modulation and Heterogeneity

Rahat Alam, Igor Bado

Breast cancer is the second leading cause of cancer death in women. The primary cause of mortality is associated with the metastasis process of cancer cells to other organs. It has become obvious that epigenetic alteration plays a crucial role in breast cancer progression through the modulation of various signaling pathways. Additionally, the plastic nature of cancer cells is essential to their adaption in different metastatic sites. However, implementing epigenetic therapies in the clinic has been challenging due to the ubiquitous expression of many epigenetic factors. Here, we evaluate a library of epigenetic drugs to

determine their impact on receptors commonly targeted in breast cancer. These include estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). We coupled an immuno-fluorescence imaging approach to bioluminescence imaging to capture receptor heterogeneity and the proliferation index of each epigenetic drug. So far, we have assessed 460 drugs that target various factors, including Epigenetics, JAK/STAT, DNA Damage, Angiogenesis, Cell Cycle, PI3K/Akt/mTOR and Cytoskeletal Signaling targeting and histone deacetylases (HDACs), DNA methyltransferases (DNMTs), histone methyltransferases (HMTs), aurora kinase PARP, Sirtiin, EGFR, JAK, Plm pathways. We also analyzed the expression of key epigenetic markers (e.g., EZH2) and stemness-associated factors (e.g., SOX9). As a result, we found BIO, an inhibitor of GSK-3 and JAK, increases ER expression. Similarly, Curcumin, an inhibitor of p300 histone acetyl-transferase and Histone deacetylase (HDAC) promotes a broader range of factors, including Her2, Sox9, and ER. Interestingly, (+)-JQ1(BET bromodomain inhibitor), I-BRD9 (BRD9 inhibitor) and CUDC-101 (HDAC inhibitor) drastically reduced Ki67 level. Overall, these findings suggest a potential for combination therapy. While further investigation of these promising candidates is needed (3D, ex vivo, and in vivo testing), this investigation reveals new possibilities to overcome therapeutic resistance in breast cancer.
Keywords: Breast cancer, epigenetics, drug screening.

P4-10-28: Efficacy, safety and biomarker results of AC699, a chimeric estrogen receptor degrader, in patients with advanced or metastatic breast cancer

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Background: Endocrine therapy is the mainstay of therapy for patients with estrogen receptor positive (ER+) advanced or metastatic breast cancer. The combination of endocrine therapy along with cyclin dependent kinase 4/6 (CDK) inhibitors has improved progression free survival (PFS) in the frontline metastatic setting to over 24 months. However, in later lines of therapy, monotherapy treatment with selective estrogen receptor degraders (SERDs) result in PFS of <4 months and limited objective response rates (ORR) of <15%, demonstrating high unmet medical need. AC699 is a chimeric degrader that functions by binding to ERα, bringing it into proximity with the E3 ligase cereblon, resulting in ubiquitination and degradation of ERα. Safety, efficacy and biomarker data from an ongoing phase 1 dose escalation trial of AC699 is presented here (NCT05654532).
Methods: Patients with ER+/HER2- locally advanced or metastatic breast cancer who had received at least two prior lines of endocrine therapy or at least one prior line if combined with a CDK4/6 inhibitor were enrolled in this study, which followed a standard 3+3 dose escalation design. Additional patients were backfilled at selected cleared dose levels. Patients were treated with AC699 dosed orally, once a day, in 28-day cycles, and followed for response with imaging assessments performed every 2 cycles. The relationship between ESR1 mutational status and anti-tumor activity was investigated as an

exploratory analysis. Changes in circulating tumor cell (CTC) count and circulating tumor DNA (ctDNA) were also assessed.
Results: Thirty-five female patients with a median age of 60 year received AC699 at five dose levels ranging from 100 to 600 mg. The population was heavily pre-treated with a median of 5 (range 1-10) prior lines therapy, including 3 (range 1-8) in the metastatic setting. All patients had received a prior CDK4/6 inhibitor. Twenty-eight (80%) patients had measurable disease while 7 (20%) had non-target evaluable bone lesions only. Twenty-two patients with measurable disease had a follow up imaging assessment and were evaluable for response. The ORR was 23% (5/22) with 4 confirmed partial response and 1 unconfirmed partial response. The clinical benefit rate (CBR), defined as patients who achieved complete response, partial response, or stable disease ≥24 weeks was 30% (8/27). In the subgroup of evaluable patients with a confirmed ESR1 mutation (defined as a variant allele frequency [VAF] of >1% assessed in their baseline ctDNA sample), the ORR was 56% (5/9), the CBR was 55% (6/11) with 1 patient still receiving AC699. In patients who had an ESR1 mutation at baseline, 10 had a ctDNA sample at the end of cycle 1, and all 10 had a decrease in the VAF of the predominant ESR1 variant. In the entire population, 70% (19/27) of patients had a decrease in CTC count following initiation of study treatment.
Adverse events (AE) occurred in 77% (27/35) of patients with nausea (17%), dehydration (14%), fatigue (14%), hot flush (14%), and neutrophil count decreased (14%) being the most common. Treatment related AEs occurred in 37% (13/35) of patients, all of which were Grade 1 or 2, including hot flush (14%), nausea (11%) and neutrophil count decreased (9%). No dose limiting toxicities, dose reductions or discontinuations occurred due to treatment related AEs, and the maximum tolerated dose was not reached.
Conclusions: AC699 monotherapy given orally once daily was tolerable in this ongoing trial. Preliminary results demonstrated the highest objective response rates to our knowledge in a post-CDK4/6 population compared to previously reported phase 1 trials. In addition, a vast majority of patients had an initial response to therapy as demonstrated by a decrease in CTC count, even in those who progressed shortly thereafter. A phase 2 study will begin enrolling in early 2025.

P4-10-29: Repurposing hypertensive drugs for breast cancer therapies

Maninder Khosla, Shengli Dong, Suresh K. Alahari

Background: Triple-negative breast cancer (TNBC) is a challenging disease to treat in patients due to a lack of well-defined molecular targets and the highly proliferative and invasive nature of these tumors. There exist several targeted therapies, but only a fraction of patients responds to these therapies and these patients typically develop resistance and relapse. The development of a new therapeutic strategy for the treatment of TNBC is desperately needed. Work in our lab has previously identified Nischarin as a potential tumor suppressor of breast cancer. The binding of Nischarin to imidazoline receptors leads to inhibition of tumor cell migration, invasion, tumor growth and metastasis. Moxonidine is

a clinically approved centrally acting drug used to treat hypertension and is believed to function through imidazoline receptors. In addition, rilmenidine, a moxonidine like compound behaves the same way, and here we repurpose these drugs for breast cancer therapies. Our data for the first time show that moxonidine upregulates Nischarin expression as well as decrease tumor cell growth in vitro. In addition, rilmenidine, has also been shown to downregulate TNBC growth with a lower IC50 as compared to moxonidine.
Results: Our data reveal that a dose dependent increase in Nischarin protein expression in TNBC cell line MDAMB231 treated with moxonidine as well as rilmenidine. Additionally, analysis of cell viability through MTT assays suggested that increased treatment of moxonidine or rilmenidine leads to reduced mitochondrial activity in multiple TNBC cell lines. Nischarin positive BC patients have increased overall survival (OS). We downloaded mRNA expression (level 3RNAseq v2) and survival information for 921 patients with breast invasive carcinoma from TCGA portal. In addition, publicly available RNAseq data from TCGA suggests decreased Nischarin expression in TNBC as compared to other breast cancer subsets (LumA, LumB and normal tissue). These data suggest that Nischarin expression is low in TNBC, and it is important to induce the expression in TNBC.
Conclusions: Triple-negative breast cancer is an incredibly deadly subset of a highly heterogenous disease. These tumors are highly refractory to clinically approved therapies due in part to their highly proliferative and metastatic capabilities. Nischarin has been shown to downregulate the proliferation of cancer cells as well as inhibit their metastatic potential. Using clinically approved hypertensive drugs moxonidine and rilmenidine as drugs to regulate TNBC tumor development by increased Nischarin expression can serve as a fundamental basis for a novel new therapeutic strategy.

P4-10-30: Injectable novel hydrogel formulation for bacterial encapsulation

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Background information: Nowadays, temperature-sensitive hydrogels are widely used for the targeted delivery of drugs to solid tumor sites, as they can become solid at body temperature and retain drugs only at the tumor site. They are biodegradable, biocompatible, and have pores for the sustained release of drugs, which makes them perfect scaffolds for trapping bacterial cells. Mesoporous hydrogel with genetically engineered bacteria that synthesizes anticancer drugs inside can be injected into subcutaneous levels of the human body to kill rapidly differentiating cancer cells.
We synthesized a chitosan-β-glycerophosphate-hyaluronic acid hydrogel, which was then encapsulated with genetically engineered E. coli bacteria. The main goal of this study was to create a scaffold for genetically engineered bacteria and assess its effectiveness regarding pore size, release behavior, and cytotoxic activity.
Methods: Chitosan-β-glycerophosphate-HA hydrogel was synthesized via physical crosslinking and sol-gel transition was examined using rheometer. SEM imaging showed

correlation between pore size and concentration of hyaluronic acid. MTT assay assessed cytotoxicity levels towards E.coli cells and release was monitored via Bacterial Release Test.
Results: Sol-gel transition appeared in 8 min and the rheometer identified solidification temperature as 36.62 °C. With increasing concentrations of hyaluronic acid from 0.5% to 2%, the pore size decreases from 56 um to 3.94 um. Viability of E.coli cells reduced to 20.9045% which indicates that hydrogel does not have significant adverse effects on bacterial cells. Release in 72 hours showed sustained release bacteria, which confirmed that hydrogel can be used as a drug delivery system.
Conclusion: Novel temperature sensitive drug delivery systems for bacterial encapsulation can be time and resource saving for drugs released by bacterial strains, chitosan-β-glycerophosphate-hyaluronic acid hydrogel characterizations showed stronger ability to be used as scaffold and further in-vivo studies are needed.

P4-11-01: Retrospective analysis of outcomes of HR-positive/HER2-negative early-stage breast cancer patients from adjuvant GEICAM studies and El Álamo IV registry

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Background: Endocrine therapy (ET) with or without abemaciclib is the current standard of care for intermediate- and high-risk hormone receptor (HR)-positive/HER2-negative early breast cancer (EBC) patients (pts). The NATALEE study, with 33.3-months follow-up (F-U), showed that the addition of 3 years of ribociclib to ET improves the 3-year (y) invasive Disease-Free Survival (iDFS) by 3.1% compared with ET alone in that population. Obtaining data on the long-term risk of relapse can be of great help in interpreting trial results and making decisions about new adjuvant drugs.
Methods: Retrospective analysis of 8819 HR-positive/HER2-negative EBC pts recruited from El Álamo IV registry (pts diagnosed between 2002 and 2005) and 5 adjuvant GEICAM studies (9805, 9906, 2003-02, 2003-10 & 2006-10) carried out from 1999 to 2010. In order to estimate their long-term outcomes, pts were divided into 3 cohorts (C), 2 of them according to the NATALEE study definition of risk of recurrence: C1 and C2: high- and intermediate-risk and one (C3) of low-risk (stage I and T2N0 plus one of the following features: histological G1 or G2 or GX with Ki-67 <20%).
Results: 20.0% of pts were high-risk, 36.0% intermediate-risk and 44.0% low-risk. Prior chemotherapy in neo/ adjuvant setting was used in 75.5% of patients (88.9%, 88.1% and 59.1% in cohort 1, cohort 2 and cohort 3, respectively). The median exposure time to adjuvant ET was 5 years (range: 0-15 years), similar in all cohorts. Selective Estrogen Receptor Modulator (SERM) were administered in 67.4% of patients, either alone (36.1%)

or with aromatase inhibitors (31.3%) mainly in sequence. With a median F-U of 10.7 years from adjuvant ET start, 10-y iDFS, 10-y distant DFS (dDFS) and 10-y overall survival (OS) were 77.8%, 80.9% and 86.1% in the overall population, respectively. 10-y iDFS were 57.8%, 77.3% and 87.2%, 10-y dDFS were 60.5%, 80.5% and 90.5%, and 10-y OS were 70.1%, 86.6% and 93.0%, in C1, C2 and C3, respectively. In C1, invasive relapse rate (IRR) and distant disease rate (DRR) had a peak in years 2-3 and a second peak in year 7, while in C2 and C3 the rates increased steadily (being higher at all timepoints in C2). In C1, death rate (DR) had a soft peak in years 3-4 and years 7-8 while in C2 and C3 they increased steadily (also being higher at all timepoints in C2).
Conclusions: Intermediate-risk pts have a 10-y iDFS of 77.3%, and 10-y OS of 86.6%. IRR and DRR had a peak in years 2-3 and a second peak at year 7-9 in the 3 cohorts, higher for high-risk pts. Longer F-U of the NATALEE study is needed to see the potential benefit of adjuvant ribociclib in intermediate-risk pts.

P4-11-02: Characterization of BTX-9341, a bifunctional degrader of CDK4 and CDK6 for HR+/HER2- breast cancer.

Hannah Majeski, Kirti Kandhwal Chahal, Angela Pasis, Casey Carlson, Qiao Liu, Arvind Shakya, Akinori Okano, Shenlin Huang, Aparajita Chourasia, Leah Fung

CDK4/6 inhibitors (CDK4/6i) such as palbociclib, abemaciclib and ribociclib are used to treat HR+/HER2- breast cancer, but patients can develop resistance via many mechanisms, several of which converge on upregulation of the cyclin D-CDK4/6 signaling node. This has been shown to limit the effectiveness of CDK4/6i in ER+ breast cancer with up to 20% of patients exhibiting innate resistance and up to 70% of patients developing acquired resistance after 3 years on therapy (Scheidemann, 2021). To address acquired resistance, we sought to apply a degrader approach. We utilized our PRODEGY platform of Cereblon (CRBN) binders to synthesize CRBN mediated CDK4/6 bifunctional degraders and identified BTX-9341 as a development candidate.
Breast cancer cell lines treated with BTX-9341 for 6 hours demonstrated up to 85% degradation of CDK4 and CDK6 with DC50s <1nM. BTX-9341 showed specificity for CDK4/6, with minimal off-target binding or degradation. CDK4/6 phosphorylates retinoblastoma protein (Rb) which releases the transcription factor E2F, inducing the expression of genes including CDK2 and Cyclin E which promote cell cycle progression. To determine the effect of BTX-9341 on downstream signaling, we examined Rb phosphorylation by in-cell western, and E2F target gene expression by qPCR and western. BTX-9341 was potent in all assays, with phospho-Rb IC50s <30nM, and E2F target gene downregulation at concentrations as low as 10nM. These downstream effects were sustained for 72 hours with BTX-9341 treatment, whereas rapid recovery was seen with CDK4/6i treatment. We used a 2D colony formation assay (CFA) to assess inhibition of proliferation. BTX-9341 potently inhibited cell proliferation with CFA IC50s of 20-50nM while CDK4/6i had CFA IC50s of 50-1000nM. This increased activity was due to CRBN mediated target degradation, as demonstrated by a shift in CFA IC50 values in a CRBN

knockout cell line towards the values seen with the inhibitors. Combining BTX-9341 with selective estrogen receptor degraders (SERDs) resulted in a synergistic anti-proliferative effect in HR+ breast cancer cells. In a palbociclib-resistant HR+/HER2- cell line model BTX-9341 maintained a low CFA IC50 (<150nM) while CDK4/6i displayed IC50s > 1μM. [SG1] BTX-9341 showed enhanced inhibition of Rb phosphorylation and a stronger synergistic effect with SERDs in CFA assays than CDK4/6i in this palbociclib resistant model. BTX-9341 displays excellent pharmacokinetic properties which allowed for oral dosing in xenograft studies. In several breast cancer xenograft models, BTX-9341 showed dose-dependent tumor growth inhibition, tumor regression at higher dose levels, and was effective with multiple alternate dosing regimens.
These results demonstrate the excellent activity of BTX-9341 as a single agent and in combination with SERDs, particularly in comparison to CDK4/6i. BTX-9341 mediated downregulation of CDK2 and cyclin E1, which are known to drive resistance to CDK4/6i, was more pronounced and more sustained than that mediated by CDK4/6 inhibitors including approved inhibitors such as palbociclib and inhibitors under clinical development such as PF-07220060. BTX-9341 maintained single agent activity and synergistic activity with SERDs in a CDK4/6i resistant model. These results indicate that utilizing a degrader such as BTX-9341 may be more effective in a post CDK4/6i setting than switching to a new CDK4/6 inhibitor. Based on this data, we have initiated a Phase 1 clinical trial with BTX-9341 as a monotherapy and in combination with fulvestrant, for HR+/HER2- breast cancer patients who have progressed on CDK4/6i therapy.
Reference: Scheidemann, Erin R, and Ayesha N Shajahan-Haq. “Resistance to CDK4/6 Inhibitors in Estrogen Receptor-Positive Breast Cancer.” International journal of molecular sciences vol. 22,22 12292. 14 Nov. 2021, doi:10.3390/ijms222212292

P4-11-03: Prediciting high risk hormone receptor (HR) positive Her-2 negative early breast cancer (BC) by artificial intelligence (AI) algorithms

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Background: Genomic tests are needed in addition to clinical/pathological factors to optimize adjuvant treatment decisions in most patients with HR positive Her-2 negative early BC. We aimed to develop an AI-based model to predict high risk HR positive Her-2 negative early BC by using clinical/pathological data and Oncotype DX scores. Methods: 234 patients who underwent Oncotype Dx test at our center between 2008 and 2023 were included. Clinical/pathologic characteristics and OncotypeDx scores were analysed by an ensemble of XgBoost, Random Forest, LightGBM, and Decision Tree machine learning algorithms to predict high risk disease. The dataset was split into two subsets: 75% for the training and 25% for the test set. The final model is chosen to maximize the precision of high-risk patients by using the data associated with high risk (HR) Oncotype-Dx scores and also the clinical/pathological data from patients who had distant metastases (DM) with low and intermediate recurrence score (RS). Results: Median follow up was 84 months and

median age was 47. Among 234 patients % 65 had stage I, % 22 had node positive, % 78 had grade 2 disease. Fourteen %, 68% and 18% of patients had HR, intermediate (IR) and low risk (LR) Oncotype Dx scores respectively, % 72 had clinically LR disease. Six %, 21% and 88% of patients with LR, IR and HR RS received adjuvant chemotherapy. There were 25 events: 13 DM, 2 local/regional relapse, 1 second primary, 9 deaths (7 due to BC). Two patients with HR, 8 patients with IR and 3 patients with LR Oncotype Dx scores had DM. The training (n: 175) and test set (n: 59) had similar risk level distributions. There were 6 DM, 2 deaths due to BC in the test set. Among 6 patients with DM, one patient had LR Oncotype Dx and IR AI-based score, another patient had IR Oncotype Dx and HR AI-based score, 4 patients have concordant scores (3 had IR, 1 had HR in both Oncotype Dx and AI-based model). Oncotype Dx and AI-based predicted scores were concordant in 5/11 (45%) patients with HR, 28/39 (%72) patients with IR and 1/9 (11%) patients with LR Oncotype Dx scores in the test set. There were no DMs in the AI-based low risk group vs one patient with DM in the Oncotype DX LR group. Conclusion: Our model might be used to predict high/intermediate risk patients and its performance needs validation with additional new datasets before its clinical implementation.

P4-11-04: Correlative analysis of Breast Cancer Index with Body Mass Index for prediction of extended endocrine therapy benefit in the BCI Registry study

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Background: Hormone receptor-positive (HR+) breast cancer patients derive only modest benefit from extended endocrine therapy (EET) while facing an increased risk of late distant recurrence (DR). The Breast Cancer Index (BCI) is a validated genomic assay for providing individualized risk of overall (0-10y) and late (5-10y) DR and predicts the likelihood of benefit from EET. The predictive component of BCI, the HOXB13/IL17BR ratio [BCI (H/I)] with a high status predicting EET benefit, has been validated in several randomized controlled trials including MA.17, Trans-aTTom, IDEAL, and NSABP B-42.
Previous studies suggest that breast cancer survivors who are overweight or obese based on Body Mass Index (BMI) are at an increased risk of cancer recurrence, and higher all-cause mortality. This analysis aims to investigate the correlation between BCI and BMI in patients enrolled in the BCI Registry study.
Methods: The BCI Registry study is a prospective study to evaluate the long-term clinical outcome, decision impact and medication adherence in early-stage, HR+ breast cancer patients. BMI scores were categorized as underweight/normal (BMI < 25), overweight (25 ≤ BMI < 30), obese (30 ≤ BMI < 40), and severely obese (BMI ≥ 40). BCI score, BCI (H/I) and categorical groups based on default cut-points were calculated as previously described. Spearman's correlation coefficient (R) was used to estimate the correlation between BCI (H/I), BCI risk score, and BMI as continuous variables. Kruskal-Wallis test was used to assess pairwise comparisons between BMI groups and BCI categories.

Results: BCI and BMI results were included from 1664 breast cancer patients (74.8% T1; 53.5% grade II; 79.5% N0). BCI (H/I) classified 1025 patients (61.6%) as BCI (H/I)-Low and 639 (38.4%) as BCI (H/I)-High. BCI scores classified 771 patients (46.3%) as low-risk and 893 (53.7%) as high-risk for late DR. BMI classified 417 patients (25.0%) as underweight/normal, 552 (33.2%) as overweight, 584 (35.1%) as obese and 111 (6.7%) as severely obese. When analyzed as continuous variables, no correlation with BMI was observed for BCI (H/I) (R=-0.04) nor BCI score (R=0.007). In addition, no significant relationship was observed between BMI and BCI (H/I) categories (p = 0.16) as well as between BMI and BCI prognostic groups (p =0.51).
Conclusions: BCI (H/I) and BCI risk scores exhibited no correlation with BMI categories. BCI (H/I) consistently stratified patients into low- and high-likelihood for extended endocrine benefit independent of BMI categories, suggesting that BMI is not a reliable indicator for predicting recurrence risk or benefit from EET.

P4-11-05: Aromatase inhibitors mediate specific gut-bug interactions to shift the microbiome and decrease inflammation during adjuvant treatment in breast cancer patients.

Katherine Cook, Katherine Ansley, Haley Westervelt Mabry, Emily Douglas, Akiko Chiba, Alexandra Thomas

Background: Recent studies implicate the gut microbiome as a potential risk factor for breast cancer and a feature modifying anti-cancer drug therapeutic response. However, whether orally administered endocrine-targeting therapies such as aromatase inhibitors (AI) used post-surgery in the adjuvant setting to reduce estrogen receptor-α (ER+) breast cancer recurrence, modify the gut microbiome is unknown. We measure the shift in the gut microbiome and plasma inflammation marker as part of the ongoing Oral Aromatase Inhibitors Modify the Gut Microbiome Effecting Estrogen Bioavailability clinical trial (NCT05030038).
Methods: Eligibility requirements include women with a breast cancer diagnosis after surgical and radiation treatment before being prescribed adjuvant AI therapies. After consenting for trial, women undergo a baseline fecal collection and blood draw before starting their aromatase inhibitor. Patients provided a 4-weeks and 12-weeks fecal sample, along with a 12-weeks blood sample. Fecal DNA was isolated and 12M read depth metagenomic microbiome sequencing was performed. Inflammatory cytokines and other microbial-derived metabolites were measured in plasma using ELISA and metabolomics approaches.
Results: Samples from 10 women with matching fecal and plasma specimens were analyzed for microbiome and inflammatory cytokines. Administration of AI did not significantly modify α-diversity or β-diversity measurements. However, we did observe that AI therapy resulted in a significant enrichment of Oscillospiraceae_us (0.8% baseline vs. 1.4% 12-weeks), Parabacteroides merdae (0.4% baseline vs. 0.9% 12-weeks), and Ruthenibacterium lactatiformans (0.2% baseline vs. 1.3% 12-weeks) proportional abundance. Furthermore,

oral AI therapy administration significantly reduced the proportional abundance of Faecalibacterium prausnitzii (2.1% baseline vs 0.7% 12-weeks). Administration of AI also resulted in a significant decrease in plasma interferon (IFN)γ, interleukin (IL)-2, IL-5, IL-10, and sCD137.
Conclusions: Increased Oscillospiraceae family members (often considered commensal short-chain fatty acid (SCFA) producers) are observed with AI administration; However, AI therapy is associated with decreased F. prausnitzii (a butyrate-producing bacteria) proportional abundance, suggesting a community shift in SCFA-producing populations to potentially modify gut health. This is further supported by increased R. lactatiformans proportional abundance associated with AI therapy. R. lactatiformans produce lactate and acetate which may influence anti-inflammatory outcomes. Further studies are needed to determine potential drug-microbiome interactions that may mediate efficacy.

P4-11-06: Neoadjuvant anlotinib plus nab-paclitaxel based chemotherapy in patients with HER2-negative breast cancer: A prospective, single-arm, single-center, phase II clinical study

Huimin Meng, Yidi Wang, Songpeng Li, Mengxuan Li, Jing Kong, Jing Fan, Ting Wang

Background: Antiangiogenic agent plus neoadjuvant chemotherapy confers an improvement in pathological complete response (pCR) rate among patients with HER2-negative breast cancer. Anlotinib is a novel multi-target tyrosine kinase inhibitor that effectively inhibit VEGFR, FGFR, c-KIT, c-MET, and RET. This phase II study aims to evaluate the efficacy and safety of anlotinib combined with chemotherapy as neoadjuvant treatment in HER2-negative breast cancer.
Patients and methods: Eligible patients (pts) were women, aged 18-70 years, ECOG status 0-2, previously untreated HER2-negative breast cancer. The HER2-negative was defined as immunohistochemistry of 0, 1+, and 2+/ISH-. Pts were required to have a palpable primary tumor at least 20 mm in diameter in the breast as assessed by physical examination. Pts received 5 cycles of anlotinib (12 mg qd, d1-14; q3w) plus 6 cycles of nab-paclitaxel (200 mg/m2, q3w), anthracycline (pirarubicin 50 mg/m2) and cyclophosphamide at 500 mg/m2, followed by surgery. The primary endpoint is pCR rate (ypT0/Tis ypN0) and the secondary endpoints include objective response rate (ORR), disease-free survival (DFS), overall survival (OS), and safety.
Results: From Aug 2021to Jun 2024, a total of 31 pts were enrolled and 1 pts discontinued the study due to HER2 amplification. The median age was 46 years (range, 31-68) and the ECOG score of all patients was 0. Estrogen receptor status was ≥10% in all 30 pts and progesterone receptor status was ≥20% in 25 pts (25/30, 83%). Lymph node metastasis was observed in 64% (19/30) of the pts. At the data cut off time of 30th Jun 2024, 28 pts received at least one dose of study treatment and 27 pts underwent surgery. The pCR rate (ypT0/Tis ypN0) was 14.8% (4/27). The bpCR rate (ypT0/is) and apCR rate (ypN0) were both 18.5% (5/27). There were more patients with RCB-2 (12/27), and fewer patients with RCB-0 (4/27), RCB-1 (3/27), and RCB-3 (7/27). According to the preoperative evaluation, 4

pts (4/28, 14.3%) achieved complete response (CR) and 22 pts (22/28, 78.6%) achieved partial response (PR). The ORR was 92.9% (26/28, 95% CI, 76.5%-99.1%). The grade 3 AEs were hematological toxicity in 3 cases and headache in 1 cases.

Conclusion: Anlotinib plus nab-paclitaxel based chemotherapy as neo-adjuvant therapy in patients with HER2-negative breast cancer showed favorable objective response rate and manageable toxicity. The enrollment is still ongoing.
Clinical trial information: NCT05558722.

P4-11-07: The Oncotype DX® test to guide adjuvant chemotherapy treatment decisions for early node-negative HR+/HER2- breast cancer patients in Japan: a cost-effectiveness analysis

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Background: For patients with hormone-receptor-positive (HR+), human epidermal growth factor receptor 2-negative (HER2-), axillary lymph node-negative (N0) early-stage breast cancer, adjuvant chemotherapy treatment decisions are traditionally determined by clinico-pathological features. While the use of clinico-pathological features may provide useful prognostic information, it is not able to predict the benefit of adjuvant chemotherapy, which may lead to over- or undertreatment. The Oncotype DX Breast Recurrence Score® test has been reimbursed in Japan since September 2023, and to date, it is the only multigene assay with the ability to predict chemotherapy benefit validated by evidence from randomized clinical trials. The aim of the study was to estimate cost-effectiveness of guiding chemotherapy treatment decisions using the Oncotype DX® test compared to using clinico-pathological risk alone among women with N0 HR+/HER2- early breast cancer from a Japanese healthcare perspective.
Methods: A previously published cost-effectiveness model built in Microsoft Excel estimated the cost-effectiveness of the Oncotype DX test compared to treatment decisions based on clinico-pathological risk alone over a lifetime horizon. This model used a decision tree coupled with a Markov model and was adapted to a Japanese healthcare payer perspective, complying with Japanese cost-effectiveness analysis guidelines. The N0 population was studied as a whole, as well as in subgroups based on age and clinico-pathological risk, defined according to tumour size and histologic grade. The modelled proportions of patients assigned to adjuvant chemotherapy and supportive treatment was informed by eight clinical experts in Japan to reflect current country-specific clinical practice. The Markov part of the model included health states representing recurrence-free, distant recurrence, death, as well as acute myeloid leukaemia (AML) and congestive heart failure (CHF) as latent adverse events of chemotherapy. The probability of distant recurrence of breast cancer conditional on test result category was derived from pivotal trials. Costs of testing,

adjuvant and supportive treatment, disease management, and short-term adverse events were informed using Japanese cost sources. To assess uncertainty, deterministic and probabilistic sensitivity analyses were performed.
Results: Using the Oncotype DX test was a dominant strategy compared to using clinico-pathological risk alone, with lower costs (-¥1,629,239) and additional health benefit (0.163 quality-adjusted life years, QALY) in the overall N0 population. This result was mainly driven by a reduced rate of distant recurrence for patients correctly treated with adjuvant chemotherapy after using the Oncotype DX test, which led to substantial cost savings and QALY gains. Probabilistic sensitivity analysis showed the Oncotype DX test was dominant against clinico-pathological risk alone in 99.4% of 5,000 simulations. Similar results were reported across subgroups; the Oncotype DX test was consistently cost-effective across all age and clinical subgroups considered, and was dominant (cost-saving and more effective) in 3 of 4 subgroups; in the subgroup aged≤50 with high clinical risk, the incremental cost-effectiveness ratio was ¥397,541/QALY.
Conclusion: Using the Oncotype DX test to guide chemotherapy treatment decision for women with early-stage N0 HR+/HER2- breast cancer is expected to be cost saving and a cost-effective strategy in Japan. It is expected to yield substantial net costs savings in most of the subgroups considered while increasing QALYs gained from avoiding chemotherapy adverse events and distant recurrence.

P4-11-08: Comparative analysis of clinician and AI decision making in HR+/HER2- early breast cancer.

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Background: Growing use of artificial intelligence (AI) has shown potential utility in assist imaging analysis, patient monitoring and treatment planning. Of note, pretrained Chatbots AI such as CHAT-Generative Pretrained Transformer 4o (GPT-4o) are emerging as groundbreaking tools to potentially answer clinically relevant questions and predict treatment decisions in oncology. However, the decision-making capability of these tools may substantially vary among specific clinical areas. Moreover, advantages and limitations of their use in daily clinical practice still appear unclear.
Methods: In this study, we evaluated CHAT GPT-4o performance compared with clinician recommendations for the adjuvant treatment of patients (pts) with hormone receptor-positive, HER2-negative early-stage breast cancer (HR+/HER2- eBC). Specifically, we analyzed the indication for multigene testing, type of test, and pre- and post-test treatment recommendations. Additionally, we evaluated CHAT GPT-4o prediction of multi-gene test results. Clinicians' pre-test and post-test decisions relative to consecutive HR+/HER2- eBC pts who underwent Oncotype DX test as per clinical practice (n=380) at the University of Naples Federico II were collected. GPT-4o and clinicians' decisions were expressed as

treatment strategy [chemotherapy (CT) followed by endocrine (CT-ET) vs ET alone], type of CT, and type of ET. Inter-decisions reliability was expressed as agreement rates and Cohen's kappa coefficient values. GPT-4o genomic testing prediction was expressed as percentage probability that the test would indicate high risk [Recurrence Score (RS)> 25] or low risk (RS≤ 25) in postmenopausal pts, and high risk (RS > 25), intermediate risk (RS 16-25) and low risk (RS≤ 15) in premenopausal pts. Genomic test prediction was evaluated using the Area Under the Curve (AUC) metric to determine accuracy in identifying high-risk and low-risk pts. The statistical association between predicted and observed results was analyzed by Chi Square test.
Results: Genomic testing was proposed by GPT-4o for all pts of our cohort. Among multigene assays, GPT-4o recommended the Oncotype DX for all cases. The agreement between clinician and AI pre-test decision (CT-ET vs ET) was 68% (kappa 0.391, $p < .001$), whereas the agreement in post-test decision was 93% (kappa 0.810, $p < .001$). Notably, the agreement between clinicians' pre-test and post-test treatment recommendations was 55% (kappa 0.198, $p < .001$), whereas concordance between pre-test and post-test treatment recommendations provided by AI was 68% (kappa 0.281, $p< .001$). Before Oncotype DX results, clinician recommended CT for 60.1% of pts, with docetaxel-cyclophosphamide (59.1%) as the preferred chemotherapy regimen. On the contrary, pre-test recommendation by GTP-4o included ET in 60.6% of cases, especially aromatase inhibitors (69.1%). The AI prediction of Oncotype DX RS results was associated with an AUC of 0.746 and 0.805 among postmenopausal and premenopausal pts, respectively. GTP-4o predicted with higher accuracy high genomic risk compared to low genomic risk in postmenopausal pts (89.4 % vs 45.5%, $p< 0.001$). On the contrary, in premenopausal pts AI showed lower accuracy in predicting low and intermediate genomic risk as compared with high genomic risk (55.9% vs 41% vs 66.7%, $p< 0.001$).
Conclusions: Without Oncotype DX results, the agreement in treatment recommendations between GTP-4o and clinicians was modest, and this discordancy deserve deeper investigation. Pre-test indications provided by GPT-4o were less impacted by the multigene assay results, maybe due to the capability of AI to better approximate Oncotype DX results. After Oncotype DX results, the agreement between AI and clinicians was extremely high indicating the key role of multigene testing in guiding treatment decisions.

P4-11-09: Epigenetic Alterations Affecting Cell Morphogenesis and Tumor Plasticity Pathways in Primary Tumors of Breast Cancer Patients with High Nodal Involvement

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Introduction: Survival rates for patients with breast cancer have improved significantly with widespread screening and effective systemic therapies. However, patients who present with advanced nodal stage at diagnosis have higher rates of distant relapse and require more aggressive therapies than patients with pathologic N1 disease (pN1). Understanding

the molecular alterations that occur in primary breast cancer (BC) that drive progression to a higher nodal stage (>pN1) could uncover potential therapeutic targets for patients with regionally metastatic BC. Given that epigenetic mechanisms represent that crucial layer of dynamic change that regulates genome-wide gene expression, we assessed the DNA methylation profiles of primary BC at various nodal stages.
Methods: We conducted an exploratory study using a well-annotated, retrospective cohort of ER+/HER2- treatment-naive primary BC from patients with clinically positive nodes and complete pathologic nodal data treated at Duke University (n=49, 30 cases pN1 vs. 19 cases >pN1). DNA was extracted from paraffin-embedded samples, and DNA methylation profiling was performed using the Illumina EPIC BeadChip v1 arrays. The ChAMP package was utilized to correct batch effect, and the Wilcoxon test was used to identify Differentially Methylated Sites (DMS). Tumor content purity was assessed using the LUMP algorithm and Gene Ontology enrichment of the DMSs was examined with the GOmeth tool. Data analysis and visualization were performed using the R packages ggplot2 (v. 3.4.4), circlize (v. 0.4.16), and gplots (v. 3.1.3.1), with data management facilitated by the tidyverse (v. 2.0.0) collection.
Results: Tumor purity was similar between the two groups ($p>0.05$). The comparison revealed distinct DNA methylation patterns in 962 genomic regions between pN1 and >pN1 tumors (differential methylation > 15%, $p<0.01$); 683 regions were hypermethylated and 279 were hypomethylated in the >pN1 group. There was a significant enrichment of biological processes associated with morphogenesis among the hypermethylated sites, with 22 of the 43 affected molecular pathways linked to cellular morphogenesis. Additionally, epigenetic alterations were predominantly found in pathways related to the response to bone morphogenic protein (BMP). Expression-methylation Quantitative Trait Loci (emQTLs) were linked to epithelial to mesenchymal transition (EMT) genes in >pN1 tumors. Finally, higher methylation of the protocadherin cluster genes (PCDHGA) and regions encompassing DUSP6 and KRT7 genes were identified in tumors of higher nodal stage. These findings suggest inherent or acquired epigenetic alterations in primary BC associated with higher nodal stage.
Conclusions: Understanding the mechanisms underlying lymph node invasion in breast cancer progression is crucial for improving the clinical management of patients with BC. This study indicates that ER+/HER2- primary tumors from patients with higher nodal involvement have an epigenetic predisposition to activate morphogenic differentiation and EMT more efficiently than patients with lower nodal involvement. Deeper insight into these alterations could contribute to more accurate prognostic tools, the development of novel therapeutic approaches, and potentially help avoid more aggressive axillary procedures.

P4-11-10: Long-term Outcome of Slowly Proliferating Luminal Type ILC versus IDC

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Introduction: Invasive lobular carcinoma (ILC) ranks as the second most common histopathological subtype of breast cancer, following invasive ductal carcinoma (IDC). Prior studies have demonstrated that while ILC patients initially exhibit better survival rates within the first 5 years, their long-term outcomes are comparable to, or worse than, those of IDC patients. However, the lower prevalence of ILC relative to IDC and their distinct clinicopathological characteristics complicate direct comparisons between the two groups. This study aims to evaluate the long-term outcomes of luminal type A IDC versus ILC.
Methods: This retrospective cohort study analyzed data from January 2008 to December 2015 at Samsung Medical Center. The study included patients with patients with primary breast cancer who underwent surgery and were histologically diagnosed with either IDC or ILC, were hormone receptor-positive, HER2-negative, and had a Ki-67 index ≤20. Patients with mixed histopathology, those undergoing palliative surgery, and those treated with neoadjuvant chemotherapy were excluded. Cohort analyses were performed in various subsets: real-world setting, patients receiving more than 2 years of endocrine therapy, and propensity score-matched cohorts.
Results: In the real-world setting, of the 3439 patients, 3156 had IDC and 283 had ILC. With a median follow-up of 110 months (range 0-195 and 1-190 months, respectively), ILC patients exhibited worse long-term outcomes. Although breast cancer-specific survival (BCSS) did not show a significant difference ($p=0.081$), analysis by 5-year periods indicated significantly poorer survival for ILC patients beyond 5 years (5 to 10 years, $p=0.004$; 10 to 15 years, $p=0.014$). Disease-free survival (DFS) and distant metastasis-free survival (DMFS) were significantly worse in the ILC group ($p<0.001$ and $p=0.005$, respectively). In the second analysis, excluding patients with less than 2 years of endocrine therapy, 2919 IDC and 269 ILC patients remained. With median follow-ups of 112 months (range 17-195 months) and 110 months (range 19-190 months) respectively, the ILC group showed poorer long-term DFS and DMFS ($p<0.001$ and $p=0.007$, respectively). However, the ILC group exhibited a significantly higher incidence of synchronous contralateral breast cancer, pathologic T and N stages, and a lower nuclear grade compared to the IDC group ($p<0.001$, $p<0.001$, $p=0.040$, $p<0.001$, respectively). The third analysis used propensity score matching to control for confounding variables, including age, BMI, menopausal status, BRCA status, synchronous contralateral breast cancer, pathologic T and N stages, TNM stage, nuclear grade, histologic grade, adjuvant chemotherapy, and duration of endocrine therapy. In this analysis, long-term survival outcomes did not significantly differ between groups (BCSS $p=0.248$, DFS $p=0.265$, DMFS $p=0.429$).
Conclusion: The study reveals that in the real-world setting, the long-term outcomes of ILC are worse compared to IDC. However, when adjusted for clinicopathological parameters, the long-term outcomes of ILC do not significantly differ from those of IDC.

P4-11-11: Real world data on breast cancer prognostication in India using CanAssist Breast- an indigenous test validated in global studies.

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Objective: Data on prognostication of Asian HR+/HER2/neu- early stage breast cancer patients using Western prognostic tests is limited and intriguing. Asian patients do get diagnosed almost a decade earlier and typically in Stage II thus the underlying tumor biology could be different. CanAssist Breast (CAB)- an immunohistochemistry and artificial intelligence based prognostic test was developed on Indian patient's tumors and validated in retrospective global studies in India, US, Spain, Germany, Austria, Italy and via prospective-randomized completed TEAM trial in The Netherlands. CAB is included in Asian geriatric Society's guidelines as well. Since mid 2016, CAB is in clinical use in India as well as Sri Lanka, Bangladesh, UAE and Turkey. In this abstract we assess the usefulness of CAB in our day to day treatment planning in India.

Methods: We analysed use of CAB on total 643 consecutive patients in our clinical practice from mid 2016 till December 2023. Specifically, we analyzed how does CAB segregate patients under/over 48 years ie pre/post-menopausal, N0, N1 patients. Patients with varying levels of tumor size, tumor grade and expression of ER and PR.

Results: Over all CAB segregated 66% patients as 'low risk' and 34% as 'high risk' for distant recurrence. Median age of the patient was 58 years and median tumor size was 2.5cm. Majority (75%) of patients were over 48 years of age. The low:high risk segregation in pre and post-menopausal was similar at 68:32 and 65:35. The current data is represented from North, South and West of India and in each of the regions as well the distribution of low:high risk proportions was similar. 33% patients had T1 tumors, 63% had T2 tumors however the low: high risk proportions in T1 versus T2 were significantly different at 84:16 and 59:41 respectively. Majority (75%) of patients had lymph node negative disease (N0) and the low:high risk proportions were 75:25 and 35:65 in N0 and lymph node positive (N1) disease. G1 tumors represented 12% of the total while 64% were G2 and 24% had G3 tumors. Low risk percentage across the three tumor grades was significantly different at 92%, 77% and 24% respectively. 88% of patients had ER and PR positive disease while 5% had ER+/PR- disease and for 7% PR status was unknown. 88% of patients had high ER expression (>70%) while 10% had intermediate (10-70%) and 1% had low ER (1-9%) expression. 46% patients had T2N0, 27% had T1N0, 7% had T1N1 and 17% had T2N1 disease. 99% were HER2/neu negative. CAB was used to plan treatment for all of these patients. We have treatment details for 77% of the patients. 94% of low risk patients did not get chemotherapy and 84% of high risk patients got chemotherapy. For the 6% low-risk patients who got chemotherapy, we believe the potential reasons could be N1 disease, G3 disease, high Ki67 index, Her2+ disease and pre-menopausal patients.

Conclusion: CAB is able to segregate the patients into low or high risk in line with clinical parameters. CAB has helped 94% of low-risk patients to avoid chemotherapy. CAB represents tumor biology of younger patients and coupled with world-wide validation it presents as a cost-effective, ideal alternative to western prognostic tests to patients in Asia.

P4-11-12: CLEAR-B - Adjuvant therapy effectiveness in patients with primary breast cancer: A retrospective registry study

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Objectives: Adjuvant endocrine treatment for premenopausal patients with early hormone receptor positive HER2 negative (HRpos/HER2neg) breast cancer (BC) is supposed to depend on the estimated risk profile. Patients with a high recurrence risk should be offered aromatase inihibtors (AI) and ovarian function suppression (OFS) and patients with a low recurrence risk tamoxifen (TAM) with or without OFS. However utilization of these treatment options is diverse even in high risk patients. Aim of this retrospective study was therefore to assess the treatment patterns and outcomes of endocrine treatment variability in a real world setting.
Methods: CLEAR-B is an anonymized retrospective study that uses prospectively collected data generated in the course of the certification process of breast centers in the period from January 2016 to June 1019 and from January 2022 to December 2023. Eligible were premenopausal patients with early HRpos/HER2neg BC an intermediate high and very high recurrence risk. Patient and disease characteristics are documented along with information about recommended and performed adjvuant treatments as well as information about recurrence and death.
Results: The first patient was documented in February 2023. A total of 3.000 patients will be documented until August 2024 at 75 study sites in Germany. An interim analysis showed that the majority of documented patients is treated with tamoxifen despite an increased recurrence risk. Changes in therapy patterns and patient/disease characteristics will be analyzed according to patient risk profiles and in the two assessment periods (2016-2019 vs. 2022-2023). Additionally the context of healthcare provision (treatment centers vs. de-central healthcare) will be investigated.
Summary: Awareness of treatment standards along with efficacy data and quality of life data is important to make informed therapy decisions about adjuvant endocrine treatment together with the patients. A full analysis of treatment patterns over time and outcomes will be presented at the conference.

P4-11-13: High Progesterone Receptor (PR/PGR) Expression Determined by APIS Breast Cancer Subtyping Kit Correlates with Oncotype Dx Recurrence Score

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Introduction: Expression of ER, PR, HER2, and Ki67 serve as valuable prognostic and predictive markers, with PGR expression believed to predict favourable outcomes in ER+ HER2- breast cancer (BC). Traditionally, the expression of these markers is determined by immunohistochemistry (IHC), which is well established for ER, PR and HER2, but for Ki-67 faces challenges in standardization. Several quantitative gene expression tests, like Oncotype Dx, aim to overcome these challenges but are often limited by their costs.
The APIS Breast Cancer Subtyping Kit offers an accurate and cost-efficient method for determining ER/PR, HER2, and Ki67 expression. This study seeks to assess the relationship between Oncotype Dx recurrence score (RS) and PGR expression as determined by the APIS kit.
Methods: Tissue from a cohort (N=153) of ER+/HER- BC patients diagnosed between 2020 and 2022 at Cantonal Hospital Basel-Land and Basel University Hospital was included in this study. Each patient had a valid Oncotype DX RS score on file and received at least one line of adjuvant therapy. PGR expression was determined using the APIS kit, following instructions for use. Spearman correlation tests were applied to the data to examine the association between the tests. The model classification explorer (JMP v 16.0) was used to predict PGR expression cut-offs for RS and plot a receiver operator curve (ROC) for the data.
Results: A moderate Spearman's correlation coefficient of ρ = -0.4876 between the APIS kit and the Oncotype Dx RS was observed. ROC curve for PGR prediction of oncotype score was (0.782 (95% CI 0.717-0.847)). When stratifying RS scores by low (<26) and high risk (>26) a PGR ΔCt of above 3.06 was calculated to identify the low-risk group. The specificity for low-risk RS was 93.5% (95% CI 79.3-98.2%), with a positive predictive value of 0.949.
Conclusions: This study demonstrates that PGR expression, as assessed by APIS Breast Cancer Subtyping Kit, could potentially predict low-risk tumours identified by Oncotype Dx reliably, offering a cost-effective pre-screening tool. Additional validation is necessary to affirm its diagnostic accuracy.

P4-11-14: Prescribing patterns and real-world clinical usage of adjuvant abemaciclib for early-stage, high-risk, hormone receptor positive breast cancer

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Background: The FDA approved abemaciclib in October 2021 for use in conjunction with hormone therapy for adjuvant treatment of early-stage, high-risk, hormone receptor positive (HR+), human epidermal growth factor receptor 2 (HER2)-negative breast cancer (BC) based on the results of the monarchE trial (NCT03155997), which demonstrated that adding abemaciclib improved invasive disease-free survival. Given its recent FDA approval, the prescribing practices and real-world uptake of abemaciclib have not been extensively reported. The objective of this retrospective study was to determine the rate of adjuvant abemaciclib prescribing for high-risk HR+ patients (pts) and to determine the pt and provider factors that were associated with its prescribing. We also sought to describe the

clinical experience (e.g., dose reductions, toxicities) of pts on adjuvant abemaciclib.

Methods: Data regarding demographics, clinicopathologic characteristics, and adjuvant treatment details were obtained from the University of Pittsburgh Medical Center Cancer Registry on pts with lymph node (LN)-positive, stage I-III HR+/HER2- BC, diagnosed between 1/1/2021-8/31/2023. Pts were included in our analysis if they initiated hormone therapy on or after 7/12/2021. High-risk status was determined by monarchE inclusion criteria for two cohorts: 1) AJCC nodal status N2+, N1 and tumor grade 3, or N1 and T3+ (i.e., cohort 1) or 2) N1 and Ki-67 (%) ≥20 (i.e., cohort 2). Under the 2021 FDA approval guidelines, pts were eligible for abemaciclib if their tumors were N2+ or N1 and Ki-67% ≥20 and either grade 3 or T3+. Additional information regarding abemaciclib was extracted from the electronic health records. We evaluated the relationship between tumor-, provider-, and pt-level factors and receiving prescription for abemaciclib (outcome) using Pearson's χ-square tests and multivariable logistic regression models, adjusted for age at diagnosis, race/ethnicity, nodal status, tumor size, and grade.

Results: The entire high-risk cohort included 469 pts, and 272 (58%) were eligible for abemaciclib under 2021 FDA approval guidelines. Median age was 61 years (range 24-93), the majority of pts were white (93%) and most (61%) were treated at UPMC Hillman Cancer Center community sites. 320 (68%) pts had N1 and 149 (32%) pts had N2+ disease. Overall, 163/469 pts (35%) were prescribed abemaciclib – 120/272 pts (44%) eligible under 2021 FDA guidelines. Of pts that met monarchE inclusion criteria for cohort 2, 20% (29/144) received abemaciclib. Abemaciclib prescriptions significantly increased by year of diagnosis with 28% and 42% of pts diagnosed in 2021 and 2022, respectively, receiving a prescription ($p=0.012$). In multivariable logistic regression analysis, age at diagnosis, nodal status, and tumor size were associated with receiving a prescription for abemaciclib ($p < 0.05$). Among pts in our cohort who were not prescribed adjuvant abemaciclib and had documented reason for not being prescribed (n=80), the most common reason was the pt did not want it (n=44, 55%). Of pts who were prescribed abemaciclib (n=163), 47% (n=77) had dose modified, 81% (n=132) experienced one or more toxicities, and 20% (n=32) discontinued treatment. The most common reason for dose modification and discontinuation was diarrhea. At study cutoff of 4/1/2024, 80% of pts who started abemaciclib either completed the planned two years of treatment or continued on the medication.

Conclusions: In this single institution, retrospective study, adjuvant abemaciclib prescribing was low. Considering the benefit of adjuvant abemaciclib in pts with high-risk HR+/HER2- BC, further research is required to address barriers to its prescribing and to improve our understanding of factors that influence physician and pt decisions.

P4-11-15: Variability in physician treatment decisions for HR+ / HER 2 negative Early Breast Cancer in young patients: a Latin-American survey

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Introduction: Breast cancer in young people (age ≤ 40), represents 5% of all breast cancer cases. Population outcomes for this group are often derived from subgroup analyses of randomized or retrospective trials. Limited data about physicians' knowledge, attitudes and practices as they relate to this diagnosis. The aim of our study was to evaluate the decision-making process in Latin-American oncologists for the treatment of young women with early breast cancer.
Methods: A survey was designed with 30 items. Principal topics included: use of ovarian suppression, indication for adjuvant chemotherapy, fertility, use of genomic tests and recommendations for management of treatment-associated symptoms.
Content validation was undertaken by Argentinian oncologists of SUMA (Argentinian Group for the treatment and research of breast cancer).
The study was conducted via an online questionnaire, sent by email to professional societies and to oncologists involved in the management of breast cancer patients.
Descriptive analyses were included. Chi square test was performed to evaluate relationships between physician characteristics (country, gender, years of expertise, whether the physician worked in a tumor specific clinic or not) and responses. The level of statistical significance was set at $p < 0.05$, two-tailed.
Results: A total of 329 Latin American oncologists from 17 Latin-American countries participated in the electronic survey.
The responding oncologists included 74.5% who treated patients with any tumor type (ALLT), 14.6% specialized in breast and gynecological tumors (BRGY), and 10.9% focused exclusively on breast tumors (BREX). Working in a tumor-specific clinic was associated with differential utilization of several treatments or diagnostic tests, including ovarian suppression (OFS) for therapeutic purposes during neo/adjuvant chemotherapy (74.7% ALLT, 91.6% BRGY, 86.1% BREX; p=0.031), selective recommendation for OFS with endocrine therapy during the adjuvant setting (51.4% ALLT, 66.6% BRGY, 75% BREX; $p < 0.001$), and routine evaluations of FSH levels during treatment with OFS (70.5% ALLT, 86.8% BRGY, 86.2% BREX; p=0.038). Genetic counseling was always offered in young patients by 47.8%, 72.9% and 69.4% of physicians with ALLT, BRGY and BREX clinics, respectively (p=0.014).
The use of genomic platforms for decision-making also differed: 55.6% of BREX oncologists reported always using these platforms in cases of young women compared to 38.8% of oncologists who treat all types of tumors ($p <0.001$). Specifically, chemotherapy was recommended to patients with recurrence score higher than 20 by 49%, 77.8% and 60.4%

of oncologists with ALLT, BRGY and BREX clinics, respectively (p=0.003)
The reported practices did not differ among professionals from different countries .
Conclusions: According to the results of our survey the approach to managing early HR+ HER2- negative breast cancer in young women differ depending by professional focus. Full results will be presented. Further research is needed to understand the basis for these differences. However, given the heterogeneity of responses, our study highlights the need for specific guidelines for the management of breast cancer in young patients.

P4-11-16: Improvement of Pathologic Complete Response Prediction with Plasma Cytokine Profiles in Hormone Receptor-Positive Early Breast Cancer Treated with Neoadjuvant Chemotherapy

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Background: Neoadjuvant chemotherapy (NCT) is the standard of care for most cases of HER2-positive early breast cancer (BC) and is also used in selected patients with HER2-negative hormone receptor (HR)-positive BC. Immune biomarkers, especially stromal tumor infiltrating lymphocytes (sTIL), have been shown to be useful for prediction of pathologic complete response (pCR). However, this prediction is less accurate both in HER2+ and HER2- HR-positive BC. The interplay between the peripheral and the tumor microenvironment immune compartments might have implications for the search of new predictive immune biomarkers. Plasma levels of cytokines and growth factors may allow the identification of differential immune and inflammatory activation states in BC patients, potentially contributing to chemotherapy response.The aim of this study was to determine if circulating plasma cytokines might improve prediction of pCR in early HR+ BC treated with NCT.
Methods: Pre-treatment plasma levels of cytokines were determined in a prospective single-center cohort of women with hormone receptor (HR)-positive BC treated with NCT. Bead-based multiplex assays and Luminex technology was used to simultaneously obtain the plasma concentrations of 29 cytokines and growth factors. The outcome variable was pCR. After pre-processing of plasma cytokine values (log transformation, asignment of half the limit of detection to non-detectable values, treatment of outliers and imputation of missing values with an iterated random forest algorithm), cytokine variables were discretized into high vs low concentration using k-means clustering. In order to identify the information that each variable could add to prediction of pCR, a minimum-redundancy maximum-relevance mutual information algorithm was applied to all relevant clinical and pathological variables and to pre-treatment dichotomized cytokine levels. The first four variables in the mutual information relevancy matrix, together with HER2 status, were included in multivariable logistic regression models for pCR. Goodness of fit of the models were compared with the likelihood ratio test (LLRT). AUC (area under cuve) of ROC

(receiver-operating curve) was calculated to evaluate model performance.We used R version 4.2.3 (package varrank) for statistical analysis and data visualization.
Results: We included 63 patients with HR-positive early BC in the study. Median age: 49 (range: 31-76); 39,7% grade III; 33,4% stage III; 39 patients HR+/HER2- (61,9%) and 24 HR+/HER2+ (38,1%). All HER2+ patients received antiHER2 treatment combined with NCT. After NCT, pCR rate was 28.6%; ypN0 rate was 57.1%. Maximal relevance for pCR corresponded to grade 3, CD28 baseline levels, TNFa baseline levels, Ki-67 (continuous variable), negative progesterone receptor (PgR) and HER2 amplification. Higher plasma levels of TNFa and lower levels of CD28 were significantly associated with pCR. A logistic regression model for pCR based only in clinical and pathological variables (Grade 3, odds ratio [OR] 11.1, 95%CI 2.67-61.5; Ki67, OR 1.05, 95%CI 1.01-1.10; HER2+, OR 4.37, 95%CI 0.93-26.70) was improved (LLRT, p=0.005) after inclusion of baseline cytokine levels into the model. In the final multivariate model, OR was 11.0 for TNFa (95%CI, 1.86-120.0, p=0.02) and 0.19 for CD28 (95%CI, 0.02-0.96, p=0.08). The ROC-AUC of the first model was 0.87, while the model including cytokine levels had a ROC-AUC of 0.92.
Conclusions: In HR-positive early BC, circulating cytokine TNFa and CD28 provided predictive value for pathologic complete response after neoadjuvant chemotherapy, beyond the information provided by classic clinical and pathological variables. Validation of these findings in a larger cohort and further research on the relationships between tumor and circulating immune biomarkers of chemotherapy response might improve the current selection approach of HR+ BC patients for primary systemic treatment.

P4-11-17: Prognostic significance of complete cell cycle arrest compared to the CPS + EG scoring system in patients with HR+/HER2- breast cancer receiving neoadjuvant chemotherapy

Sohyun Moon, Sung Gwe Ahn

Background: Pathologic complete response (pCR) after neoadjuvant chemotherapy (NAC) is not predictive in HR+/HER2- breast cancer patients. Complete cell cycle arrest (CCCA) is regarded as a biomarker associated with response to endocrine therapy and recurrence-free survival (RFS). The CPS + EG scoring system has been developed and validated to predict a prognosis in patients receiving NAC. We evaluated a prognostic influence of CCCA compared to the CPS+EG score in patients with HR+/HER2- breast cancer who had residual tumors after NAC.
Methods: Between January 2007 and June 2021, we retrospectively identified 1,419 HR+/HER2 breast cancer patients who underwent NAC in two academic institutes in South Korea. Among these, 918 with Ki67 index in surgical specimens with residual cancer were included. We obtained the CCCA value, which is Ki67 less than or equal to 2.7%. The clinicopathologic stage (CPS) + EG scores were obtained, and the cutoff for high risk was defined as ≥3. The primary endpoint of this study is recurrence-free survival (RFS).
Results: In 918 patients, the CCCA rate was 60.1% (552/918) with a 7-year RFS of 75.8% (95% CI, 71.3-80.6%) in the CCCA group compared to 63.9% (95% CI, 58.0-70.4%) in the

non-CCCA group. The group with CCCA showed significantly longer 7-year recurrence-free survival (RFS) rates compared to the non-CCCA group in both CPS + EG score-high patients (75.9% vs. 62.6%) and CPS + EG score-low patients (84.2% vs. 78.9%). In multivariable analyses, achieving CCCA was demonstrated as a prognostic factor for RFS (hazard ratio 0.488, 95% CI, 0.361-0.660) independent of stage, age, grade, and the CPS-EG score. Conclusions: Our findings show that achieving CCCA is associated with improved survival among patients with HR+/HER2- breast cancer who do not attain a pathologic complete response after NAC and suggest that a prognostic significance of CCCA is independent of the CPS-EG score.

P4-11-18: Efficacy of anthracycline- and taxane-containing neoadjuvant chemotherapy for luminal HER2-negative BRCA-mutated breast cancer

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Introduction: Germline mutations in the BRCA1/2 genes occur in 5-10% of patients with luminal HER2-negative (ER+HER2-) breast cancer (BC) and are associated with a more aggressive course of the disease. Data on the effectiveness of neoadjuvant chemotherapy (NACT) in this group is limited. The aim of the study was to assess the rate of pathological complete response (pCR) in BRCA mutated ER+HER2- BC patients (pts) receiving NACT.
Materials and methods: This retrospective study included pts with ER+ (ER>10%) HER2- stage II-III BC and germline BRCA1/2 mutations (mut) 4th or 5th pathogenicity class. Pts received anthracycline- and taxane-containing NACT (4 cycles AC every (q) 2 or 3 weeks (w) then 4 cycles docetaxel 75 mg/m2 q3w or 12 paclitaxel 80 mg/m2 ± carboplatin AUC2 weekly) in a single center from September 2017 to December 2023. All the pts from the center's database meeting the criteria mentioned above were included in the study.
Results: Thirty one pts were included, 14 (45.2%) with BRCA1 mut, 16 (51.6%) - BRCA2 mut, one (3.2%) had both. Mutations were detected by PCR in 17 (54.8%) pts and by NGS in 14 (45.2%). AC q3w and q2w received 11 (35.5%) and 20 (64.5%) of pts respectively, docetaxel and paclitaxel – 17 (54.8%) and 14 (45.2%), 6 pts (19.4%) received carboplatin. Mean age was 40.5 years (27-58), 29 pts (93.5%) were premenopausal. Most of them (71%) had a locally advanced stage, 58% - cT4 and 83.9% - cN+. More than 2/3 (74.2%) of tumors were G2, 25.8% - G3, 87.1% had ER expression >50%, 67.7% - progesterone receptors >20%. Median ki67 was 49.6% (17-85). Luminal B subtype identified in 29 (93.5%) cases. The pCR rate was 45.2% (N=14), in the subgroup of BRCA1mut pts - 64.3% (N=9), BRCA2mut - 31.3% (N=5) (p=0.07).
Discussion: Anthracycline- and taxane-containing NACT in BRCA mut ER+HER2- BC achieved pCR in 45.2%, which substantially exceeds the rate of pCR in luminal BC - 16-18%. BRCA mutation could be an independent factor in the choice of neoadjuvant/adjuvant therapy due to the high chemosensitivity of these tumors.

P4-11-19: An analysis of sexual dysfunction symptoms in hormone receptor positive breast cancer patients during adjuvant endocrine therapy in a single Brazilian center

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Background: Hormone receptor positive (RH+), HER 2 negative (HER 2-) early-stage breast cancer (BC) patients undergo adjuvant endocrine therapy (ET) during a period ranging from five to ten years, and the treatment impact on sexual life hasn't been properly addressed as it should. In this study performed in a single Brazilian center we aim to show the impact of prolonged ET on the sex lives of early stage BC patients in ET.
Methodology: 105 women with an early-stage RH+, HER 2 - invasive BC undergoing adjuvant ET for at least 6 months were included. We separated sexually active and inactive patients, with sexually active being defined as having engaged in sexual intercourse at least once in the past four weeks. Those with an active sex life took the Female Sexual Function Index questionnaire (FSFI) and sexual dysfunction was indicated by a score of <=26.55 on the FSFI, while > 26.55 indicated an absence of sexual dysfunction. Data collection was performed using RedCap software. The data analysis was done using the RStudio program.

Results: Patients average age was 58.7 years, 57.8% were married, 58.9% were white and 51.0% had a college degree. Prior to BC diagnoses, 65.4% of the women were sexually active and only 42.3% have engaged in sexual relations in the four weeks before answering the questionnaire. Regarding the stage of the disease, 42.9% and 41% of the patients were in stages I and II, respectively, while only 16.2% were in stage III. The sexual function score indicated sexual dysfunction in 77.3% patients (SD = 21.8), with the main determinant factors being the domain of sexual desire (SD =3.1) and pain during sexual intercourse (SD = 3.2). The patients reported a moderate level of overall satisfaction with their sexual lives (SD =4.4), orgasm (SD=4,0) and lubrification (SD=4.1).

Conclusion: There was a notable decline in the quality of the sexual lifes of patients in ET. This analysis represents an urgent symptom which is little discussed by the professionals and poorly managed during ET.

P4-11-20: Development of a method for detecting genetic mutations in early breast cancer using Plasma-Safe-SeqS technology and its clinical application.

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Background: The detection of circulating tumor DNA (ctDNA) in blood has attracted attention and research as a way to assess the risk of recurrence and treatment effectiveness

in early-stage breast cancer (EBC). However, detecting ctDNA in blood in EBC is challenging due to its extremely low leakage, and it requires an ultra-sensitive test for clinical use.
In this study, we used a gene panel test for ctDNA in blood using the latest technology to investigate the effectiveness of mutation identification in EBC. This study also compared gene mutation identification using this method with next-generation sequencing (NGS) using tissue samples.
Methods: Among patients with hormone-positive and HER2-negative early breast cancers, which account for 60-70% of breast cancer patients, 12 patients at high risk of recurrence (Patients with four or more positive nodes, or one to three nodes and either tumor size ≥ 5 cm, histologic grade 3, or central Ki-67 ≥ 20%) were enrolled.
Blood samples for ctDNA were collected at the time of diagnosis, and for the NGS gene panel, Sysmex's Plasma-Safe-SeqS technology (PSS) was used to detect six breast cancer-related genes (AKT1, ERBB2, ESR1, KRAS, PIK3CA, and TP53) from ctDNA.
The panel can detect mutant allele frequency values of 0.05%. OncoGuide™ NCC Oncopanel was used to confirm the presence of genetic mutations in the tissue at the time of diagnosis and to measure the gene mutation identification rate.
The Institutional Review Board of Showa University Hospital issued approval 22-208-A.
Results: The OncoGuide™ NCC Oncopanel identified genetic mutations in 6 out of 12 patients , with 2 patients also found to have mutations in PSS. The gene mutations in these two cases were AKT1 and PIK3CA, respectively.
The two cases identified by PSS were both cT4 with large tumor volumes and skin invasion. One had skin and muscle invasion and was 95 cm3 in size, and the other had skin invasion and was 132 cm3 in size. On the other hand, the four cases identified only in the OncoGuide™ NCC Oncopanel were AKT1 in one case, PIK3CA in one case, and TP53 in two cases.
The detection rate of genetic mutations in operable breast cancer using ctDNA was 33%. The two cases identified by PSS were both cT4, with large tumor volumes and skin invasion. On the other hand, the reason why PSS could not identify four cases could be that the tumor volume was small and the amount of ctDNA leaked into the blood was low.
Tumor reduction rate after neoadjuvant chemotherapy by genetic mutation was as follows: two cases with AKT1 mutations averaged 81% (range: 71%-91%), one case with PIK3CA gene had 100% reduction, and two cases with TP53 gene averaged 41% reduction (range: 39%-43%). Conversely, the six patients without genetic mutations had an average reduction rate of 85 % (range: 19%-100%).
There were no differences in clinical pathological factors between the two cases with TP53 mutations and the other 10 cases, other than TP53 mutations. These two cases had a poorer response rate to chemotherapy than others, suggesting that TP53 is considered an independent predictor of reduced response to chemotherapy.
Conclusions: In this study, we found that PSS could be utilized in perioperative breast cancer treatment in clinical practice. In addition to the postoperative blood sampling described above, we are currently conducting (1) postoperative blood sampling and (2) postoperative blood sampling after completion of adjuvant therapy. We aim to ascertain whether this mutation can serve as a predictive marker for response to postoperative

chemotherapy or recurrence, especially in hormone-positive breast cancer patients at high risk of recurrence.

P4-11-21: The use of adjuvant Abemaciclib in HR+, HER2- early breast cancer: a multi-institutional UK experience.

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Background: The landscape for the treatment of HR+ HER2- early breast cancer continues to evolve with an increasing focus on targeted therapies. This study explores real world experience of the use of adjuvant Abemaciclib.

Methods: This is a retrospective study of 138 patients (pts) who received adjuvant Abemaciclib in combination with hormonal treatment between September 2022 until February 2024 at Nottingham University Hospital and University Hospitals of Leicester NHS Trust. Pts' demographics, menopausal status, histological types, high-risk disease status, toxicities and reasons for discontinuation were reviewed.High-risk disease was defined as ≥ 4positive axillary lymph nodes or 1-3 positive axillary lymph nodes (LNs) with either tumour size ≥5cm or histological grade 3. Data analysis was conducted using SPSS statistics, version 28.0.ResultsThe cohort was predominantly females (99.3%) with an average age of 57 years. The majority were White-British (77.5%) and postmenopausal (69.6%). Histology characteristics showed most of patients were invasive ductal carcinoma (70.3%) with half of the cohort having tumours measuring 2-5 cms and having 1-3 positive axillary LNs.Overall toxicities was reported in 60.9% of pts. Treatment related neutropenia and diarrhoea were mostly grade 1-2 .Neutropenia occurred at Grade 1 in 13.8% (19 pts), at Grade 2 in 8% (11 pts), and at Grade 3 in 5.8% (8 pts). Diarrhea was noted in 39.9% (55 pts) at Grade 1, 18.8% (26 pts) at Grade 2, and 5.1% (7 pts) at Grade 3.Other toxicities reported included fatigue (35.2%),skin rash (11.1%), and nausea (9.3%). About 58% of pts required at least 1 dose reduction. Sixteen percent of pts discontinued treatment due to toxicity. Five (3.6%) pts progressed on treatment.
Conclusions: Our study`s results, despite its smaller size, align with the outcomes observed in the larger phase 3 trial. In comparison to the MonarchE study, our pts experienced lower incidences of G2/3 neutropenia and all grades of diarrhoea. The discontinuation rate due to intolerability was similar in this cohort compared to the phase 3 study (16%) vs (18.5%).The data is still immature for making definitive comments on survival benefits.

P4-11-22: Prognostic Value of high-risk Oncotype Recurrence Score (RS) and MammaPrint (MP) assay in Premenopausal African American (AA) Patients with Hormone-Positive (HR+), Node-Negative Breast Cancer (BC): A Study of the National Cancer Database (NCDB).

Devashish Desai, Prashanth Ashok Kumar, Dongliang Wang, Abirami Sivapirasgasam

Background: It has been hypothesized that RS score has lower prognostic accuracy in AA, compared to Caucasians. This study evaluates this difference using the NCDB, focusing on premenopausal, node-negative patients with RS ≥ 26 or MP high risk. Methods: The 2021 NCDB PUF was used to include premenopausal female BC patients aged 18-50 years. Inclusion criteria were N0, M0 patients with T1-4, RS ≥ 26 or MP high risk, estrogen and/or progesterone receptor-positive, and HER2-negative. Patients were stratified by their recorded race (Caucasians and AA). Univariate analysis was used after distributing the groups based on chemotherapy receipt. Kaplan-Meier analysis (KM) was used to evaluate survival rates. Results: 8,842 patients had RS ≥ 26 [Caucasians-7,412(83.83%), AA-1,430(16.17%)] of which 7,699 (87.07%) received chemotherapy [Caucasians-6495(84.36%), AA-1204(15.64%)] and 7,910 (89.46%) received hormonal therapy [Caucasians-6,663(89.89%), AA-1,247(87.20%)]. In the high-risk MP category, 1,524 patients were identified [Caucasians: 1,278 (82.99%), AA: 262 (17.01%)]. Among these, 1,259 (82.61%) received chemotherapy [Caucasians: 1,051 (82.24%), AA: 208 (79.39%)] and 1,365 (89.57%) received hormonal therapy [Caucasians: 1,141 (89.28%), AA: 224 (85.50%)]. The KM survival curve showed similar survival rates in Caucasians and AA among RS ≥ 26 patients who received chemotherapy [5 years – Caucasians 95.9(95.3-96.5)%, AA 95.1(93.4-96.4)%; 10 years – Caucasians 90.1(88.8-91.3)%, AA 89.2(86.0-91.7)%]. Numerically, survival rates were lower in AAs among RS ≥ 26 patients who did not receive chemotherapy [5 years – Caucasians 94.9(92.9-96.4)%, AA 90.4(84.4-94.2)%; 10 years – Caucasians 88.0(83.9-91.1)%, AA 80.2(66.1-88.9)%]. In the high-risk MP group, the 5-year survival rate with chemotherapy was 97.0% (95.5-98.1) for Caucasians and 93.9% (88.4-96.9) for AAs. Without chemotherapy, the 5-year survival rate was 95.5% (88.6-98.3) for Caucasians and 94.7% (68.1-99.2) for AAs. Conclusions: Our study shows that among high genomic risk, node-negative, premenopausal patients, the survival rates are numerically similar between Caucasians and African Americans (AAs) when chemotherapy was administered. However, when chemotherapy was omitted, there were numerical differences in the survival rates within the high-risk RS population. This trend was not observed with the MP cohort. The analysis also highlights that approximately 13% of patients with RS ≥ 26 and 17% of high-risk MP patients did not receive chemotherapy.

P4-11-23: Treatment Response to Neoadjuvant Endocrine Therapy in Hormone Receptor-Positive, HER2 Negative Early Breast Cancer Patients in Los Angeles County

Gene Yoshikawa, Krixie Silangcruz, Lauren Lewis, Katie La Barbera, Andrew Hwang, Charity Huang

Background: There has been increasing interest in utilizing the role of neoadjuvant endocrine therapy (NET) before definitive surgical resection in patients with hormone receptor (HR) positive human epidermal growth factor 2 (HER2) negative non-metastatic breast cancer [1]. Numerous studies have demonstrated that postmenopausal breast cancer patients who receive NET have improved outcomes compared to those who receive upfront surgical resection with curative intent [2][3][4]. In this study, we conducted a real-world retrospective analysis of response rates to NET in premenopausal and postmenopausal underserved women in a Los Angeles (LA) County hospital with HR-positive/HER2-negative breast cancer. In addition, this study was conducted during the COVID-19 era, when efforts were heightened to minimize non-essential interactions with the healthcare system. This context underscores the importance of exploring alternative treatment strategies such as NET, which could potentially reduce the need for immediate surgical interventions while maintaining effective management of HR-positive/HER2-negative breast cancer in underserved populations.
Methods: We conducted a retrospective analysis of 56 HR-positive/HER2-negative early breast cancer patients from a safety net hospital in LA County on NET. The primary endpoint was treatment response, determined by change in tumor size at the time of surgery. Secondary endpoints included various clinical and pathological factors, as discussed in the results section.
Results: The objective clinical response was 48.2%, and the median time on NET for all patients was 123 days. Overall, there was a reduction in median tumor size from 17.83mm before treatment to 13.69mm after treatment (p=0.012), with a combined median size reduction of 2mm. Median tumor size reduction was 2mm in non-Hispanic patients compared to 1.5mm in Hispanic patients, though this difference was not statistically significant (p=0.543). Median tumor reduction was 4mm for premenopausal women and 1.5mm for postmenopausal women but was also not statistically significant. No significant association was seen between histological subtype and response to NET. The median clinical and pathologic stages were IA and IB, respectively. No differences between ethnic groups were noted (p=0.884). The mean OncotypeDX score was 14.83 in Hispanic patients and 15.77 in non-Hispanic patients without a correlation via linear regression. Breast-conserving therapy (vs. mastectomy) showed no association with ethnicity (p=0.884) or menopausal status (p=0.383).
Conclusion: This retrospective chart review underscores the effectiveness of NET in decreasing tumor size among HR-positive/HER2-negative early-stage breast cancer patients, notably in an underserved demographic. There was no association between histological subtype and response to NET. Additionally, there were no significant differences between ethnic groups in OncotypeDX scores or breast-conserving therapy

decisions, suggesting standardized treatment approaches and similar clinical characteristics across diverse patient groups. Despite challenges such as delayed presentation and concurrent health issues, the significant reduction in tumor size post-treatment indicates a favorable response and suggests that NET has the potential to enhance surgical outcomes independent of menopausal status. Moreover, the observed difference in tumor size reduction between non-Hispanic and Hispanic patients highlights the need for further investigation into the factors influencing treatment response among diverse patient groups.

P4-11-24: Real-world data on high and intermediate-risk hormone receptor (HR)-positive HER2-negative early breast cancer patients prior to adjuvant CDK1/6i era

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Background: Patients with high- or intermediate- risk HR-positive HER2-negative early breast cancer (EBC) have increased risk of disease relapse. Combination of endocrine therapy (ET) with CDK4/6 inhibitors have shown to improve clinical outcomes in these settings based on MonarchE and NATALEE studies. The objective of this study was to determine the distant disease-free survival (DDFS), invasive disease-free survival (iDFS) and overall survival (OS) of HR-positive HER2-negative EBC cancer patients prior to the use of adjuvant CDK4/6 inhibitors.
Methods: This retrospective study retrieved real-world patient data from the database of the Hong Kong Breast Cancer Foundation. They were consented for data collection from 11 public and 4 private hospitals/clinics in Hong Kong. This study was approved by the regional ethics committee of individual units. Patients who underwent definitive surgery for EBC between January 2006 and December 2011 were studied. The inclusion criteria of MonarchE and NATALEE were applied. Patients were divided into 3 groups: group 1, those that satisfied both MonarchE and NATALEE criteria; group 2, those eligible for NATALEE criteria but not MonarchE; and group 3, remaining patients, who had stage 1 cancers. Patients background characteristics were collected. DDFS, iDFS and OS were evaluated using the Kaplan–Meier method. Descriptive statistics were used to report the clinical outcomes of the 3 groups.
Results: A total of 3541 ER-positive HER2-negative EBC were included. 923 (26.1%) in group 1, 962 (27.2%) in group 2 and 1656 (46.8%) in group 3. 1633 (46.1%) patients were premenopausal, 1684 (47.6%) were postmenopausal while 224 (6.3%) had unknown menopausal status at diagnosis. 1900 (53.7%) had node-negative disease. 899 (25.4%) had grade 3 cancers. High tumour Ki67 (defined as >/=20%) was reported in 825 (23.3%) patients. 2467 (69.7%) received radiotherapy, 2241 (63.3%) underwent chemotherapy. 3366 patients received ET (95.1%); information on ET of 12 (0.3%) patients were unknown. For group 1 vs group 2 vs group 3, the 10-year DDFS were 75.2% vs 90.3% vs 95.8%, the 10-year iDFS were 69.7% vs 84.2% vs 89.4% respectively, while the 10-year OS

were 79.9% vs 90.2% vs 94.5% respectively.
Conclusions: The present study confirmed that in the real-world clinical setting, patients who satisfied both MonarchE and NATALEE eligibility criteria had the worst prognosis, while patients who satisfied NATALEE but excluded from MonarchE also had poorer outcomes when compared with stage 1 EBC patients. Further studies to determine which patients would benefit most from adjuvant CDK4/6 inhibitor treatment in the real-world setting is warranted.

P4-11-25: Enhancing Care of HR+/HER2- Early Breast Cancer Patient At Risk of Recurrence Through an Expansive Curriculum

Urvi Patel, Nabil Dorkhom, Victoria Phoenix, Jordan Schwartz, Katie Lucero

Introduction: Although therapies for patients with HR+/HER2- early breast cancer (EBC) have been available for several years, a number of clinical gaps remain, minimizing the optimization of care in this patient population. In an effort to overcome these gaps, a large educational curriculum was developed to improve the learner's ability to determine which patients are at high-risk for recurrence and how to implement and maintain the patient on appropriate therapy.
Methods: The curriculum included 11 online CME activities that were distributed between October 2023 and May 2024. The goal of this curriculum was to enhance oncologist's knowledge, competence, and confidence along three core learning areas: identification of patients with HR+/HER2- EBC at high risk for recurrence, selection of optimal treatment for high-risk patients, and improvement of patient's adherence to therapy. Educational impact was assessed with repeated pairs design. Oncologists were asked pre-education and post-education knowledge/competence and confidence questions. These questions were based on the identified education gaps and designed to assess if the learning objectives were met. Data were collected from October 2023 to May 2024. Statistical significance was assessed using McNemar's test ($P < .05$ level).
Results: A total of 20456 global oncologists participated in these activities, of which 3564 completed the activities. The assessment questions and the outcomes data were divided into three core learning areas, three levels of learning, and by geographical area to determine impact of education.
IDENTIFICATION OF RISKGeographical Area/Learning Level (n) Pre% Post% Abs Change% P ValueKNOWLEDGEUS
(117) 44 47 3 <.05OUS
(217) 52 61 9 <.001COMPETENCEUS
(271) 65 75 10 <.001OUS
(374) 67 75 8 <.001CONFIDENCEUS
(376) 19 24 5 <.001OUS
(84) 28 36 8 <.001
TREATMENT SELECTIONGeographical Area/Learning Level (n) Pre% Post% Abs Change% P ValueKNOWLEDGEUS

(190) 67 70 3 --OUS
(231) 76 80 4 <.01COMPETENCEUS
(381) 46 61 15 <.001OUS
(497) 50 61 11 <.05CONFIDENCEUS
(410) 12 20 8 <.001OUS
(84) 22 33 17 <.001
ADHERENCE TO THREAPYGeographical Area/Learning Level (n) Pre% Post% Abs Change% P ValueKNOWLEDGEUS
(56) 67 69 2 --OUS
(105) 73 77 4 <.05COMPETENCEUS
(165) 58 64 8 <.05OUS(245)
56 64 9 <.05CONFIDENCEUS
(130) 17 21 4 <.001OUS
(80) 21 39 18 <.05
Conclusions: The results of these activities have led to a significant increase in the knowledge, competence and confidence of oncologists through all segmentations of the core learning areas, learning levels, and geographical areas assessed. Despite this positive growth many gaps still remain, and further education is needed to minimize those gaps.

P4-11-26: Efficacy of neoadjuvant endocrine therapy versus chemotherapy in ER+ HER2-negative breast cancer: a single-center matched-cohort study. Preliminary results.

E.I. Kovalenko, Tatiana Titova, Yaroslav Zhulikov, Vladimir Fedko, Maxim Khoroshilov, Alexander Petrovskiy, Elena Artamonova

Introduction: The role of neoadjuvant endocrine therapy (NET) is still not well defined. The comparative trials with combination chemotherapy (CT) demonstrated similar rate of clinical, radiological response and breast conserving surgery. However, there are no direct comparative studies to date in terms of the pathological response of modern neoadjuvant (NA) anthracycline- and taxane- (A-T) containing regimen versus NET. It still remains unclear what role plays NET in de-escalating or sparing CT. Materials and methods. The aim of the study was to assess the rate of pCR, RCB 0+1 and axillary clearance (conversion of N+ to N0) of NET in comparison to A-T NACT in stage II-III breast cancer (BC) in postmenopausal patients. RCB 0-1 as an end point was chosen due comparable long-term results in ER+HER2- BC as per meta-analisys. The study included patients with early ER+HER2- BC who received NET or NACT in a single center from Aug 2018 to Nov 2023. Results: A total of 206 patients were included, of which 96 received NET with aromatase inhibitors (AI), 110 – CT (4 cycles doxorubicine/cyclophosphamide (AC) every 2 (dose-dence) or 3 weeks followed by 4 x docetaxel every 3 weeks or 12 x paclitaxel weekly). After propensity matching analysis to adjust for selection bias 69 patients in each treatment group were included in the final analysis. The majority of patients (94,2-91,3% in each group) had stage III disease, median age was 64,6-58,2 y.o., median ki67 34,2-39,5%. The

pCR rate was 1,4% in the NET group vs 10,1% in the CT group (p=0.06), RCB 0+1 rate 4,3% vs 20,2% (p=0,008), axillary clearance – 3,5% vs 27,6% (p 0,001). Following surgery 37 (53,6%) patients from the NET group received adjuvant CT: 14 (37,8%) - A-T, 7 (18,9%) - 6 cycles of docetaxel/cyclophosphamide (TC), 9 (24,3%) – 4 cycles of TC, 5 (13,5%) – other CT, 32 (46,3%) received no CT. 3-year event-free survival (EFS) was 92,8% vs 87,0% (p=0,6). Conclusion: Our preliminary results suggest that NET demonstrates significantly lower rate of RCB 0+1 and axillary clearance compared to A-T NACT. However, it does not translate into differences in EFS so far, probably due to adjuvant CT, which was administered to 53,6% of patients. NET allowed to avoid CT in 46,3% of patients or de-escalate it (to 6 or 4xTC) in 23,2%.

P4-11-28: Breast Cancer Recurrence Risk Stratification with the 21-Gene Recurrence Score Assay - the Portuguese National Experience

Diana Pessoa, Joana Luís, Diogo Alpuim Costa, Mário Fontes e Sousa, Idília Pina, Débora Cardoso, Isabel Andrade, Joana Simões, Ana Ferreira, Renato Cunha, Diogo Branco, José Luís Passos Coelho, for the Portugal "21-Gene Recurrence Score Assay" National Cohort Study

Introduction: The 21-gene recurrence score assay (RSA), a genomic tool to personalize therapy, avoiding over and undertreatment of breast cancer (BC) patients (pts), is available in Portugal since 2012. We aimed to characterize the population of pts with BC whose tumors were tested from 2012 until Dec 2021, analyzing prescription patterns and invasive disease-free survival (iDFS).
Methods: Observational, multicenter nationwide retrospective cohort study of pts with RH+/Her2-neg invasive BC treated by upfront surgery, in whom the RSA was performed. The cohort was identified by the national representative of the RSA manufacturer and clinical data was retrospectively collected from medical records by independent investigators.
Results: 1119 pts from 36 centers were preregistered. We weren't able to retrieve data from 7 patients; 21 pts were excluded due to insufficient clinical-pathological information; 7 were excluded for insufficient tissue, and 1 was staged pN2. Thus 1083 pts (91%) were included in the analysis: pN0 - 832 pts (76,8%), pN1 - 238 pts (22%) and pNx -13 pts (1,2%). In pN0 group 57.2% of pts were postmenopausal. Tumors were mostly no special type (NST, 76.6%), G2 (82.9%), with median (med) expression levels of ER 100%, PR 80% and Ki67 25%, pT1 (66.1%), Luminal B like (72.7%) and MINDACT clinical low risk (64.7%). The med RS was 17 (range 0-61). After publication of the TAILORx trial there was an increase in the number of tests per unit of time (248 vs 584); pts and tumor characteristics were similar but for pts > 50 yo with RS ≤ 25 (n= 412) there was an increase in the percentage of pts that didn't receive adjuvant chemotherapy (ACT, 79.3% vs 96%); for pts ≤50 yo with tumor RS 16 to 25 (n=137) there was an inversion in the percentage of pts treated with ACT (before TAILORx CT 62.2%; after 30%); after TAILORx, 22 of 70 pts ≤50 yo (31.4%) with tumor RS 16-25 who did not receive ACT, received adjuvant ovarian suppression. In pN1 group 64.7% pts were postmenopausal. Tumors were mostly NST

(76.5%), G2 (77.3%), with med expression levels of ER 100%, PR 80% and Ki67 17.5%, pT1 (61.8%), Luminal B like (55.5%), high MINDACT risk (85.7%) and with only one positive lymph node (81.9%). The med RS was 15 (range 0-63). 237 of the 238 pts with pN1 disease had the RSA requested before publication of the RxPONDER trial results. 74% of pN1 premenopausal pts with RS ≤ 25 did not receive ACT; 8 of these 54 pts (14.8%) received ovarian suppression. With a med follow-up of 29 months and 16 pts (1,5%) lost to follow up, there were 24 (2%) invasive disease recurrences (17 pN0, 6 pN1 and 1 pNx): 14 distant, 6 locoregional, 3 both and 1 contralateral. Among pN0 pts, 9 pts were premenopausal (med tumor RS of 17, range 7-21), and 2 had received ACT; of the 8 postmenopausal pts (med tumor RS of 25.5, range 10-44), 3 pts had received ACT. 6 pN1 pts recurred - 3 premenopausal with tumor RS of 9, 14 and 15 (1 treated with ACT) and 3 postmenopausal with tumor RS of 23, 28 and 29 (the last 2 treated with ACT).
Discussion/Conclusion: In this national cohort, the RSA was requested in mostly hormone receptor (HR)-strongly positive, good prognosis, BC with low risk of early recurrence disease. Pts characteristics are similar to the general characteristics of TAILORx and RxPONDER studies. The analysis of TAILORx suggested potential benefit of ACT in women ≤ 50 yo with tumours RS 16-25; however it did not result in more frequent prescription of ACT after July 2018. There was a high proportion of pN1 premenopausal pts with RS ≤ 25 that did not receive ACT (74%) but almost all pts were tested before the publication of RxPONDER. These results show that real-world studies at a national level are fundamental to evaluate clinical practice, analysing adherence to treatment recommendations and measuring pts outcomes.

P4-11-29: Clinical Impact of 18F–fluoro–17b–fluoroestradiol (18F–FES) PET in Patients with Estrogen Receptor-Positive Breast Cancer

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Background: Estrogen receptor (ER) status is an important factor in risk assessment for predicting response to systemic therapy for breast cancer. Currently, the gold standard for determining ER status in metastatic breast cancer is tissue immunohistochemical (IHC) testing. However, some patients have metastases that can be difficult to visualize using standard imaging techniques, particularly in the setting of invasive lobular carcinoma (ILC). ILC tends to proliferate in a single-file, sheet-line, lowly FDG-avid pattern as opposed to the more discrete, FDG avid masses which are typical of invasive ductal carcinomas (IDC). Additional clinical challenges include evaluating the ER expression of areas that are either difficult to biopsy, or difficult to interpret IHC results such as decalcified bone tissue. 18-FES PET is a functional imaging modality that can help identify lesions expressing ER as well as inter-lesion heterogeneity in expression. In this study, we evaluated the clinical impact of 18-FES PET in diagnosing metastatic disease and the utility in patients who had discordant biopsy results.

Methods: We performed a retrospective analysis of 40 patients with ER+ early breast cancer (EBC) who underwent 18-FES PET imaging between March 2022 and March 2024 at UCLA. After IRB approval, electronic medical records were reviewed and abstracted for demographics, disease, and treatment.
Results: Of 40 patients included, mean age was 61 years, 21 patients had ILC, 14 had IDC, and the rest had IDC with lobular features. ER percentage was > 70% in all patients. Eight (20%) postmenopausal patients had newly diagnosed metastatic disease by 18-FES PET; 4 (50%) with ILC and 4 (50%) with IDC. Metastatic sites of ILC included bone (2), ureter (1) and chest wall (1) while those of IDC included bone (4). Two of the eight patients were asymptomatic, one had clinical recurrence in the chest wall, three had elevated tumor markers, and two had suspicious findings on prior imaging (CT, FDG PET) that prompted obtaining 18-FES PET. Plasma circulating tumor DNA (ctDNA) testing was obtained along with FES PET in two patients; one had detectable ctDNA, but the other did not. Out of six patients with metastatic site avidity on FES-PET, one did not have feasible site to biopsy, but five patients had confirmatory biopsy. Three of them had ILC with metastases to bone (2) and ureter (1), and two had metastatic IDC to bone. For the other two patients (both with ILC and osseous metastases), negative pathologic ER expression was demonstrated despite positive FES activity; one with mild and one with intense radiotracer uptake. However, endocrine therapy was started despite biopsy findings and both patients had significant responses to endocrine therapy. Of the 32 patients (80%) with negative FES PET, no evidence of metastatic disease was observed at subsequent follow-ups.
Conclusion: FES PET was helpful in diagnosing metastatic disease and in assessing ER status in patients with ER+ EBC and suspected recurrence when the location of biopsy was challenging, particularly in setting of bone metastases in which IHC for ER expression is less reliable. FES positivity was associated with response to endocrine therapy when ER expression by IHC was negative in bone tissue. Prospective trials are needed to further characterize the role of FES PET in early diagnosis of metastatic disease and potential impact on survival for patients with ER+ breast cancer.

P4-11-30: Assessing the APIS Breast Cancer Subtyping Kit for RNA-Based Risk Stratification in Older Patients: Swiss Cohort Findings

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Introduction: ER, PR, HER2, and Ki67 serve as valuable prognostic and predictive markers in breast cancer (BC). APIS Breast Cancer Subtyping Kit accurately detects these markers mRNA expression, along with a with a novel four-gene proliferative signature (PS).
Objectives: We evaluate the kit's ability to distinguish between Luminal A and B subtypes and compare PS ability to predict risk of recurrence with Oncotype DX (ODx) and PAM50 tests in two cohorts of hormone receptor positive (HR+) patients, <65 and ≥65 years of age.
Methods:

Tissue of ER+/HER- BC patients (<65 N=33 and ≥65 N=28) diagnosed at Cantonal Hospital Basel-Land and Basel University Hospital (2020-2022) was included. All had received adjuvant therapy and had ODx scores available. All specimens underwent testing with PAM50 (Prosigna®). The % agreement of subtype calls among IHC, the APIS kit and PAM50 was assessed. Spearman correlation tests were applied to the data to examine concordance between recurrence tests.
Results: IHC and the APIS kit had high subtype call agreement in both patents aged <65 (73%) and patients ≥65 (68%). Strong overall agreement was observed in the ≥65 cohort between PAM50 and the APIS kit (82%) compared to the <65 cohort (61%). Agreement was lower between IHC and PAM50 for both cohorts, with 54.6% overall agreement for the <65 cohort and 57.2% for the ≥65 cohort.
Significant association was observed between the APIS kit's PS and ODx and PAM50 scores. Spearman's correlation coefficient between PS and ODx was 0.4542 (|ρ|=.0001*) for <65 cohort and 0.3799 (|ρ|=.0042*) for ≥65 cohort. Between PAM50 and PS, p = 0.3912 (|ρ|=.0244*) for <65 cohort and p = 0.5778 (|ρ|=.0013*) for ≥65 cohort. Proliferation Score was significantly higher for patients ≥65 (p=0.029). No association between age and PAM50 or ODx scores was observed.
Conclusions: The APIS Breast Cancer Subtyping Kit shows improved subtype call agreement with PAM50 in patients ≥65, indicating that molecular testing may be a more suitable method for determining proliferation and distinguishing between luminal A and B subtypes. Significant correlation between the APIS kit's PS, PAM50 and ODx indicates that APIS kit has the potential to accurately identify patients at risk of recurrence, although additional studies are needed to validate these results.

P4-12-01: Correlation between the Oncotype DX Recurrence Score® categories and progression-free survival of patients with primary metastatic estrogen-receptor positive and HER2-negative breast cancer

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Background: The Oncotype DX Recurrence Score® assay is a 21-gene biomarker validated to provide individualized prognostic estimates of distant recurrence risk and predictive information on the benefit of chemotherapy in patients with early-stage, ER+, HER2- BC. In ER-positive, HER2-negative primary metastatic BC (PMBC), endocrine therapy (ET) is the preferred option for first-line therapy, unless there is a high visceral burden of disease in which case chemotherapy (CT) alone or chemoendocrine therapy (CET) may be considered. There are many unanswered questions related to the optimal management of endocrine responsive PBMC, including optimal drug sequencing, and the potential for individualized treatment on the basis of predictive markers. Studies are warranted to understand individualized treatment response based on PMBC tumor biology and treatment naivety. The primary objective of this retrospective study is to evaluate the association of the Recurrence Score® (RS) result with progression-free survival (PFS) in a cohort of German patients with ER+, HER2- PMBC who received treatment independent of the RS result.

Methods:
Patients diagnosed with ER+/HER2- PMBC between 2008 and 2018 were included in this retrospective analysis.
The RS result was collected retrospectively and as such was not used for clinical decision-making.
Descriptive analyses were used to characterize patient and tumor characteristics. T-tests and Wilcoxon rank sum tests were used to compare continuous characteristics between RS result groups (0-25 and 26-100). Chi-square and Fisher's exact tests were used to compare categorical characteristics between RS result groups.
Kaplan-Meier estimates and log-rank tests were used to compare PFS and 2-year overall survival (OS) between RS result groups.
Multivariable Cox regression models adjusted for age, grade, treatment, and RS result were used to examine the association between RS result with PFS and OS.

Results: A total of 75 patients were included in this analysis (47 had a RS result of 0-25 and 28 had a RS result of 26-100). Mean (SD) age differed between RS groups (p = 0.03); 69.3 (12.6) years for RS 0-25 and 60.1 (17.4) years for RS 26-100. First-line systemic therapy also differed between RS groups (p = 0.004); for patients with RS results 0-25, 38 (80.9%) received ET, 5 (10.6%) received CET, and 3 (6.4%) received (CT). For RS results 26-100, 13 (46.4%) patients received ET, 11 (39.3%) received CET, and 4 (14.3%) received CT.
For RS results 0-25, patients were older, post-menopausal, and had less CT assigned as treatment. The RS result 26-100 cohort consisted of younger, more pre-menopausal women, with a greater percentage assigned CT alone or CET.
The RS 0-25 group had significantly improved PFS (p=0.013) with a median of 35 months compared to 12 months in the RS 26-100 group.
The difference in 2-year overall survival rates between the RS 0-25 group (79%) vs. RS 26-100 group (63%) did not reach statistical signifiance (p=0.18).
In multivariable Cox regression models, the RS result was prognostic for PFS and 2-year OS (p=0.002 and 0.010, respectively).

Conclusions: In this retrospective analysis, there were differences in treatment assignments based on clinical risk of PMBC that were consistent with treatment recommendations according to RS outcomes in EBC.
The binary RS result cut offs (RS ≤25; RS >25) were prognostic for PFS and OS in this retrospective analysis of patients with ER+/HER2- PMBC.
These findings suggest that the Oncotype DX® assay also reflects tumor biology in PMBC, and genomic assays standardly used in early breast cancer might warrant further prospective investigation in the treatment-naïve advanced setting.

P4-12-02: Patterns and predictors of disease progression and their impact on survival in patients receiving Cyclin D Kinase 4/6 inhibitors (CDK4/6i) for advanced Breast Cancer (aBC).

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Background: CDK4/6i-based therapy is the standard 1st line treatment for hormone receptor-positive (HR+), and human epidermal receptor-negative (HER2-) aBC, but most patients eventually progress. There is a lack of evidence on patterns of progression (POP), the clinicopathological factors that predict them, and their impact on survival after disease progression on CDK4/6i-based treatment.
Methods: In this single-center, retrospective study patients with HR+ HER2- aBC treated from January 2016 to June 2023, with at least 3 months of follow-up, and documented progression on CDK4/6i based therapy were investigated. Based on the European Society for Radiotherapy and Oncology/European Organization for Research and Treatment of Cancer consensus, patients were divided into 4 patterns, repeat oligo-progression (RO, oligo-progression with a history of oligometastatic disease), induced oligo-progression (IO, oligo-progression with a history of poly-metastatic disease)- collectively called oligo-progressive disease (OPD) and de-novo poly-progression (DP, poly-progression with a history of oligometastatic disease), repeat poly-progression (RP, poly-progression with a history of poly-metastatic disease)-together called diffusely progressive disease (DPD). Kaplan Meier survival analysis was used for time-to-event data & odds ratio (OR) was calculated with logistic regression for identifying predictors of POP.
Results: Among 200 patients who received CDK4/6i-based therapy during this period, 151 met the inclusion criteria. At the time of diagnosis of metastatic disease, the median age was 59 years, 43 (28%) had de-novo aBC & 98 (65%) had visceral disease. Palbociclib was the most common CDK4/6i used in 142 (94%) patients and the median number of lines of prior treatment received at the time of CDK4/6i start was 0 (range 0-3). At a median follow-up of 58 months, the median time to progression, next-line progression-free survival (nlPFS), and overall survival (OS) were 12 months, 7.5 months, and 56 months, respectively. Overall, 47 (31%) had OPD including 34 (22%) with RO and 13 (9%) with IO. Moreover, 104 (69%) had DPD including 93 (61%) with RP and 12 (8%) with DP. In multivariable logistic regression analysis, high tumour grade (OR 0.31, p=0.03), HER2-low (HER2 being 1+ or 2+ on immunohistochemistry but negative by in-situ hybridization) status (OR 0.34, p=0.03) and progression with in 6 months of the start of CDK4/6i based therapy (OR 0.19, were associated with lower chances of OPD while body mass index of ≥25kg/m2 at diagnosis was associated with higher chances of DPD (OR 1.8, p=0.02). The nlPFS was significantly different among the 4 POP (1-year nlPFS 52% in RO versus 39% in IO versus 30% in DP versus 26% in RP; p=0.01). The 3-year OS also differed significantly among the 4 POP (92% in RO versus 83% in IO versus 66% in DP versus 59% in RP; p=0.02).
Conclusion: The four distinct POP after disease progression on CDK4/6i based therapy significantly affect next line PFS and OS. High tumour grade, HER-low status and early progression within 6 months of CDK4/6i based treatment are associated with lower chances of oligo-progressive disease.

P4-12-03: Vepdegestrant, a PROteolysis TArgeting Chimera (PROTAC) Estrogen Receptor (ER) Degrader, Plus Abemaciclib in ER-Pos/Human Epidermal Growth Factor Receptor 2 (HER2)-Negative Advanced or Metastatic Breast Cancer: TACTIVE-U Prelim Phase 1b Results

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Background: Vepdegestrant (ARV-471), an oral PROTAC ER degrader, was well tolerated and showed evidence of clinical activity in the first-in-human phase 1/2 study in heavily pretreated patients (pts) with ER+/HER2- advanced breast cancer (NCT04072952). In xenograft models, vepdegestrant plus the cyclin-dependent kinase (CDK)4/6 inhibitor abemaciclib showed greater tumor growth inhibition vs fulvestrant plus abemaciclib. The open-label, phase 1b/2 TACTIVE-U umbrella study is evaluating the safety, efficacy, and pharmacokinetics (PK) of vepdegestrant in combination with other anticancer treatments in pts with ER+ advanced or metastatic breast cancer. Here, we report preliminary phase 1b results from the ongoing TACTIVE-U sub-study A (NCT05548127) investigating vepdegestrant plus abemaciclib.
Methods: Eligible pts were aged ≥18 years with confirmed ER+/HER2- advanced or metastatic breast cancer and had received up to 2 lines of prior therapy, including a CDK4/6 inhibitor-based regimen in any setting (pts with prior CDK4/6 inhibitor dose reductions due to adverse events [AEs] were excluded). Following a 7-day PK lead-in with abemaciclib administered alone, pts received vepdegestrant 200 mg orally once daily and abemaciclib 150 mg orally twice daily (both continuously). The primary endpoint of the phase 1b portion of the study was dose-limiting toxicities (DLTs) during the first cycle. Secondary endpoints of phase 1b are assessments of safety (type, frequency, and severity of AEs), PK (plasma concentrations of study drugs), and antitumor activity (objective response, clinical benefit rate).
Results: As of April 4, 2024, 16 female pts (median age: 56 years [range: 38–79]) received vepdegestrant plus abemaciclib, with 9 pts treated for ≥5 cycles; 12 remain on treatment. All pts (100%) received prior CDK4/6 inhibitors (ribociclib [n=8], palbociclib [n=7], and abemaciclib [n=1]), 14 (88%) prior aromatase inhibitors, 11 (69%) prior chemotherapy, and 5 (31%) prior fulvestrant. There were no DLTs or treatment discontinuations due to treatment-emergent AEs (TEAEs). TEAEs led to dose reduction of vepdegestrant and abemaciclib in 1 pt (neutropenia) and dose reductions of abemaciclib in 6 pts (fatigue [n=2] and fatigue/muscular weakness, neutropenia, diarrhea, and anemia [n=1 each]). Treatment-related AEs (TRAEs) to either vepdegestrant or abemaciclib that occurred in ≥20% of pts were diarrhea (69%), nausea (44%), fatigue (44%), and decreased appetite (25%). The majority of TRAEs were grade 1/2; grade 3 TRAEs were neutropenia or neutrophil count decreased in 5 pts and fatigue in 2 pts. There were no grade ≥4 TRAEs. Observed PK of vepdegestrant and abemaciclib was consistent with historical data reported in single-agent clinical studies. The exposure of abemaciclib (Cmax and AUCtau) increased slightly (15%

and 13%, respectively) when dosed with vepdegestrant compared with abemaciclib alone. Conclusions: The safety profile of vepdegestrant plus abemaciclib in pts with ER+/HER2- advanced or metastatic breast cancer was generally consistent with the known profiles of each agent, and no DLTs were observed. Neutropenia was manageable with dose modifications. The impact of vepdegestrant on abemaciclib exposure was minor and indicated no significant drug interaction. These data support further evaluation of this combination at the full standard doses of vepdegestrant 200 mg once daily and abemaciclib 150 mg twice daily in the phase 2 portion of this sub-study. Additional ongoing sub-studies for TACTIVE-U are evaluating vepdegestrant plus ribociclib (sub-study B; NCT05573555) and samuraciclib (sub-study C; NCT06125522).

P4-12-04: Real-world effectiveness of palbociclib plus an aromatase inhibitor in HR+/HER2- MBC patients living in disadvantaged neighborhoods

Filipa Lynce, Xianchen Liu, Benjamin Li, Lynn McRoy, Connie Chen, Raymond Liu, Hope S. Rugo

Background: Palbociclib (PAL) in combination with endocrine therapy (ET) has been approved for hormone receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-) advanced/metastatic breast cancer (MBC) since February 2025. Despite extensive real-world data in a variety of populations, little is known about the use and effectiveness of PAL combination in MBC patients living in disadvantaged neighborhoods. The current study aimed to compare overall survival (OS) and real-world progression-free survival (rwPFS) of first-line PAL plus an aromatase inhibitor (AI) vs AI alone in HR+/HER2 MBC patients living in disadvantaged neighborhoods in the US.

Methods: This is a retrospective observational study of HR+/HER2- MBC patients in the Flatiron Health longitudinal database. The database contains electronic health records from >280 cancer clinics, representing >3 million actively treated cancer patients in the US. Patients were included in this analysis if they had HR+/HER2- MBC, were ≥18 years, started PAL + AI as first-line therapy between February 2015 and June 2022 (index period), and lived in disadvantaged neighborhoods. Neighborhood disadvantage was measured with the Yost index, a composite measure of neighborhood social economic status. The Yost index score ranges from 1 (the first quintile) to 5 (the fifth quintile), with higher scores indicating higher socioeconomic status of the neighborhoods. Neighborhood disadvantage was defined as a Yost Index score of 1-2. Patients were retrospectively assessed from start of PAL+AI to December 2022 (data cutoff date), death, or last medical activity, whichever came first. OS was defined as months from start of PAL+AI to death. rwPFS was defined as months from start of PAL+AI to death or disease progression, evaluated based on clinical assessment or radiographic scan/biopsy. Stabilized inverse probability of treatment weight (sIPTW) was used to balance baseline demographics and clinical characteristics. 1:1 propensity score matching (PSM) was performed as sensitivity analysis.

Results: Of the 723 patients who were eligible for the analysis, 394 and 329 patients received PAL+AI and AI, respectively. Median follow-up was 27.2 months for PAL+AI and 25.7 months for AI treated patients. Compared with AI group, PAL+AI group was younger (median age 66.0 vs 69.0 years) and had higher proportions of de novo MBC (39.9% vs 24.0%) and lung/liver metastases (32.0% vs 19.8%). After sIPTW, baseline demographic and clinical characteristics were generally well balanced between PAL+AI and AI groups. After sIPTW, median OS was 57.1 (95%CI=47.2-70.8) months in PAL+AI group vs 38.2 (95%CI=29.6-48.0) months in AI group (HR=0.70, 95%CI=0.55-0.90, p =0.0053). Median rwPFS was 19.1 (95%CI = 15.8 – 24.2) months in PAL+AI group and 14.0 (95%CI=10.7-19.7) months in AI group (HR=0.66, 95%CI=0.52-0.84, p =0.0007). Sensitivity analysis with 1:1 PSM showed consistent results.

Conclusions: This real-world data analysis demonstrated that first-line palbociclib plus AI was associated with prolonged OS and rwPFS in HR+/HER2- MBC patients living in disadvantaged neighborhoods. These findings support use of first-line palbociclib in combination with endocrine therapy in this population.

P4-12-05: Prescription patterns and preferences for CDK4/6 inhibitors among oncologists in Latin America

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Currently, three CDK4/6 inhibitors (abemaciclib, palbociclib, ribociclib) are FDA-approved as first-line agents for the treatment of metastatic breast cancer (MBC). However, no specific recommendations favor one over the others, and usage is variable between oncologists. This study aims to investigate the preferences and prescription patterns of CDK4/6 inhibitors among oncologists in Latin America.
We conducted an online survey targeting Latin American medical oncologists with experience in treating MBC with CDK4/6 inhibitors. The survey was distributed in June 2024 through email lists obtained from the databases of Latin American oncology societies and oncology-focused social media groups. Descriptive statistics were employed to summarize the collected variables.
A total of 116 medical oncologists practicing in 20 Latin American countries completed the online survey. The majority of respondents were from Mexico (45%), followed by Guatemala (11%), Argentina (8%), and Colombia (8%). Regarding their practice scope, 86% of oncologists did not exclusively treat breast cancer, while 14% did. Most oncologists (56%) primarily practiced in public healthcare settings, whereas 41% mainly practiced in private settings.
Ninety-seven oncologists (84%) had used all three approved CDK4/6 inhibitors. When

asked about their general preference, 66 (57%) preferred ribociclib, 35 (30%) preferred abemaciclib, and 15 (13%) preferred palbociclib. This preference was not impacted by country of practice, time practicing oncology, scope of practice, patient workload, or site of primary care.
The main reason for choosing ribociclib was its benefit in overall survival, noted by 77% of respondents. Abemaciclib was mainly favored due to industry-sponsored accessibility programs (62%) and palbociclib was mainly selected due to its price (38%). Regarding specific clinical scenarios, among premenopausal women, ribociclib was selected by 80% of respondents. In cases of visceral disease, visceral crisis, and bone-only disease, ribociclib was chosen by 67%, 66%, and 50% of respondents, respectively. Primary endocrine resistance (47%) and central nervous system disease (53%) were selected as scenarios in which abemaciclib was favored. When managing patients with a history of heart or liver disease, abemaciclib was preferred by 53% and 47%, respectively. Lastly, for geriatric patients and male patients, palbociclib was preferred by 58% and 50%, respectively. Ribociclib emerged as the most favored CDK4/6 inhibitor, followed by abemaciclib and palbociclib, each preferred for specific clinical scenarios. Our findings highlight the importance of overall survival in guiding oncologists' preferences for prescribing CDK4/6 inhibitors. This observation is noteworthy considering that pivotal trials evaluating these agents focused on progression-free survival, with overall survival as a secondary endpoint. This work was conducted on behalf of the Latin American Breast Cancer Association (LABCA).

P4-12-06: Real-world clinical practice PIK3CA mutation testing patterns in patients with hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2+) metastatic breast cancer (mBC)

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Background: With the advent of targeted therapy for PIK3CA-mutated, HR+, HER2− mBC, evidence-based guidelines recommend PIK3CA biomarker testing at initial mBC presentation and at progression on first-line mBC therapy. Real-world information on PIK3CA testing patterns and results may provide insights into unmet testing needs.
METHODS: This retrospective cohort study included US adult patients from the Flatiron Health oncology database who were diagnosed with HR+, HER2− mBC between January 2017 and April 2023, and received any systemic oncologic therapy. Eligible patients were grouped into 3 cohorts according to the year of mBC diagnosis: 2017–2018, 2019–2020, or 2021–2023. Patients were followed through October 2023. PIK3CA testing patterns, timing, biopsy types, and results were assessed. Summary statistics were used to describe baseline characteristics at mBC diagnosis. The Kaplan–Meier method was used to evaluate time from mBC diagnosis to the first PIK3CA test. A multivariate Cox regression analysis was used to identify factors associated with PIK3CA testing.
RESULTS: Of 7,018 eligible patients (median age: 66 y; 53% aged ≥65 y; 99% female), 38% were initially diagnosed with de novo mBC, and 81% were treated at a community practice.

During the study period, 2,903 patients (41%) had undergone PIK3CA testing. In the 2017–2018, 2019–2020, and 2021–2023 cohorts, 6%, 24%, and 33% of patients, respectively, were tested within 6 mo of mBC diagnosis, and the median times to first test were 52.9 mo, 35.0 mo, and 23.0 mo, respectively. Next-generation sequencing (NGS) was the most frequent first test method overall (88%), increasing in use from the 2 earlier cohorts (both 84%) to the 2021–2023 cohort (95%). The next most frequent first test method overall was polymerase chain reaction (8%), followed by other (4%). NGS was the most frequent second/subsequent test method across all cohorts (91%–94%). Of patients who underwent NGS, liquid and tissue biopsies were used in 37% and 63%, respectively, as the first test, and in 55% and 45%, respectively, as the second/subsequent test. The likelihood of PIK3CA testing (all methods) was higher since 2019 vs before 2019 (HR 2.20; 95% CI: 2.02–2.40; p<0.0001); in patients aged <50 y and 50–<65 y, respectively, vs ≥65 y (HR 1.33; 95% CI: 1.19–1.49; p<0.0001; HR 1.36; 95% CI: 1.25–1.48; p<0.0001); in patients with recurrent vs de novo disease (HR 1.28; 95% CI: 1.18–1.38; p<0.0001); in academic vs community practice (HR 1.29; 95% CI: 1.03–1.61; p=0.03); and in the Northeast vs West (HR 1.20; 95% CI: 1.05–1.37; p=0.01). Among tested patients, 43% (1,255/2,903) had a disease-documented PIK3CA mutation, which was more prevalent in patients aged ≥65 y (48%) vs <50 y (36%) or 50–<65 y (44%) and in White vs African American patients (47% [858/1,830] vs 34% [107/314]; p<0.0001).
Conclusions: PIK3CA testing rates have increased over time and the median time to testing relative to mBC diagnosis has decreased in recent years in patients with HR+, HER2− mBC. Testing rates varied by age, de novo vs recurrent disease, geographic region, and practice type, suggesting major differences in practice patterns. The presence of a PIK3CA mutation was more prevalent in patients aged ≥65 y; however, testing rates were significantly lower in these patients, who represent more than half of the HR+, HER2− mBC population, underscoring an unmet need for diagnostic testing in this age group. Given the increasing number of agents that target the PI3K/ATK pathway, increased NGS testing and reduced time to testing may positively affect treatment decisions.

P4-12-07: Cyclin-dependent kinase 4/6 inhibitors dose reduction in patients with endocrine resistant advanced breast cancer experiencing early disease progression

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Background. Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) in combination with endocrine therapy represent the gold standard for treating patients with HR-positive/HER2-negative advanced breast cancer. However, endocrine resistance poses a risk factor for early disease progression. Escalating therapy strategies may lead to increased toxicity, resulting in dose reductions and treatment discontinuation. Notably, previous chemotherapy and concurrent radiotherapy are associated with myelotoxicity, which

constitutes the primary adverse event in the majority of patients treated with CDK4/6i. In this study, we aimed to assess the rates of CDK4/6i dose reductions in patients experiencing early disease progression, considering the context of endocrine resistance, prior chemotherapy, and concurrent radiotherapy.
Methods. We conducted a retrospective analysis of patients diagnosed with advanced breast cancer who received CDK4/6 inhibitor (CDK4/6i) treatment at our cancer center between 2018 and 2024.
Results. A total of 491 patients received treatment with CDK4/6 inhibitors (CDK4/6i). Among these patients, 242 required dose reduction due to toxicity. Patients without CDK4/6i dose reduction had a median progression-free survival (PFS) of 23.8 months, compared to 31.6 months for patients with dose reduction (difference not significant, p = 0.19). The 24-month PFS for patients without CDK4/6i dose reduction was 49.8% (95% CI 42.3-57.2%) versus 60.0% (52.8-66.5%) for patients with dose reduction. The median overall survival (OS) in patients without CDK4/6i dose reduction was 39.5 months, compared to 46.5 months for patients with dose reduction (difference not significant, p = 0.77). The 36-month OS for patients without CDK4/6i dose reduction was 56.5% (95% CI 47.2-64.7%) versus 62.7% (54.3-69.9%) for patients with dose reduction. However, in the group of patients diagnosed with early disease progression, defined as progression within 12 months from CDK4/6i commencement, dose reduction rates were significantly lower than in patients with longer response (41% compared to 55.1%, p=0.03). No significant differences in the rates of previous chemotherapy and concurrent radiotherapy were observed between these two groups. Higher rates of endocrine resistance were observed in patients experiencing early disease progression (66.7% of patients compared to 40.2% of patients, p<0.001).
Conclusions. Cyclin-dependent kinase 4/6 inhibitors dose reduction is safe, and treatment with a reduced dose remains efficacious in patients with advanced breast cancer. Lower dose reduction rates were observed in endocrine-resistant patients experiencing early disease progression. Therefore, considering treatment escalation in this population with a worse prognosis may be a feasible approach. However, further studies are necessary to address this specific patient group.

P4-12-09: Cytotoxic treatment and survival after CDK4/6 inhibitors in patients with luminal metastatic breast cancer before the era of antibody-drug-conjugates: Results from the Austrian AGMT_MBC-Registry

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Background: The recently published data from the DESTINY-Breast06 trial may lead to the replacement of conventional first-line chemotherapies by the antibody-drug-conjugate (ADC) trastuzumab-deruxtecan (T-DXd) in a relevant proportion of patients with luminal HER2-low or ultra-low metastatic breast cancer (MBC). While T-DXd yielded a clinically relevant improvement in progression-free survival (PFS), the question was raised if the ADC should still be administered preferentially as second cytotoxic treatment line in light of the tolerability profile and the absence of positive overall survival (OS) results. This analysis of the Austrian Study Group of Medical Tumor Therapy (AGMT) MBC-Registry describes the chemotherapy landscape before the availability of ADCs in early treatment lines in patients with luminal HER-negative MBC with a focus on the attrition rate from first- to second treatment line.
Patients and methods: The AGMT_MBC-Registry is a multicenter nationwide ongoing retrospective and prospective registry for MBC patients in Austria. For this analysis patients with known hormone-receptor (HR) and HER2 status, a CDK4-6 inhibitor treatment before the first cytotoxic treatment for MBC were included. Patients with ADC treatment in the cytotoxic first- or second-line were excluded.
Results: As of 26-June-2024, 1,605 patients with luminal HER2-negative MBC were included in the registry. Out of them, 751 fulfilled the conclusion criteria. Overall, 427 patients (57%) were still alive at data cut-off. Nearly half of the patients (46%) had HER2-low disease. CDK4/6 inhibitors were primarily used in first-line (81%), and less frequent in second-line (14%) or later lines (5%). A total of 389 patients (52%) were still on endocrine treatment without any chemotherapy treatment for MBC. Overall, 264 patients (35%), were treated with at least one line of chemotherapy for MBC. In the cytotoxic first-line, most patients received capecitabine (46%; median time to next treatment [TTNT] 8.4 months, 95% CI 7.0-11.0), followed by taxanes (33%; median TTNT 7.9 months, 95% CI 6.3-9.2), and other cytotoxic agents or combination regimens (21%; median TTMT 4.9 months, 95% CI 4.1-7.1). First-line chemotherapy was combined with bevacizumab in 75% of patients. Median overall survival (OS) calculated from the start of the cytotoxic first-line was 17.2 month (95% CI 15.0-20.3). Out of 169 patients who discontinued cytotoxic first-line treatment, 113 patients (67%) started a cytotoxic second-line. The most frequently used agents were taxanes (29%; median TTNT 5.7 months, 95% CI 5.5-8.3), capecitabine (20%, median TTNT 5.4 months, 95% CI 3.2-9.7), eribulin (12%, median TTNT 6.4 months, 95% CI 3.7-9.9) and others (39%, median TTNT 4.8 months, 95% CI 3.7-6.4). Median OS calculated from the start of the cytotoxic second-line was 11.5 (95% CI 9.4-14.0). Bone only-disease was documented in 6% and 4% of patients at the start of cytotoxic first-, and second-line treatment, respectively.
Conclusion: Median OS of patients with luminal HER2-negative MBC commencing first-line chemotherapy after a CDK4/6 inhibitor is relatively short at around 17 months in our real-world data analysis. Furthermore, only two thirds of first-line chemotherapy patients received any further chemotherapy line. This underlines the importance to implement treatments with proven survival benefit as early as possible.

P4-12-10: UNIQUE MECHANISM OF ACTION OF ELACESTRANT SENSITITIZES ESR1-MUTANT BREAST CANCER TO NOVEL THERAPEUTIC COMBINATIONS

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Estrogen receptor (ER) signaling is the main driver of tumorigenesis in ER+ breast cancers by inducing proliferation and survival through genomic and non-genomic means. Therefore inhibition of ER signaling has been a mainstay of treatment for decades. While primary ER+ breast cancer has a relatively good initial prognosis, ~30% of patients will develop treatment-refractory metastatic disease. In addition, up to 30% of advanced or metastatic tumors will acquire a mutation of ESR1, the gene which encodes ER. These mutations often make tumor cells less responsive to ER-targeting compounds. Elacestrant, a new-generation endocrine therapy, was found to out-perform the standard of care Fulvestrant in the EMERALD clinical trial [NCT03778931], specifically in patients with ESR1-mutant tumors. Preclinical studies have determined Elacestrant is a selective ER degrader, similar to Fulvestrant and it has been postulated that the increase in efficacy is the result of differences in affinity for mutated ER or increased bioavailability of Elacestrant. However, our studies determined that while Elacestrant reduces tumor burden in ESR1-mutant ER+ breast cancer Patient-Derived Xenograft (PDX) models, it does not reduce ER expression. Additionally, cells treated with Elacestrant in vivo demonstrate a unique transcriptomic profile, indicative of ER modulation rather than degradation. Elacestrant similarly reduced tumor burden in PDX models expressing only the wild-type ESR1 allele but exhibited a reduction in ER expression, consistent with previous reports. This suggests that the SERM-phenotype may be restricted to ESR1-mutant cells. High throughput screening of ESR1-mutant models with and without Elacestrant shows potentiation by compounds which target the cell cycle and the PI3K pathway, consistent with current clinical practices. Screening also determined topoisomerase inhibitors and Src family inhibitors to be synergistic with Elacestrant in ER-mutant cells. Ongoing studies include determining the mechanism of action of Elacestrant synergistic combinations and determining the role of SERM activity in treatment response in the metastatic setting.

P4-12-11: Large Oncosomes Loaded with circPEX13 Enhance Osteoclast Differentiation and Facilitate Breast Cancer Bone Metastasis

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Breast cancer (BC) demonstrates a pronounced tendency to metastasize to bone, leading to severe skeletal complications and mortality. Currently, early precise diagnosis and individual treatment for BC patients with bone metastasis are lacking in clinical practice. A critical stage in the metastatic process involves the establishment of pre-metastatic niches (PMNs) conducive to tumor cell colonization before clinical metastasis occurs. Tumor-derived extracellular vesicles (EVs) play a pivotal role as key mediators in selectively

modulating the microenvironment of distant organs to generate PMNs that promote organ metastasis. Our research found that BC with bone tropism significantly downregulated BRMS1L, leading to a reduction of its inhibitory effect on EIF4A3, a pre-mRNA splicing factor that promotes pre-mRNAs circularization by directly binding to their flanking regions. We next identified a novel circular RNA, circPEX13, highly enriched in large oncosomes (LOs), atypical large cancer-derived EVs. These LOs significantly supported bone-directed BC cell metastasis by promoting osteolytic PMN formation, which was crucial for facilitating BC cell growth in bone metastasis. Mechanistically, circPEX13 translocated to the nucleus and bound to the promoter region of NFATc1. Concurrently, circPEX13 acted as a sponge for HDAC1, thereby relieving HDAC1-mediated transcriptional repression of NFATc1.Based on these findings, we discovered that treatment with Zotatifin reduced circPEX13 expression and effectively suppressed BC bone metastasis in BC patient-derived xenograft models. In conclusion, these findings unveil a plausible mechanism whereby LOs secreted by bone metastatic BC cells mediate bone tropism by establishing PMNs in bone, and suggest a potential strategy for treating BC patients with bone metastasis.

P4-12-12: Endocrine Therapy with or without Cyclin-dependent Kinase 4/6 Inhibitor for HR-Positive, HER-Negative, advanced breast cancer following progression on a prior CDK4/6 Inhibitor plus Endocrine Therapy: A Systematic Review and Meta-Analysis

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Background: The combination of Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) and endocrine therapy (ET) is the standard medical care for patients with hormone receptor-positive (HR+), human epidermal growth factor receptor 2–negative (HER2-), advanced breast cancer (ABC). However, no consensus exists regarding a subsequent treatment with CDK4/6i and ET. We conducted a meta-analysis comparing CDK4/6i plus ET versus ET alone in patients who progressed on a prior CDK4/6i plus ET.
Methods: The Medline, Embase, and Cochrane databases, as well as the American Society of Clinical Oncology (ASCO) conference abstracts were scrutinized for randomized, controlled trials comparing maintenance or alternation of CDK4/6i in combination with ET versus ET alone in patients with HR+/HER2-, ABC following progression on a prior CDK4/6i plus ET. The primary outcome was progression-free survival (PFS). The secondary outcome was the overall response rate (ORR). Statistical analysis was performed using Review Manager (Cochrane Collaboration).
Results: Four randomized clinical trials were selected after our search, including 905 patients. The mean age was 57 years, and visceral disease was present in 60.1% of patients. Palbociclib was the most common previous CDK4/6i (82%). There was a statistically significant PFS improvement for CDK4/6i plus ET compared to ET alone (hazard ratio [HR], 0.77 [95% CI, 0.62 to 0.97]; P = 0.03). There were no significant differences in ORR between groups (risk ratio [RR]: 1.61, 95% CI: 0.76 to 3.38, P = 0.21).

Conclusion: CDK4/6i combined with Endocrine Therapy demonstrated significant PFS improvement compared to Endocrine Therapy alone in patients with HR+/HER2-, advanced breast cancer with disease progression on previous CDK4/6i plus ET treatment.

P4-12-13: CDK4/6 inhibitors (iCDK4/6) in breast cancer (BC): Is it possible to predict response?

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Background: Up to 20% of luminal BC patients presents recurrent disease in the first 10 years. The development of iCDK4/6 has changed treatment paradigms because of their significant improvement in treatment outcomes. Despite extensive translational research developed until now, no predictive biomarker has been identified so far. There is also a lack of evidence for iCDK4/6 efficacy in specific populations (such patients carrying pathogenic germline) and situations (as treatment sequencing, combinatorial strategies, and the role of post-iCDK4/6 therapy).
Methods: We performed a retrospective study of luminal BC patients treated with 3 available iCDK4/6 (abemaciclibe AB, Ribociclib RIB and Palbociclib Pb) between 2018 to 2024, with the aim of reviewing clinical, histological, and genomic profile searching for predictive variables of better responses
Results: A total of 383 patients were included. The median age of patients was 60 years old. 38% were pre-menopausal women. Median PFS and OS were 30 months (m) and 56m, respectively.The majority(65%) of patients received iCDK4/6 in the first line (1L), 15% in the 2nd line (2L), 20% in subsequent lines of treatment. PFS and OS and progressively declined in subsequent lines- 1L: 36/62m; 2L: 22/52m, others 14/33m (p<0,01).
There was no difference in PFS (p0.96) or OS (p=0,42) between iCDKs (AB 24/43m, RIB 25/45m, PB 27/45m). Patients with visceral metastasis presented worse PFS/OS (respectively, 28vs32m, p0,009 ;and 49 vs 60m, P=0,01). A higher Ki67 (>70%) was predcitive of worse outcome (PFS 15 vs 29 m p 0,009 / OS 34 vs 56m p0,017). Her2 low tumors perfomed as well as tumors with no Her2 expression (p=0,48).
Among 92 who underwent germline testing, 13 patients had a BRCA mutation(mt). There was no statistical difference in PFS (p 0.41) and OS P0,027 for this group. 129 patients underwent PIK3CA somatic sequencing (38% presented a PIK3CAmt). The PFS and cumulative OS rate were worse in PIK3CAmt patients (PFS 23x32m, p0,096 and OS 54 vs 57m, p 0.005).
Conclusion: highlighted variables with potential predictors of response to iCDK4/6. These data can serve as generators of hypotheses that must be validated in a larger cohort.

P4-12-14: Updated efficacy and safety of CDK4/6 inhibitors plus endocrine therapies in elderly HR+/HER2- metastatic or advanced breast cancer: patient-level network meta-analysis

Yun-Sheng Tai, Henry, WC Leung, Shyh-Yau Wang

Background: Breast cancer (BC) is the most common cancer in women worldwide. More than 80% of invasive BCs cases are newly diagnosed among women aged 50 years or older, and mainly present with an estrogen receptor ER-positive and HER2-negative subtype disease. About 91% of deaths occur in this age population. Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors showed a significant increased survival benefits in terms of progression-free survival and overall survival (OS), but the evidence on treating older women with metastatic BC is limited. Therefore, we comprehensively evaluated the efficacy and safety of cyclin-dependent kinase 4/6 (CDK4/6) inhibitors combined with endocrine therapy (ET) in older women with HR+/HER2- metastatic or advanced BC.Methods: We comprehensive search on PubMed and EMBASE database from Jan 2018 to Dec 2024 for phase II or III randomized controlled trials (RCTs) investigating modality treatments for HR+/HER2- metastatic or advanced BC. Kaplan–Meier curves for progression-free survival (PFS) and overall survival (OS) were reconstructed to retrieve individual patient-level data to strengthen the comparison of the benefits of all modality treatments of interest. Each study was pooled in a fixed-effects or randomized-effects model based on the individual study quality in this network meta-analysis (NMA). We also performed a subgroup analysis and reported the incidence of ≧grade 3 adverse events in elderly patients (≧65 years). The primary endpoint was pooled PFS, OS and the comparable safety. The modality treatments were ranked using SUCRA scores.Results: We identified 10 phase II and III randomized controlled trials with 7 modality treatments that met the inclusion criteria. Among them, 1823 patients with PFS and 2132 patients with OS results were included in the analysis. In terms of PFS, ribociclib+letrozole (Let) ranked highest among all other treatment modalities, followed by palbociclib+fulvestrant(ful). For OS, palbociclib plus ful is the best treatment compared to other treatment options of interest. The ranking probabilities of palcociclib plus fulvestrant (97.52%) and ribociclib plus let had the highest probability of achieving superior OS and PFS (90.89%) for elderly women with mBC. In terms of safety, palbociclib plus endocrine (letrozole or fulvestrant) (55.7%), abemaciclib plus letrozole or fulvestrant (28.7%) were associated with relatively high ≧grade 3 adverse events (AE) compared to placebo plus endocrine therapy. Ribociclib plus Let (15.2%) had a relatively lower rate of ≧ grade 3 adverse events (AEs) than the other two CDK4/6 inhibitors.Conclusion: Ribociclib plus letrozole is considered effective for PFS and a relatively safe treatment option for older women with HR+/HER2- subtype metastatic or advanced BC. However, given the limited reliable evidence, comprehensive and well-designed randomized controlled trials with a large sample size are needed to confirm our results. KEYWORDS: Breast cancer, elderly patients , Abemaciclib, Ribociclib, Palbociclib, Network meta-analysis.

P4-12-15: Key Factors Affecting Clinical Investigators Use of Oral SERDs in Current Management of ER-Positive, HER2-Negative, ESR1-Mutated (ER+/HER2-/ESR1+) Metastatic Breast Cancer That Has Relapsed After Treatment w/ a CDK4/6 Inhibitor/Endocrine Therapy

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Background: The January 2023 FDA approval of the novel SERD elacestrant (E) provided an important new therapeutic consideration for patients with relapsed ER+/HER2-/ESR1+ metastatic BC. More so, a host of promising data sets and ongoing studies evaluating this agent and others in the class suggest that SERDs will continue to play an important role in the disease. Relevantly, it is poorly understood how E is currently being employed in the clinic and for which types of patients clinicians favor or avoid this agent versus other available treatments.
Methods: In February 2024, 20 US-based and international CIs with a specialized interest in BC management were recruited from a proprietary database to complete a case-based survey focused on their approach to treatment for ER+/HER2-/ESR1+ advanced BC that has relapsed following treatment with a CDKi/ET in various types of patients. A modest honorarium was offered for participation. For each case question one or more variables (eg, age, time on CDKi, PIK3CA mutation status, site/extent of metastases) were altered. A number of other general queries about available and investigational SERDs were also included.
Results: For a 65-year-old woman with ER+/HER2-/ESR1+ BC who experiences a recurrence of minimally symptomatic bone metastases 18 months after treatment with CDKi/fulvestrant (ful), 19 of 20 CIs would recommend E. When the duration of benefit from the CDKi/ful was reduced to 12 months, 17 of 20 CIs continued to favor the SERD. However, when the duration was further reduced to 6 months, only 4 of the CIs selected E as their preferred approach.
Similarly, for a 65-year-old woman with ER+/HER2-/ESR1+ BC who also has a PIK3CA mutation and experiences a recurrence of minimally symptomatic bone metastases 18 months after treatment with a CDKi/ful, CIs were largely split between E (10) and capivasertib (capi)/ET (7). Again, when the duration of benefit from the CDKi/ful was reduced to 6 months, the CIs decreased their utilization of E and, in fact, none selected that agent. However, when the patient's age was increased to 80 years and the duration of CDKi benefit was maintained at 18 months, more CIs (12) selected E and fewer (4) favored capi/ET.
In terms of perspectives on the investigational SERDs imlunestrant and camizestrant, at the current time, CIs largely view their efficacy and tolerability to be similar to that of E or they are unable to make distinctions in this regard based on available data and experience.
Conclusions: CIs have rapidly incorporated oral SERDs into their current management algorithms for patients with relapsed ER+/HER2-/ESR1+ advanced BC. However, short duration of benefit from prior CDKi/ET and the presence of PIK3CA mutations appear to

decrease utilization, in some instances significantly, whereas older age may increase the use of these agents. Additional and expanded efforts are needed to further validate how CIs are employing SERDs in their practices and better understand the impact of various clinical factors as well as new research findings (eg, postMONARCH) on current treatment patterns.

P4-12-16: Identification of CGT4255 an EGFR sparing, ErbB2 clinical development candidate with activity across activating mutations in systemic and CNS tumors

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Alterations in ErbB2, including amplification, overexpression, insertions, and point mutations, are established oncogenic drivers in many solid tumors. These mutations are found in approximately 3–4% of breast cancers and 3% of advanced lung cancers and have emerged as mechanisms of acquired resistance to targeted therapies. ErbB2 alterations are also found in metastatic breast and lung cancers that have advanced to the brain and remain a major clinical challenge with limited therapeutic options. Current approved ErbB2 tyrosine kinase inhibitors have inferior potency against these mutations and lack sufficient brain penetration to be an impactful treatment option for patients. Herein, we describe advanced preclinical profiling of our EGFR-sparing, ErbB2 inhibitor clinical development candidate CGT4255 with best-in-class brain penetrance and activity against prevalent mutations.

P4-12-17: Pre-clinical data and phase I design for PQ-203: a novel peptide-drug conjugate (PDC) targeting the SORT1 receptor in hormone receptor "Triple" Negative Breast Cancer (TNBC)

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Background: Hormone Receptor “Triple” Negative Breast Cancer (TNBC) is an aggressive subtype of breast cancer with limited treatment options. Sortilin (SORT1) is a cell surface receptor involved in protein trafficking and cell signaling that is overexpressed in TNBC, making it a promising therapeutic target. We designed a novel peptide-drug conjugate, PQ-203, to selectively bind SORT1 with high affinity and deliver the cytotoxic agent MMAE directly to TNBC cells.
Methods: Here we demonstrate efficacy in pre-clinical models resistant to TOPO1-targeted ADCs as well as preliminary safety results and a human phase I study design. Binding affinity and internalization assays were performed to confirm the interaction between PQ-

203 and SORT1. The efficacy and specificity of PQ-203 were evaluated in vitro and in vivo using TNBC xenograft models. Preclinical pharmacokinetic (PK) and toxicity studies were performed to determine the safety profile of PQ203.
Results: PQ203 demonstrates high binding affinity and selectivity to SORT1. In vivo xenograft models demonstrate activity compared to sacituzumab govitecan (Trodelvy) and other biologic controls. In vivo studies reveal that PQ203 effectively reduces tumor growth in TNBC xenograft models with minimal systemic toxicity observed. Pharmacokinetic characterization of PQ203 reveals rapid clearance from plasma with corresponding sustained delivery of MMAE to tumors, highlighting a potential differentiation from MMAE-conjugated antibody drug conjugates (ADCs) that have relatively longer systemic exposures. Pre-clinical safety studies confirm that PQ203 is well tolerated in rodent and non-rodent species.
Conclusions: PQ203 exhibits promising pre-clinical efficacy and safety in targeting SORT1-positive TNBC. These findings support the initiation of a phase I clinical trial to evaluate the safety, tolerability, and preliminary efficacy of PQ203 in human cancer patients. The phase I study will follow a Bayesian Optimal Interval (BOIN) dose-escalation design, aiming to establish the maximum tolerated dose (MTD) / biologically optimal dose and to assess preliminary anti-cancer activity. Future studies will explore the potential of PQ203 as a targeted therapy for TNBC and to address the urgent unmet need for effective treatments in this challenging patient population.

P4-12-18: Discovery and characterization of ETX-636, a potential best-in-class, oral, small molecule, pan-mutant-selective PI3Kα inhibitor

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PIK3CA, which encodes p110α, the catalytic subunit of phosphatidylinositol 3-kinase alpha (PI3Kα), is one of the most frequently mutated oncogenes, with activating mutations seen in ~16% of all solid tumors and up to 40% of breast tumors, including hormone receptor-positive/HER2-negative, advanced breast cancer. The most frequent gain-of-function PI3Kα hotspot mutations, H1047R, E542K, and E545K, are well-recognized oncogenic drivers. Cancer cells with PIK3CA activating mutations are dependent on PI3Kα signaling and HR-positive, HER2-negative breast cancer patients with PIK3CA mutations respond to alpelisib, an approved PI3Kα inhibitor. While PI3Kα is important for cancer cell proliferation and tumor growth, it is also a critical component of the insulin signaling pathway. Therefore, the use of orthosteric inhibitors like alpelisib, which inhibit both the wildtype and mutant proteins, often results in significant hyperglycemia limiting their clinical utility. We report the discovery and pre-clinical characterization of an allosteric, pan-mutant-selective PI3Kα inhibitor, ETX-636, which was designed leveraging our Kinetic Ensemble® platform for optimal binding properties. Compared to other allosteric, pan-mutant-selective PI3Kα inhibitors (ie RLY-2608 and STX-478), ETX-636 has stronger target

binding affinity, better on-target potency in biochemical and cellular pharmacodynamic and viability assays, and demonstrates superior anti-tumor activity in vivo. More specifically, ETX-636 inhibits both kinase (H1047X) and helical domain (E542K and E545K) mutant PI3Kα biochemical activity with single digit nM potency and 8-10-fold selectivity relative to wildtype PI3Kα protein. ETX-636 shows greater than 1000-fold selectivity over the β, δ, and γ class I PI3K isoforms in biochemical assays and the selectivity extends more broadly across a panel of ~350 kinases. In cellular assays, ETX-636 potently inhibits proliferation and PI3Kα signaling specifically in PIK3CA-mutant cell lines. Mechanistically, ETX-636 induces proteasome-dependent degradation of mutant p110α protein in vitro and in vivo. Consistent with the compound-mediated decrease of mutant p110α protein and the high binding affinity (slow off-rate) of the compound, ETX-636 achieves sustained PI3Kα pathway inhibition in cellular washout assays and in cell-derived xenograft (CDX) tumor models. Once daily, oral dosing of ETX-636 shows single agent efficacy at well-tolerated doses in both PI3Kα kinase and helical domain-mutant breast cancer CDX models, significantly inhibiting tumor growth or inducing regression, while suppressing PI3K pathway activity in a dose-dependent manner. In an ER-positive, HER2-negative, PI3Kα-mutant breast cancer xenograft model, ETX-636 is efficacious as a single agent and shows enhanced activity in combination with fulvestrant, inducing consistent tumor regression while being well-tolerated. ETX-636 demonstrates superior magnitude and duration of PI3Kα pathway inhibition in vivo, compared to known pan-mutant-selective allosteric PI3Kα inhibitors, as well as orthosteric inhibitors. At efficacious doses, ETX-636 has significantly less of an effect on blood glucose in mice compared to orthosteric inhibitors, demonstrating that ETX-636 can achieve potent anti-tumor activity by targeting mutant PI3Kα protein without significantly affecting the activity of the wildtype protein. In addition, based on pharmacokinetic/pharmacodynamic/efficacy and toxicology studies, ETX-636 is unlikely to pose a significant risk of hyperglycemia at predicted human efficacious doses. These data support clinical exploration of ETX-636 in mutant PI3Kα solid tumors and, potentially, mutant PI3Kα-driven rare diseases.

P4-12-19: Preclinical Characterization of a Novel PI3Kα H1047R Mutant-Selective Inhibitor

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PIK3CA encodes the p110α catalytic subunit of PI3-kinase alpha (PI3Kα) and is the most frequently mutated kinase in human cancer with common mutations occurring in the kinase domain (H1047R) and helical domain (E542K/E545K). The approved PI3Kα inhibitor,

alpelisib, shows promise for this targeted class of agents with improvements in progression-free survival in ER+/Her2- breast cancer patients in combination with fulvestrant. However, toxicities attributed to the inhibition of wild-type PI3Kα, such as hyperglycemia, gastrointestinal issues, and skin reactions, lead to sub-optimal target engagement due to requisite dosing modifications. A PI3K mutant inhibitor that spares WT PI3K is predicted to be better tolerated, require fewer dosing modifications, and therefore, have the potential to provide improved clinical benefit. Herein, we present the preclinical in vitro and in vivo activity of a novel, wild type sparing PI3Kα inhibitor series which is potent against the oncogenic H1047R mutation.

P4-12-20: OKI-219 enhances activity of SOC therapies in double and triple combinations in pre-clinical PI3Kα H1047R mutant breast cancer models

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Phosphoinositide 3-kinase alpha (PI3Kα) H1047R mutations are found in approximately 15% of breast cancers and lead to constitutive activation of the PI3K/AKT/mTOR signaling pathway. Targeting PI3Kα in cancer is a therapeutically proven strategy, with the approved drug alpelisib showing clinical activity alone and in combination with other therapies. However, non-mutant selective inhibitors of PI3Kα are associated with significant toxicities, such as hyperglycemia, rash, and diarrhea, due to on-target inhibition of the wild-type enzyme. OKI-219 is an orally bioavailable and brain penetrant PI3KαH1047R mutant-selective inhibitor with greater than 100-fold selectivity for the H1047R mutation over wild-type PI3Kα.

We have previously demonstrated that OKI-219 shows robust antitumor activity both as a single agent and in combination with SERDs or HER2-targeted agents in models of PI3KαH1047R-mutated cancer. Notably in these studies, no changes in insulin or glucose are observed, supporting the potential for OKI-219 to achieve therapeutically active exposures in the absence of on-target toxicity.

CDK4/6 inhibitors in combination with ER-targeted agents are a mainstay of the treatment of HR+ breast cancer, where activation of PI3Kα signaling is associated with a poorer outcome. We investigated the impact of combining OKI-219 with CDK4/6 inhibitors and endocrine agents in PI3KαH1047R-mutated models. In vitro, in the T47D ER+, PI3KαH1047R model, the combination activity of OKI-219 + fulvestrant + ribociclib or palbociclib was assessed in proliferation assays and evaluated for synergy using the Loewe additivity model. The triple combinations compared to doublet showed a synergistic effect. In the xxT47D xenograft model in vivo, we show that the addition of OKI-219 to standard of care therapies, such as fulvestrant and fulvestrant + a CDK4/6 inhibitor drives enhanced tumor regression beyond that seen with SOC agents alone. Importantly, in tumors that had progressed on alpelisib or alpelisib + fulvestrant, the replacement of alpelisib with OKI-219 drove tumor regression. Similarly, adding OKI-219 to tumors that progressed on ribociclib + fulvestrant drove tumor regressions. These results show that pre-clinically, OKI-219 can

restore antitumor activity in models that are resistant to alpelisib and other SOC therapies. Combining PI3K inhibition with SERDs and CDK4/6 inhibitors offers a rational strategy to overcome resistance and enhance antitumor efficacy. The data presented here supports the combination of OKI-219 with SERDs and CDK4/6 inhibitors as a promising therapeutic strategy for ER+ breast cancer, including in cancers that have progressed on prior PI3K inhibitors. These preclinical studies provide compelling evidence of synergistic antitumor effects and lay the groundwork for ongoing clinical trials. OKI-219 is being investigated clinically in the PIKture-01 study, a First-in-Human Study of OKI-219 in Advanced Solid Tumors and Advanced Breast Cancer (NCT06239467).

P4-12-21: Efficacy and Safety of MRG002 in Prior TKI-Treated HER2 Positive Breast Cancer Patients: A Single Arm, Open Label, Multicenter, Phase II Study

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Background: Human epidermal growth factor receptor 2 (HER2) positive breast cancer represented approximately 15%-20% of all breast cancers. Liver metastasis was commonly observed in patients treated with trastuzumab and tyrosine kinase inhibitor (TKI), thereby further reducing the overall survival to only 8-14 months. MRG002 is a novel antibody-drug conjugate composed of recombinant humanized anti-HER2 monoclonal antibody MAB802 and potent cytotoxic small molecule MMAE linked by the vc-linker. This study was designed to evaluate the efficacy and safety of MRG002 in liver metastases breast cancer patients after the treatment failure of trastuzumab and TKI.
Method: This single-armed, open-label, multicenter, phase II study enrolled patients who had received ≥2 lines of anti-HER2 treatment (including trastuzumab and TKI) and had confirmed tumor progression during or after the most recent treatment. MRG002 was administered at a dose of 2.6 mg/kg every 3 weeks until the end of treatment, initiation of new anti-tumor therapy, withdrawal of informed consent, or death.
Result: A total of 102 females were enrolled in this study, with a median age of 53 years (range: 26-73). Forty-four patients had an ECOG score of 0, and 58 scored 1. Forty-one patients (40.2%) were immunohistochemistry (IHC) 3+, and 61 (59.8%) were IHC 2+/ISH+. All patients had liver metastasis, 55 (53.9%) had bone metastasis, and 37 (36.3%) had lung metastasis. All patients had received HER2-mAb and anti-HER2-TKI drug treatment (Pyrotinib 94 cases, 92.2%). The median treatment line was 3 (range: 2-10), wherein 39 patients (38.2%) received two treatment lines, 28 (27.5%) received three, and 35 (34.3%) received ≥ four lines.
As of July 19, 2024, the median follow-up time was 14.8 months (range: 0.4-27.2), and 7 patients (6.9%) had a treatment duration of nearly 2 years. The Independent Review Committee (IRC) evaluated the objective response rate (ORR) based on RECIST v1.1 as 60.8% (95% CI: 50.6-70.3), the disease control rate (DCR) as 86.3% (95% CI: 78.0-92.3), the

median progression-free survival (mPFS) as 8.6 months (95% CI: 6.9-11.9), and the median duration of response (mDoR) as 9.4 months (95% CI: 6.2-16.8). The investigator assessed the ORR as 56.9% (95% CI: 46.7-66.6), the mPFS as 7.5 months (95% CI: 5.7-8.0), and the mDoR as 6.8 months (95% CI: 6.0-8.9). Subgroup analysis showed that the ORR of IHC 3+ and IHC 2+/FISH+ patients were 70.0% and 60.7%, respectively. The mPFS were 11.9 months and 7.7 months, respectively.
The most common treatment-related adverse events (TRAEs) included 70.6% white blood cell count decreased, 64.7% neutrophil count decreased, 62.7% AST increased, and 54.9% ALT increased. TRAEs were mainly Grade 1-2 and recovered after treatment. TRAEs ≥ Grade 3 included 31.4% neutrophil count decreased, 13.7% white blood cell count decreased, and 6.9% peripheral neuropathy. No new safety signals were found, and the adverse events were manageable.
Conclusion: MRG002 showed a potential efficacy in trastuzumab and anti-HER2-TKI treatment failed HER2-positive liver metastasis breast cancer patients with a good tolerance and safety profile. The emergence of MRG002 presented a promising treatment option for patients.
Clinical trial information: NCT05263869

P4-12-22: Ergosterol Peroxide affects the VCP/ANKZF1 complex spatial relationship and interaction in IBC cells

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Inflammatory breast cancer (IBC) is a highly aggressive and lethal type of breast cancer. Furthermore, patients with the triple-negative breast cancer subtype have tumors that lack the estrogen receptor, progesterone receptor, and HER2 expression. These tumors display greater invasiveness, and consequently, patients exhibit increased metastasis. Due to the lack of targeted therapies, IBC patients typically undergo multimodality therapy with cytotoxic chemotherapy like taxanes and anthracyclines, radiation, and surgery. There is an urgent need for selective therapies for IBC and natural products have gained significant attention for their potential in breast cancer therapy. In our laboratory, we work with Ergosterol Peroxide (EP), a bioactive compound extracted from the Ganoderma lucidum mushroom. Our studies indicate that EP is a unique endoperoxide that upon entrance into the cancerous cell, deploys its warhead via selective interactions with intracellular proteins, without any effect in non-cancerous cells. The Valosin-containing protein (VCP) / Ankyrin repeat and zinc finger domain-containing protein 1 (ANKZF1) complex plays a vital role in maintaining cellular proteostasis and ensuring proper protein synthesis and degradation processes. ANKZF1 interacts with VCP and translocates to the mitochondria under cellular stress conditions. We believe that EP affects this complex to induce cancer cell death. We hypothesize that EP targets the VCP/ANKZF1 complex, impairing the ability of IBC cells to clear damaged proteins and causing mitochondria dysfunction to induce IBC cell death. To validate the interruption of VCP/ANKZF1 interactions, we used Proximity Ligation Assays (PLA) with antibodies for VCP and ANKZF1 in the IBC cell model, SUM149. Veh, EP, and NMS

873 (VCP inhibitor, positive control) were administered, to detect changes in PLA signals for VCP and ANKZF1. To understand the VCP/ANKZF1 spatial relationship, under Veh, EP, and H2O2 (positive control) effects in SUM149, we used a colocalization analysis by Immunocytochemistry (ICC). Results were visualized with Gen 5 Image Prime 3.15 program in Cytation C10 Confocal Imaging Reader. The PLA preliminary results indicate that EP affects protein-protein interactions, since the intensity of green fluorescence, which indicates protein activity, is lower in the treated cells. Especially when compared to the positive control - NMS 873 the protein inhibitor. The preliminary results of ICC demonstrated that when SUM149 cells are treated with EP compound, VCP displays greater fluorescence intensity, while ANKZF1 fluorescence is diminished. In addition, cells treated with EP display a nucleus with greater size when compared to the Veh. Furthermore, ANKZF1 looks to be co-localizing with the nucleus, while VCP has an around and near localization of the nuclei when compared with the Veh. In general, EP affects VCP/ANKZF1 spatial relationship and interaction in IBC cells. Further research is needed to comprehensively understand the intricate mechanisms through which EP exerts its antineoplastic effects in IBC cells.

P4-12-23: Z-Endoxifen-PROTAC, a novel drug for chemo-sensitive and-resistant Triple Negative Breast Cancer.

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Background: In the estrogen receptor positive (ER+) breast cancer, the tamoxifen metabolite Z-endoxifen (ENDX) potently inhibits ERα and partially degrades protein kinase C beta I (PKCβI) resulting in suppression of AKT signaling and the induction of apoptosis (Jayaraman et al, NPJ Breast Cancer, 2023). Intriguingly, at higher concentrations (5 μM and higher), ENDX inhibits the viability of triple negative breast cancer (TNBC) cells and multiple non-breast cancer and ER- cell line models. We thus surmised that ENDX may elicit its anti-cancer activity through mechanisms extending beyond inhibition of ER and PKCβI, and that development of more potent ENDX derivatives would extend the clinical utility of this molecule. Therefore, we developed a series of chemically synthesized ENDX-PROTAC (EPrC) compounds with ENDX as the warhead and cereblon (CRBN) as the E3 ubiquitin ligase recruitment moiety with the expectation that degradation of ENDX targets would elicit more potent anti-cancer activity at lower concentrations.

Methods: We characterized the anti-cancer activity of multiple EPrC compounds using multiple ER+ and TNBC cell line models and performed a series of unbiased approaches to identify novel proteins for which ENDX is a substrate.

Results: Initial studies in ER+/HER2- breast cancer cell lines showed that EPrC's resulted in no greater anti-cancer effects compared to ENDX. However, three EPrC's (EPrC 7-9)

exhibited profound anti-cancer activity at nanomolar concentrations in chemosensitive (MDAMB231, BT549, MDAMB436 and MDAMB468) as well as chemoresistant (doxorubicin-resistant and paclitaxel-resistant MDAMB231) TNBC cells. Conversely, ENDX had little activity in these models even at 5 μM concentrations. The potent anti-cancer activity of EPrC 7-9 also extended to chemoresistant TNBC patient-derived organoid (PDO) models. In contrast, EPrC 7-9 displayed no effects on proliferation or apoptosis in the non-malignant MCF10A breast epithelial cell line. We further evaluated the anti-cancer activity of EPrC's and ENDX using the NCI-60 human cancer cell line panel where broad anticancer activity was additionally observed in leukemia, lung, melanoma, and ovarian cancer cells. Mechanistically, EPrC 7-9 activity was independent of PKCβ1 expression/targeting. Therefore, we performed an unbiased SILAC and TMT-based mass spectrometry (MS) analysis utilizing MDAMB231 and MCF10A cells to identify EPrC target proteins that are ubiquitinated and degraded in MDAMB231 cells but not MCF10A cells. One of the top hits from this screen was G to S phase protein 1 (GSPT1), a conserved protein that regulates protein translation termination. We validated GSPT1 as a bona fide and rapidly targeted protein by EPrC's with near complete GSPT1 elimination occurring within four hours of treatment. The necessity of EPrC-mediated GSPT1 degradation to the anti-cancer activity of these molecules was confirmed using CRBN knockout MDAMB231 cells where EPrC 7-9 completely failed to inhibit cell proliferation, induce apoptosis, or alter GSPT1 protein levels.

Conclusion: Taken together, we have developed a new class of PROTAC drugs with ENDX as the warhead, which display potent anti-cancer activity in chemosensitive and chemoresistant models of TNBC, as well as non-breast cancer cell lines, and implicate GSPT1 as a novel and direct target of ENDX. Efforts are ongoing to elucidate the structural basis of EPrC-GSPT1 interaction, to assess GSPT1's contribution to EPrC activity and to study the pharmacology, toxicity, and in vivo antitumor activity of these molecules.

P4-12-24: Preclinical characterization of LY4045004, a next-generation, mutant-selective PI3Kα inhibitor

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Introduction: PI3Kα mutations are oncogenic drivers found in approximately 40% of HR+ breast cancers as well as multiple other solid tumors. Approved treatments for PI3Kα mutant-driven HR+ breast cancers include direct inhibitors of either PIK3CA or downstream AKT. However, these inhibitors block PI3K pathway signaling in unmutated host tissues resulting in hyperglycemia, rash, and GI toxicity. Herein we describe the preclinical profile of LY4045004, a next-generation, mutant-selective inhibitor of PI3Kα.

The compound exhibits potent inhibition across the most common PI3Kα mutations in breast cancer (H1047R, E545K, and E542K) while sparing wild type (WT) PI3Kα. Strong efficacy in multiple PI3Kα-mutant breast cancer models is demonstrated while avoiding the hyperglycemia and insulin increases characteristic of non-selective inhibitors.
Methods: PI3Kα biochemical potency was measured using ADP Glo kinase assay. PI3Kα on-rate and off-rate were measured using Transcreener FI assay. PI3Kα cell potency was measured using cell viability and signal transduction assays. Tumor growth inhibition, pharmacokinetic (PK), and pharmacodynamic effects were assessed in in vivo studies using PI3Kα mutant cell-derived xenograft (CDX) and patient-derived xenograft (PDX)-models. Plasma insulin and C-peptide levels were measured using ELISA.
Results: LY4045004 is a potent, allosteric inhibitor of both kinase and helical PI3Kα mutations in enzyme and cell-based assays, inhibiting growth and signaling responses in multiple mutant-driven breast cancer cell lines. The compound is selective for mutant PI3Kα over WT PI3Kα in both enzyme and cell-based assays and exhibits a very slow off-rate from PI3Kα. LY4045004 demonstrates favorable in vitro ADME properties and excellent PK with high oral bioavailability across preclinical species. In vivo, orally administered LY4045004 demonstrated dose-dependent tumor regressions in PI3Kα H1047R-driven and E545K-driven breast cancer models both as monotherapy and in combination with fulvestrant without inducing hyperglycemia (no significant increase in insulin or C-peptide) or body weight change. Tumor pharmacodynamic analyses confirmed strong pathway inhibition at doses that caused regressions. Additionally, LY4045004 demonstrated tumor regressions in PI3Kα H1047R and E545K PDX models. LY4045004 was well-tolerated at efficacious doses in mice both as a single agent and in combination, and well-tolerated in rat, dog, and cynomolgus monkey toxicology studies.
Conclusions: The favorable potency, selectivity, and PK properties of LY4045004 are predicted to result in efficacy with improved tolerability in patients with prevalent PI3Kα helical- and kinase-domain mutant HR+ breast cancers. Global regulatory submissions are planned in the first half of 2025.

P4-12-25: TARGETING BRCA-DEFICIENT CANCER CELLS WITH SMALL MOLECULE RAD52 INHIBITORS

Shiva Ostadrahimi, Matthew J. Rossi, Sarah F. DiDomenico, Saiful Amin, Alexander V. Mazin

A crucial bulwark of genome stability is the DNA repair system. The homologous recombination (HR) pathway is essential for the repair of the most harmful DNA lesions, like DNA double-strand breaks. Deficiencies in this pathway result in genomic instability and contribute to tumorigenesis. BRCA1 and BRCA2 (Breast Cancer type 1 and 2 susceptibility proteins) play critical roles in HR. Germline mutations in the BRCA1 and BRCA2 genes are responsible for nearly 50% of all familial breast and ovarian cancers. BRCA1/2-deficiencies coerce tumor cells to depend on alternative DNA repair pathways. Recent studies have demonstrated that inactivation of RAD52 protein induces lethality (synthetic lethality) in BRCA1/2-deficient cancer cells, without affecting normal cells. By

exploiting this synthetic lethality relationship, targeting RAD52 with inhibitors may provide an exceptionally selective method for eliminating cancer cells. Focusing on DNA repair proteins involved in synthetically lethal relationships in cancer cells has become an important strategy in the development of specific cancer therapeutics.
Our laboratory has developed small molecule inhibitors targeting RAD52. We have successfully created small molecule inhibitors of RAD52 (RAD52i) and have shown their ability to inhibit the biochemical activities RAD52 including DNA annealing and DNA strand exchange. Using the GFP-based recombination assays, we show that small molecule inhibitors of RAD52 disrupt the homologous DNA repair (HDR) and single strand annealing (SSA) repair pathways in human cells. Our compounds exhibited a notable reduction in cell viability in BRCA deficient cells. Furthermore, our small molecule RAD52i exhibited considerable efficacy in a mouse xenograft model cells markedly suppressing growth of human BRCA1-mutated cancer cells. We aim to develop small molecule RAD52i as novel cancer therapeutics.

P4-12-26: Preclinical study of newly developed anti-breast cancer drug targeting sphingosine-1-phosphate utilizing a novel drug delivery system

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Background: Drug resistance is one of the major causes of treatment failure in advanced breast cancer (BC), and the development of drugs with new mechanisms of action is desirable to overcome this situation. Sphingosine-1-phosphate (S1P) is a lipid mediator that has a variety of physiological functions involved in regulation of cell survival, inflammation, and immune systems. We have reported that cancers highly express S1P-producing enzyme and that S1P plays an important role in cancer progression and metastasis. FTY720, an S1P receptor functional antagonist, has been approved by U.S. Food and Drug Administration for multiple sclerosis. FTY720 has been shown to suppress cancer progression by inhibiting S1P signaling, however, its immunosuppressive side effects have hindered its development as an anticancer agent. Now, we have newly developed prodrug-FTY720 (pro-FTY), a cancer-specific S1P signaling inhibitor, utilizing a novel drug delivery system that reacts with acrolein in cancer cells to generate the active form. The aim of this study was to evaluate the efficacy and safety of pro-FTY by in vitro and in vivo preclinical experiments.
Methods: The expression of acrolein in surgical specimens from BC patients was tested using a dedicated fluorescent probe. Eight BC cell lines including two multi-drug resistant cell lines and a normal mammary cell line were used to determine the IC50 values of paclitaxel, doxorubicin, FTY720, and pro-FTY for these cell lines. Patient-derived organoids (PDOs) and patient-derived xenografts (PDXs) were established from BC patients, including those who developed multiple-drug resistance after treatment. The IC50 values of pro-FTY and other drugs in BC PDOs were determined by 3D Cell Tite-Glo Assay. PDX mouse models were used to test the efficacy of pro-FTY, and the adverse effects were evaluated by blood analysis.

Results: We confirmed that acrolein was strongly expressed in BC tissue and was barely expressed in normal mammary tissue utilizing surgical specimens. IC50 values revealed that FTY720 and pro-FTY consistently inhibited survival of all the BC cell lines, including multidrug-resistant cells which showed resistance to paclitaxel or doxorubicin. Although FTY720 inhibited survival of normal mammary cell lines, pro-FTY did not, suggesting that pro-FTY does not act on normal mammary cells that do not contain acrolein. Pro-FTY showed consistent efficacy against multidrug-resistant PDOs, while paclitaxel and doxorubicin failed to show sufficient efficacy against multidrug-resistant PDOs. Intravenous treatment with pro-FTY showed significant tumor suppression in PDX mouse models created from multidrug-resistant PDOs. Importantly, lymphopenia was not observed in the mice treated with pro-FTY, while it was observed in the mice treated with FTY720. Finally, mass spectrometry analysis of pro-FTY-treated mice showed that FTY720 accumulated in the tumors, while it was barely detectable in the blood.
Conclusion: Pro-FTY reacts with acrolein in the cancer cell to produce FTY720, which accumulates there, and exerts specific anticancer effects on BCs that are resistant to conventional anticancer drugs. Pro-FTY does not cause adverse events, including immunosuppression, avoiding effects on normal cells.

P4-12-27: Exploring Stimulation Response of Cisplatin Prodrugs and Integrating Photothermal-Photoacoustic Dual-Modal Theranostics in Triple-Negative Breast Cancer through Ozone-mediated Photothermal Catalytic Nanosystem

Dan Zheng, Ting Luo, Hubing Shi, Tianyue Xu, Linlin Song

Background: The combination of chemotherapy with immune checkpoint inhibitors shows a synergistic anti-tumor effect. Nevertheless, the issues of chemoresistance and the significant toxicity associated with combined treatments pose substantial challenges. We have successfully synthesized a fluorocarbon chain-modified cisplatin precursor (PtF) in previous research, showing optimized controlled-release properties, robust anti-tumor activity, high efficiency, and well tolerated toxicity.
Methodology: In this study, we utilize the PtF prodrug as a delivery vehicle, encapsulating gold nanoparticles to construct the PLAOP system. The near-infrared (NIR)-I light irradiation was applied as activator of the photothermal and photoacoustic effect.Results: This study evaluated the photothermal effect of PLAOP upon NIR-I light irradiation, which enables photoacoustic imaging of tumors by inducing thermoelastic expansion of the gold nanoparticles. We also elucidated the role of the photothermal effect produced by NIR I-activated PLAOP in catalyzing the generation of reactive oxygen species (ROS) from ozone. Moreover, the synergistic application of NIR I with PLAOP leverages ROS-GSH modulation to govern the responsive release of platinum-based prodrugs, thereby achieving dual-mode photothermal and photoacoustic visualization in triple-negative breast cancer (TNBC). This strategy enables precise tumor imaging and ensures a controlled, high-efficacy release of both cisplatin and ROS, which not only potentiates the anti-tumor therapy but also triggers

immune cell death. This study presents a new strategy for theranostics for the combined treatment with ICBs in TNBC, where traditional therapeutic targets are lacking.

P4-12-28: Discovery and Validation of Novel Small G Protein Inhibitor OSURALi for Targeting Triple Negative Breast Cancer

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Background: Triple Negative Breast Cancer (TNBC) continues to be the deadliest subtype of breast cancer. There is an urgent need to identify novel molecular targets to be exploited. We previously identified the small G proteins Ras-Like Oncoproteins A and B, or RALA and RALB, as novel molecular targets of TNBC. The RALs, particularly RALA, were critical for tumor growth and metastasis in multiple models of TNBC. More recently, we found that TNBC cell lines are reliant on RALA and RALB expression. However, current generation RAL inhibitors are not suitable for clinical applications. There is an urgent need for better RAL inhibitors. Here, we show the discovery and validation of a novel RAL inhibitor and its efficacy in TNBC models.
Methods: Molecular docking studies were completed with drug-like and lead-like molecules from the ZINC15 database and 3D models for inactive GDP-RALA (Protein Data Bank, -2BOV). Virtual screening was performed with 500,000 molecules targeting the allosteric binding pocket of the RALA-GDP crystal structure and the homology models of RALB-GDP using Autodock. Surface Plasmon Resonance (SPR) was performed on the top 54 compounds (Reaction Biology). GTP-Pulldown assays were performed using a RAL-Activation Kits (BK040, Cytoskeleton, Inc. and #17-300 MilliPore-Sigma). Viability assays were perform using MTT (MilliPore-Sigma, 11465007001) and Growth in Low Adhesion (GILA) (G9682, Promega). Small G-Protein Assays were performed against RAS (BK008, Cytoskeleton), RAC/CDC42 (BK035, Cytoskeleton), RHOA (BK036, Cytoskeleton), and ARF (BK032-S, Cytoskeleton). For the measuring in vivo efficacy of OSURALi, MDA-MB-231 tumors in the mammary fat pad were allowed to reach ~100mm3 before being randomized into DMSO and OSURALi groups. Mice were injected with 50mg/kg OSURALi 5x/week. When early removal criteria were met, all mice were sacrificed, perfused, and organs were harvested for histological analysis.
Results: We identified a novel small molecule inhibitor we have named OSURALi. We assessed the efficacy of OSURALi against breast cancer cell lines and found OSURALi to be potent against RALA-dependent TNBC cell lines while RALA-independent SKBR3 cells and normal cell lines were more resistant to OSURALi. Current generation RAL inhibitors RBC8 and BQU57 were indiscriminately toxic to all breast cancer cell lines and normal cells regardless of their RAL-dependency. We further interrogated the drug-like properties of OSURALi. Excitingly, we found that OSURALi was well tolerated in mice and can significantly slow tumor growth and spontaneous lung metastasis in the MDA-MB-231 model.

Conclusion: Together these data suggests that OSURALi could serve as a lead compound for further drug optimization and may lead to a bona fide targeted therapy for TNBC.

P4-12-29: ETX-197/BG-68501, a potential best-in-class potent, selective, oral, small molecule CDK2 inhibitor, has anti-tumor activity in cancer models with Cyclin E amplification or deficiency in the Retinoblastoma 1 gene.

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In a normal cell cycle, there is redundancy in the role of the cell-cycle dependent kinases (CDKs) in regulating G1/S phase transition. In cancer cells, the regulation of G1/S transition can be subverted by (a) amplification and elevated expression of Cyclin E (CCNE), or (b) mutation/loss of the Retinoblastoma 1 (RB1) gene. Cancer cells with these genomic alterations have been shown to exhibit profound sensitivity to CDK2 depletion, validating CDK2 as a potential therapeutic target. Here, we report the discovery and preclinical characterization of ETX-197, a highly potent and selective small molecule inhibitor of CDK2 enzymatic activity. Based on known ligand-CDK2 structures, ETX-197 is designed to induce previously unexplored interactions within the CDK2 ATP binding pocket leading to improved potency and selectivity compared to other known CDK2 inhibitors. ETX-197 is >100-fold selective against other kinases in the CDK family and the selectivity extends more broadly against 385 other kinases. The affinity of ETX-197 for CDK2 results in tight binding (slow off-rate) and high potency in pharmacodynamic modulation and anti-proliferative activity in vitro and in vivo. Treatment of CCNE-amplified cancer cells with ETX-197 results in concentration-dependent inhibition of pRB phosphorylation, G1/S phase cell-cycle arrest and cell proliferation. In addition, ETX-197 treatment phenocopies CDK2 genetic knock-down in cells, as revealed by bulk-RNA Seq analysis of CCNE-amplified or wild-type cells, confirming that the cellular activity of ETX-197 is on-target and highly selective. In mouse xenograft studies using CCNE-amplified ovarian cancer cell line (OVCAR-3) or patient-derived tumors, ETX-197 treatment causes dose-dependent tumor growth inhibition with excellent tolerability. Interestingly, in RB1-deficient small cell lung cancer cell lines, ETX-197 treatment results in G2/M cell cycle arrest, accumulation of DNA damage, and apoptosis. Xenograft studies with small cell lung cancer cell lines and patient-derived tumor cells also show significant tumor growth inhibition with ETX-197. Additionally, ETX-197 has single-agent efficacy in a breast cancer xenograft model that had acquired resistance to a CDK4/6 inhibitor. These data suggest that ETX-197 has the potential to be a best-in class CDK2 inhibitor for the treatment of cancer with CCNE amplification or RB1 deficiency, including breast cancer that has progressed on treatment with a CDK4/6 inhibitor because of these genomic alterations. Currently, ETX-197 is being clinically developed by BeiGene in a first-in-human (FIH), Phase 1a/1b study to assess the safety, tolerability, pharmacokinetics (PK), pharmacodynamics, and preliminary antitumor activity in patients with advanced, nonresectable, or metastatic solid tumors (NCT06257264).

P4-12-30: The Impact of Beta Blocker Use on the Survival of Cancer Patients: A Systematic Review and Meta-Analysis

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Purpose: Beta blockers (BBs) have long been used for the treatment of cardiovascular and other medical conditions. More recently, the role of beta-adrenergic signaling in multiple processes driving tumor progression has been highlighted by preclinical studies, generating growing clinical interest in the potential repurposing of BBs as adjunctive anticancer agents. However, observational studies thus far have yielded mixed conclusions regarding their potential clinical use in oncology, and positive results may be inflated due to time bias (ITB). The main objective of this meta-analysis was to better characterize the association between beta blockade and survival in cancer patients, considering possible biases.
Methods: OVID Medline, EMBASE, and CENTRAL were searched from database inception to September 13, 2023 for research publications comparing the survival of cancer patients using BBs to that of non-users. In the large majority of studies, BBs were used to treat relevant patient cardiac comorbidities, not as adjunctive anti-cancer agents. Data extraction and quality assessments, using ROBINS-I, were conducted in duplicate. Hazard ratios (HRs) were analyzed using a random-effects model, with heterogeneity measured by the I2 statistic. Exploratory subgroup analyses assessed the association of BB type (selective vs. non-selective), cancer stage (early vs. advanced), and cancer type with survival. Sensitivity analysis was performed to assess the influence of ITB. The systematic review was prospectively registered (PROSPERO CRD42020200238) and not funded.
Results: We identified 79 eligible studies comprising 213 distinct analyses and 492,381 total patients. 2/79 studies were prospective (2.5%), while the remainder were retrospective. The most common primary tumor types assessed included breast (n=33), ovarian (n=30), and colorectal (n=28). Advanced disease (n=56) was more commonly studied than early/non-metastatic disease (n=26). Beta-blocker use was associated with significantly longer progression-free survival (PFS) than non-use, with a pooled HR of 0.78 ([95% CI: 0.66-0.92], I2=79.8%). This remained significant after excluding studies at greater risk of ITB (HR 0.74 [95% CI: 0.61-0.91], I2=36.6%). HRs for cancer-specific survival (CSS) and overall survival (OS) were 0.95 ([95% CI: 0.91-1.00], I2 = 77.4%) and 0.99 ([95% CI: 0.94-1.04], I2 = 84.9%).
Conclusion: This meta-analysis found that beta blockade may be associated with longer PFS among patients with cancer, irrespective of cancer type or stage; these findings remained statistically significant after excluding studies at risk of ITB. There was also a trend for BB use and longer CCS, but no difference observed for OS in the overall cohort. This lack of detriment to OS in the included observational studies is notable given that BBs are most commonly prescribed to patients with cardiovascular disease who are likely at inherently higher risk of death than non-user counterparts due to cardiac comorbidities. Ultimately, conclusions of this meta-analysis are limited by the observational nature of eligible studies. However, multiple phase II trials are ongoing, including a recently registered trial

(NCT05741164) seeking to determine the efficacy of propranolol in combination with pembrolizumab in patients with checkpoint inhibitor refractory metastatic triple-negative breast cancer. If positive, findings of these trials will lay the foundation for future larger randomized controlled trials to establish the highest level of evidence in this field.

P5-01-01: Longitudinal monitoring of quality of life in patients with de novo metastatic inflammatory breast cancer (mIBC) - a protocol in progress

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Background: Up to 30% of patients with inflammatory breast cancer (IBC) present with de novo metastatic disease. While systemic therapy is the mainstay of treatment for metastatic IBC (mIBC), loco-regional therapy may be considered due to the aggressiveness of loco-regional disease. At the same time, definitive local therapy (i.e., modified radical mastectomy and adjuvant radiotherapy) may adversely impact patient-reported outcomes (PRO).
Primary Objective: To establish a prospective registry of patients with de novo mIBC in order to monitor the impact of loco-regional disease and therapy decisions on PRO, arm lymphedema, skin toxicity, and decision regret.
Secondary Objective: To evaluate loco-regional progression-free survival (LRPFS), distant progression-free survival (DPFS) and overall survival (OS) in a contemporary cohort of patients with mIBC.
Methods and Study procedures: In this prospective registry, 50 patients with de novo mIBC treated at Dana-Farber Cancer Institute will be enrolled within 8 months of initiating systemic therapy. All enrolled participants will be administered the Lymphedema Survey (LSIDS-A), the PROMIS global health 2a Survey, the Skin Toxicity survey (Skindex 16) and the Decision Regret Scale every 6 months for up to 2 years. If a patient undergoes surgery, and the most recent set of the 3 study surveys is longer than 1 month prior to the operation date, then the patient will receive another set of study surveys 3 months postoperatively. Additionally, participant medical records will be accessed by the study staff every 6 months for up to 5 years from the time of diagnosis to ascertain loco-regional disease progression (and treatment details), distant disease progression and death from breast cancer or other causes.
Statistics: The primary PRO and secondary endpoints will be summarized descriptively and graphically over the two years since diagnosis. A set of clinical paths will be defined to sort patients into subgroups with clinically meaningful treatment categories, such as the receipt of loco-regional treatment or lack thereof, and/or disease progression. The analyses will be descriptive and estimation only, without statistical hypothesis tests.

This study activated in May of 2023 and is currently enrolling, with 13 participants enrolled as of June 30th, 2024. We anticipate reaching our target accrual of 50 participants in 2028.

P5-01-02: Is breast-conserving surgery after neoadjuvant therapy safe for locally advanced breast cancer?

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Background: Breast-conserving surgery (BCS) is being considered as an option for women with locally advanced breast cancer (LABC) who show a good clinical response to neoadjuvant therapy (NAT). Recent research indicates that breast-conserving surgery (BCS) is linked to a higher overall survival rate compared to mastectomy in this specific group. This study aims to assess the overall survival (OS) of patients with locally advanced breast cancer (LABC) who received breast-conserving surgery (BCS) following neoadjuvant therapy (NAT) and compare it to the survival of patients who underwent mastectomy (MS). Methods: This retrospective cohort study was carried out at Instituto do Câncer de São Paulo (ICESP), according to the STROBE guideline recommendations. Inclusion criteria was female patients diagnosed with locally advanced breast cancer (LABC) with an anatomic stage of T3 or T4. Exclusion criteria was male patients, contra-indication for adjuvant radiotherapy and multiple malignant neoplasia. The neoadjuvant treatment was chemo or endocrine therapy and the surgical intervention could be mastectomy (MS) or breast-conserving surgery (BCS). The study enrolled patients between January 2010 and January 2015, and their follow-up continued until December 2023. The primary outcome was overall survival of patients who underwent BCS compared to those who underwent MS. Descriptive analysis and association measures were used for statistical analysis and Kaplan-Meier curves were utilized for time-dependent variable analysis. We performed univariate and multivariate analysis in our data and adopted $p < 0.05$ as the significance level. Results: There were 414 patients with locally advanced breast cancer (LABC) who met the criteria and received neoadjuvant treatment followed by surgery. 396 (95.7%) received neoadjuvant chemotherapy, while 18 (4.3%) endocrine treatment. The average age of the patient group was 52.7±1.2(23-95) years. Out of the total of 414 patients, 64 women (15.5%) were diagnosed with stage IIB, whereas 350 patients (84.5%) had stage III breast cancer. The histological subtypes were distributed as follows: 152 (36.7%) were luminal HER2 negative, 154 (37.2%) were triple-negative, 53 (12.8%) were luminal HER2 positive, and 55 (13.3%) were HER2 positive. PCR was detected in 12.60% (52 patients) of cases. Regarding the surgical approach, breast-conserving surgery was performed on 74 patients (17.8%), while mastectomy was performed on 340 patients (82.2%). The BCS and MS groups were different in terms of clinical characteristics. The BCS group included of elderly

patients ($p < 0.001$), early-stage disease ($p < 0.001$), higher BMI ($p < 0.001$), higher number of postmenopausal women, higher rates of sentinel biopsy ($p<0.001$). The pCR rate was significantly higher in the BCS group, reaching 27% (20 patients), compared to 9.4% (32 patients) in the MS group ($p < 0.001$). During a follow-up of 8 years, the 5-year OS rates for patients undergoing BCS and MS were 78.4% and 60%, respectively (log-rank, $p < 0.032$). The univariate analysis revealed a statistically significant correlation between clinical parameters such as menopausal status, BMI, pCR, staging tumor, ki67, breast and axillary surgery, and overall survival (OS), as indicated in table 2. After the multivariate analysis, it was found that breast surgery, postmenopausal status, BMI, tumor stage, and pCR were associated with influencing overall survival. The MS was significantly associated with worse OS compared to BCS (OR 1,7; IC 1,05-2,9), along with postmenopausal status (OR 1,61; IC 1,16-2,21), higher BMI (OR 1,03; IC 1,01 – 1,06), and more advanced staging (OR 2,5; IC 1,4-4,6). On the other hand, higher PCR was associated with improved OS (OR 2,5; IC 1,3-5,0).
Conclusions: BCS after NAT is oncologically safe and significantly associated with better overall survival for women with LABC and initial anatomic stage T3 and T4, when compared to mastectomy. Additionally, lower stage, lower BMI, PCR and being pre-menopausal remained as independent factors related to better overall survival.
Keywords: Locally advanced breast cancer, neoadjuvant chemotherapy, pathological response, overall survival.

P5-01-03: Boost delivery to the tumor bed in breast cancer patients who have achieved pCR confirmed by VAB without surgery

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Purpose: We evaluate various approaches to target volume definition and boost delivery in breast cancer patients with pCR after neoadjuvant systemic therapy (NST) who were treated by radiotherapy without a surgery.
Materials and methods: A pathological complete response (pCR) was diagnosed in 20 of 27 patients included in "surgery de-escalation" prospective observation study. Clips were placed in the primary tumor volume (PrTV) before NST and during the vacuum aspiration biopsy after NST. Twenty patients with pCR underwent the whole breast irradiation and a boost to the PrTV. High-dose rate brachytherapy (HDRB) was the basic technique for boost delivery. Finally, we identified the value of fused images (computed tomography [CT] before NST with simulation CT), clips and their combination for an accurate boost delivery.
Results: A complete overlap between PrTV on pre-treatment CT with the localization of the clips on simulation CT was mentioned in 10, partial mismatch in three patients. In 12 of these 13 women, HDRB was successfully used for the boost delivery. In five cases we mentioned a marked discrepancy between the PrTV on fused images and the topography of the clips. In other two women we did not find clips on simulation CT. The fused images in

five of these seven patients showed anatomical landmarks (scar, fibrosis) used for identification of the gross tumor volume. In all 20 women with pCR (average follow-up of 16.6 months), there were no locoregional recurrences.
Conclusion: Combination of the clips with fusion of pre-NST and simulation CTs is important for an accurate boost delivery.

P5-01-04: Determination and clinical characteristics of bone pseudoprogression on advanced first-line therapy in metastatic breast cancer

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Background: Bone is the predominant metastatic site for breast cancer. Whether new osteoblastic lesions are defined as progression is currently controversial. Computed tomography (bone window) is commonly used to monitor disease progression in bone metastatic breast cancer (MBC). In this study, we aimed to assess the clinical characteristics and determination methods of bone pseudoprogression.

Methods: This retrospective analysis was conducted among 23 MBC patients with new osteoblastic lesions during the first-line systemic therapy at Jiangsu Cancer Hospital from January 2018 to December 2023. After every two cycles by computed tomography (bone window) to assess treatment response(at least twice), all the participants did not show disease progression, which we now define as bone pseudoprogression and continued on treatment until explicit disease progression (extraosseous disease progression or progressive lysis on bone lesions). The baseline and follow-up ALP were analyzed separately at the time of bone progression and pseudoprogression.

Results: The spine (78.2%) was the predominant metastatic site. The median time of appearance of bone pseudoprogression after treatment was 1.77 months. Notably, 83.3% of patients showed bone pseudoprogression during the first 3 months after treatment. Besides, the median interval of all patients between bone pseudoprogression and true disease progression was 14.27 months. There was no significant difference in HER2-positive and HER2-negative MBC patients (15.83 months versus 14.23 months, p=0.830). The incidence of stable/decreased ALP was higher at the time of bone pseudoprogression than progression (84.6% vs. 15.4%). Multiple regression analysis revealed that stable or decreased ALP was an independent predictor for bone pseudoprogression ($p < 0.001$).

Conclusions: Osteoblastic new lesions detected on CT (bone window) may represent bone pseudoprogression, which occurs mainly in the early stages of treatment. ALP is a useful serologic marker to differentiate pseudoprogression from disease progression on CT (bone window) in patients with bone metastasis. For these patients without extraosseous disease progression or progressive lysis on bone lesions, clinicians should be cautious about the appearance of new osteoblastic lesions.

P5-01-05: Clinicopathological features and outcomes of ER-low/HER2-low expression in de novo metastatic breast cancer: a preliminary cohort study

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Background: Breast cancer remains a major health problem for women worldwide, with the highest incidence and the second highest mortality rate in 2023. Breast tumors consist of distinct molecular subtypes categorized by the expression of hormone receptors (HR, including estrogen receptor [ER] and progesterone receptor [PR]) and human epidermal growth factor receptor 2 (HER2). The molecular subtype is closely related to its prognosis and also offers a basis for directed treatment. It is unclear whether ER-low-positive/HER2-low-positive (ER-low/HER2-low) biomarkers, as new biological subtypes, have different biology and clinicopathological characteristics in de novo metastatic breast cancer (dnMBC). Unfortunately, no studies on clinicopathological features of ER-low/HER2-low subtype of breast cancer have been reported to date. This retrospective cohort study aimed to investigate the clinicopathological features and prognosis of ER-low/HER2-low tumors in dnMBC patients.
Methods: Using hospital-based database, we identified patients with dnMBC diagnosed between March 2010 and December 2017,retrospectively collecting demographic data,tumor characteristics,treatment types,histopathological report and survival data.The cases were categorized according to ER, PR and HER2 status. ER-low was defined as if 1% to 10% of invasive tumor cells exhibited immunostaining for ER; HER2-low was defined as immunohistochemistry score of 1+ or 2+(negative by in situ hybridization). ER-low and HER2-low were focused,while ER-negative(ER-zero) and HER2-negative(HER2-zero) as controls.The primary outcome was overall survival (OS). It was estimated by Kaplan-Meier method and log rank test. Univariable and multivariable analyses were performed using the Cox proportional hazard model to identify statistically significant prognostic factors. All statistical analyses were performed using Stata 18.0.
Results: A total of 518 women with dnMBC who received systemic therapy met the inclusion criteria and were evaluated in this study (median age at initial diagnosis, 53 [21-82] years). Across the cohort, 338 patients (65.3%) had HR-positive/HER2-zero tumors, 115 patients (22.2%) had HER2-positive tumors, 52 patients (10.0%) had ER-zero/PR-zero/HER2-zero tumors (TNBC) and 13 patients (2.5%) had ER-low/HER2-low tumors. For ER-low/HER2-low patients, the median OS was 36 months and the 5-year OS rate was 23.1%. No significant difference was observed when it was compared separately with the OS of the clinically relevant subgroups, including HR-positive/HER2-zero(hazard ratios:0.97; 95% CI:0.508-1.856,p=.928), HR-positive/HER2-positive(hazard ratios:0.67; 95% CI: 0.335-1.366, p=.268) and HR-zero/HER2-positive(hazard ratios:0.58; 95% CI: 0.297-1.146, p=.107). Interestingly, its survival curve trajectory was close to or partially overlapped with that of the HR-positive/HER2-zero subgroup. However, it had a survival advantage compared to the TNBC subgroup(hazard ratios:0.34; 95% CI: 0.168-0.689, p=.001).
Conclusions: ER-low/HER2-low tumors are relatively small in number but is similar to

other clinically relevant subgroups in terms of biology, clinicopathological features, and prognosis, although distinct from TNBC. The findings of the present study do not support ER-low/HER2-low breast cancer as a distinct biological subtype.

P5-01-06: Discerning the consequences of type I interferon signaling in RON expressing breast cancer

Levi Fox

Metastatic breast cancers continue to drive patient mortality in United States, with a relative 5-year survival of 31%. In efforts to more effectively target aggressive breast cancers and reduce mortality, it is imperative to understand the drivers and mechanisms of metastatic breast cancer. The RON receptor tyrosine kinase is overexpressed in greater than 50% of breast cancers and is prognostic for metastatic outcomes, regardless of molecular subtyping. This presents RON as an attractive therapeutic target to effectively treat metastatic breast cancer patients. Understanding the molecular pathways regulated by RON in breast cancer may likewise identify actionable interventions. In line with this goal, we have identified that across breast cancer subtypes, RON expressing breast cancer cells suppress the production of type I interferons (IFN-I). Considering that tumor cell intrinsic IFN-I production can signal in both an autocrine and paracrine fashion, we examined the significance of IFN-I action on the tumor proper and in the host microenvironment. To accomplish this, we abrogated IFN-I signaling, through loss of IFNAR1, in breast cancer cell lines and/or in the host microenvironment using in vivo and in vitro approaches. Conversely, by treating breast cancer cell lines with IFN-I, we were able to bypass RON mediated IFN-I suppression and promote cell death. Together, these approaches of modulating IFN-I signaling have identified that RON expressing breast cancer cells are uniquely sensitive to IFN-activated host immune activity and tumor intrinsic IFN signaling. Loss of IFNAR1 in both breast cancer cells and within the host microenvironment promoted tumor growth and apoptotic evasion, while also enhancing breast cancer stemness of RON expressing breast cancer cells. Correspondingly, in vitro treatment of breast cancer cell lines with exogenous IFN-I promoted apoptosis and limited breast cancer stemness. These in vitro findings are corroborated in both spontaneous and syngeneic transplant murine models which demonstrate that IFN-I signaling limits the growth and metastasis of RON expressing mammary tumors. Together, these findings suggest that the suppression of IFN-I by RON serves to enhance tumor aggressiveness and metastatic progression, presenting IFN signaling as critical arm of RON's tumorigenic program in breast cancer.

P5-01-07: Use of Neoadjuvant Chemotherapy for Local and Regionally Recurrent Triple Negative and HER2+ Breast Cancer

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Introduction: Neoadjuvant chemotherapy (NACT) for early-stage breast cancer enables de-escalation of local therapy and tailored adjuvant therapy based on pathologic response. Data on the use of NACT for local and/or regionally recurrent (LRR) breast cancer (BC) is sparse. Herein, we evaluated use of NACT over time for triple negative (TN) and HER2+ LRR BC and impact of pathologic response on oncologic outcomes.
Methods: We identified patients with first TN or HER2+ invasive LRR BC treated surgically at our institution from 9/2008-5/2024. Patients presenting with distant metastasis, in situ recurrence or second LRR were excluded. We evaluated demographics, tumor characteristics of the index and LRR tumor, and treatment modalities. NACT use over time was evaluated with Cochran Armitage Trend tests. Associations between patient and tumor factors with biologic subtype and clinical nodal status were assessed with chi-square or Fisher's exact tests where appropriate. Cumulative incidence of distant recurrence was evaluated with death as a competing risk.
Results: 103 patients with LRRs were identified: 73 (70.9%) clinically node negative (cN0) local recurrences, 11 (10.7%) regional nodal recurrences, and 19 (18.4%) with both local and nodal recurrence. LRR tumor subtype was TN in 64 (62.1%) and HER2+ in 39 (37.9%; 28 hormone receptor (HR)+, 11 HR-). Proportion of cN0 recurrence was similar for TN and HER2+ disease (70.3% and 71.8%). Median time from primary cancer surgery to LRR was 4.4 years (IQR: 1.4,10.7, [TN 3.7 yr (IQR: 1.2, 11.3) vs HER2+ 5.4 yr (IQR:2.8, 10.5, p=NS)]. Data on use of chemotherapy for LRR was available in 96 patients. Of these 75 (78.1%) received chemotherapy, with no significant change over the study period (2008-2011: 73.3%, 2012-2015 76.9%, 2016-2019 70.0%, 2020-2024: 83.3%, p=NS). Chemotherapy was given neoadjuvantly in 50/75 (66.7%) with NACT use increasing over time (2008-2011 36.4%, 2012-2015 40.0%, 2016-2019 78.6%, 2020-2024 77.5%; p=0.003). The increase in NACT use was also observed for each tumor subtype (both $p < 0.05$). cN+ LRR were more likely to receive NACT (21/25, 84.0%) versus cN0 patients (29/50, 58.0%; p=0.037). Among 50 patients receiving NACT, 20 (40%) had a pathologic complete response (pCR, 13/29 [45%] for TN; 7/21 [33%] for HER2+). 11/29 (37.9%) cN0 LR had a pCR (cN0: 37.5% for TN, 38.5% for HER2) as did 9/21 (42.9%) cN+ LRR (53.9% for TN, 25.0% for HER2+). Overall median follow-up was 2.0 (IQR: 0.5, 5.5) years. Additional events included distant recurrence (20), isolated nodal recurrence (1), and radiation associated angiosarcoma (1). Following surgery for LRR, the median time to distant recurrence was 0.9 years (IQR: 0.5, 3.0). 5-year cumulative incidence of distant recurrence with death as a competing risk was 26.9% overall (95% CI: 17.5, 41.4) and was significantly higher at 43.5% (95% CI: 24.4, 77.6) among those with NACT not achieving pCR compared to 0% among those with pCR (p=0.001).
Discussion: Use of NACT for TN and HER2+ BC LRR has increased over time and was more common for LRR with nodal involvement. Among patients receiving NACT, pCR rate was 40% and associated with excellent clinical outcomes, with no distant recurrences observed in the pCR cohort. Conversely, nearly half of patients not achieving pCR experienced distant recurrence by 5 years. NACT for resectable LRR should be further studied in clinical trials.

P5-01-08: Perceptions and behaviors of US oncologists treating HR-positive HER2-negative metastatic breast cancer in second-line

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Background: The emergence of novel targeted therapies for hormone receptor-positive (HR+)/human epidermal growth factor receptor 2-negative (HER2-) metastatic breast cancer (mBC) offers more treatment options in second line (2L). While biomarkers can influence 2L treatment decisions, other factors such as comorbidities, co-occurring mutations, and treatment toxicities can guide treatment selection. Amid evolving treatments and clinical complexities, this interim analysis of an ongoing study aims to understand perception and behaviors of US oncologists in molecular testing and 2L treatment decisions in HR+/HER2- mBC with one or more PIK3CA, AKT1, or PTEN alterations.
Methods: US oncologists completed a cross-sectional survey in April 2024 (interim analysis data cutoff 04-23-24). Recruitment quotas ensured diverse representation of oncologists from various practice settings. Qualitative concept elicitation and desk research informed survey development. The validity of the survey was assessed via cognitive interviews (N=8). Data were summarized with descriptive statistics.

Results: This interim analysis included 152 oncologists (70 academic, 82 community), with mean 18.1 years in practice; 40.8% practiced in the Northeast. In the HR+/HER2- mBC setting, 77.6% of oncologists reported usually or almost always conducting next-generation sequencing (NGS) testing at diagnosis/prior to first-line (1L) treatment. Of patients not tested at diagnosis, 74.5% of oncologists reported usually or almost always conducting NGS testing prior to 2L treatment. When choosing 2L treatment for patients with HR+/HER2- mBC, most oncologists consider it was very important to know gBRCA (61.8%), ESR1 (57.2%), PIK3CA (55.3%), and/or PTEN (51.3%) status; fewer oncologists rated knowing AKT1 status as very important (42.8%). When presented with multiple actionable biomarkers, 68.4% of physicians selected PIK3CA mutation, AKT1 mutation, or PTEN alteration as more important for treatment choice than ESR1 mutation.

Oncologists were asked to select 2L treatments for 12 patient profiles, which varied in age (44, 56, or 64 yr old), site of metastases (liver, lung or bone only), duration of treatment with prior CDK4/6 inhibitors (CDK4/6i) in 1L (≥12 or <12 months), comorbid conditions (diabetes, pre-diabetes, or absence of these), ECOG status (0-1 or 2), and presence of biomarkers. Treatment options, based on the mutation profile, included: alpelisib+fulvestrant, capivasertib+fulvestrant, CDK4/6i+fulvestrant, chemotherapy, elacestrant, everolimus + exemestane, or others. In only 4 profiles did most oncologists agree on the 2L treatment; these profiles involved patients with one of the following biomarker alterations: PTEN and PIK3CA (51% agreement), AKT1 and PIK3CA (52% agreement), AKT1 and no ESR1 (57% agreement), and AKT1, PIK3CA, and no ESR1 (50% agreement). In these 4 cases, ≥50% selected capivasertib+fulvestrant, while the second most common treatment selected was alpelisib+fulvestrant or CDK4/6i +fulvestrant. In 8

profiles, oncologists diverged on treatment selection which involved patients with PIK3CA mutations alone or PIK3CA and co-occurring ESR1 mutations. For these patient profiles, oncologists chose various targeted treatment options with no strong consensus.

Conclusions: Our survey findings highlight the importance of biomarker testing in treatment decision-making for patients with metastatic HR+/HER2- breast cancer, particularly in choosing 2L treatment. Notably, there is a lack of consensus on treatment selection across biomarkers in determining 2L treatment approaches. This highlights the need for robust evidence to establish best practices and guide clinical decision-making.

P5-01-09: Improving ESR1 Mutations Detection in Breast Cancer Circulating Tumor DNA: Comparative Analysis of Enrichment Techniques

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Breast cancer is the most common cancer among women, with approximately 70% of breast cancer cases being estrogen receptor-positive (ER+). These cases are commonly treated with endocrine therapy. However, resistance mechanisms can arise during treatment such as the development of mutations in the ESR1 gene, occurring in 20-40% of patients. ESR1 mutations, which can significantly impact treatment efficacy, can be detected in circulating tumor DNA (ctDNA) before, and at the time of disease progression. However, these mutations are often present at low allele frequencies, requiring highly sensitive detection methods.

To address this challenge, various methods have been developed to enrich low-frequency allelic variants, thereby enhancing detection sensitivity. In this study, we assessed three enrichment methods, to enable ultra-sensitive detection of ESR1 mutations. The methods evaluated included ICE-COLD PCR, whole genome amplification using REPLI-g, and multiplex pre-amplification. Contrived samples of known variant allele frequencies were used to quantify the extent of enrichment achieved by each method. Mutation detection was performed using the APIS ESR1 Mutations Kit, capable of detecting eleven mutations within exon 5 (E380Q), 7 (S463P), and 8 (P535H, L536R/Q/H/P, Y537C/S/N, D538G). The limit of detection of the qPCR assay with each enrichment method was compared to determine their effectiveness.

Our findings suggest that enrichment methods notably enhance the sensitivity of ESR1 mutation detection in ctDNA. For instance, ICE-COLD PCR demonstrated potential for improving detection sensitivity by selectively amplifying mutant alleles, while REPLI-g and multiplex pre-amplification can increase the number of mutant copies in the reaction, without impacting amplification specificity. These advancements could enable more accurate systemic assessment and dynamic monitoring of ESR1 mutations in breast cancer patients, potentially before disease progression is evident. This capability allows for more tailored therapeutic approaches, improving patient outcomes by facilitating early intervention and more personalized treatment strategies.

In conclusion, employing enrichment techniques for low-allele frequency variants can improve the sensitivity of ESR1 mutation detection in breast cancer. This approach has the potential to enhance our ability to monitor and respond to disease progression dynamically, ultimately supporting more effective, individualized patient care.

P5-01-10: Clinical and pathologic factors associated with progression-free survival in patients with metastatic hormonal breast cancer treated with ICDK4/6

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Background: Breast cancer (BC) is the most frequent malignant tumor worldwide. The most frequent immunophenotype is luminal. 8-13% are diagnosed in metastatic stages and it is estimated that up to 20-40% of patients in local and locally advanced stages will recur. The first line of treatment in this scenario is cyclin-dependent kinase inhibitors 4 and 6 (ICDK4/6). However, in our setting real world evidence is poor.
Methods: We conducted an observational, retrospective, descriptive, cohort study in which women >18 years with histological confirmation of breast cancer with hormone receptors (HR+) and HER-2 negative (-) in metastatic stage were evaluated at the ABC medical center. The primary objective was to evaluate clinical and pathological factors associated with progression-free survival (PFS).
Results: 70 patients were treated with ICDK4/6, 54 (77%) presented recurrence while 16 (23%) presented de novo metastases, the median age was 58 years, 52 (74%) women were postmenopausal. The functional status they presented was ECOG 0 (7%), ECOG 1 (50%) and ECOG 2 (43%). Forty-five (64%) received ICDK4/6 in first, 13 (19%) in second, 9 (13%) in third and 3 (4%) in fourth or more lines of treatment. Forty (57%) had HER-2 low. The predominant number of metastatic sites was 2 in 25 (36%). Fifty (86%) used Palbociclib, 6 (9%) Ribociclib and 6 (5%) Abemaciclib. Factors that were associated with greater PFS were: using ICDK4/6 first line 23 vs 15, 20 or 4 months; HR=0.023 [95% CI; 0.004-0.136], p=<0.001; objective response rate (ORR) of 24 vs 6 months, p=0.001; absence of HER-2 low of 25 vs 17 months (p=0.011). Factors associated with longer overall survival (OS) were ORR p=<0.001, ECOG (p=0.019), INL < 4 (p=0.031), lung metastases (p=0.011), liver metastases (p=0.015), other metastatic sites (p=<0.001) and fatigue (p=0.036). HER-2 low status had a trend to lower OS (p=0.059). Adverse events (AE's) G3-4 occurred in 70%, with no differences between ICDK4/6 types, dose adjustments were required in 30%, treatment was discontinued in 19%, The most frequent toxicity with Palbociclib and Ribociclib was neutropenia (72% and 100% respectively), no grade 3-4 toxicities occurred with Abemaciclib.
Conclusions: We present a Mexican study with real-world data in luminal breast cancer patients treated with ICDK4/6. PFS, OS, ORR and toxicity are similar to previous reported in clinical trials. Factors associated with higher PFS were the line in which ICDK4/6 was received and ORR. There was no difference between ICDK4/6 types. HER-2 low status

appears to be a predictive factor in this setting.
Keywords: breast cancer, cyclin-dependent kinase inhibitors 4 and 6, progression-free survival, overall survival, HER-2 low.

P5-01-11: HER2-low is associated with longer progression-free survival in HR+/HER2- advanced breast cancer after cyclin-dependent kinase inhibitor 4/6 failure.

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Background: HER2-low is predictive for benefit in patients who receive Trastuzumab-deruxtecan (TDxd).
Formerly, low HER2 expression was considered HER2 negative according to ASCO-CAP criteria. The benefit derived from the use of TDxd has reshaped the biological understanding of HER2-low breast cancer and has triggered important efforts to decipher its prognostic and predictive significance.
At the biological level, molecular characterization studies have not identified the HER2/low BC patient group as a distinct entity.
Other studies have linked this biomarker as mirroring the activity of cross-linked intracellular signaling pathways such as PIK3CA which may confer a similar prognosis to luminal tumors. TDxd has been positioned as the first choice
of chemotherapy in HR+/HER2- patients with advanced breast cancer. The benefit in progression-free survival is striking, with a median survival of close to 12 months. The post-iCDK scenario is an ongoing clinical need where more real-life studies are required to improve decision making.
Objetive: To inform the prognosis of HER2-low expression among patients with advanced breast cancer HR+/HER2- who failed to iCDK 4/6 therapy.
Methods: We conducted an observational study based on a cohort of patients with HR+/HER2- ABC who received treatment subsequent to progression of an iCDK4/6. After screening a cohort of 160 patients, 64 progressions and 56 patients who received subsequent therapy were documented. Finally, a survival analysis was performed among patients HER2low vs HER2 0.
Results: The study included 13 patients with low HER2 and 43 with HER2 0. No significant differences were detected between the groups with respect to the type of iCDKs (Fisher's exact test p 0.47). No statistically significant differences were found between patient groups with respect to the type of subsequent treatment (CT vs. ET p 0.82). Thirty-five PFS events were observed, where 7 (53%) occurred in the HER2-low group and 28 (65%) in the HER2 zero group. No statistically significant differences were detected (p 0.52). In the survival analysis, the median PFS in subsequent treatment reached 389 vs. 153 days, with an increased probability of PFS of 174% more compared to the HER20 group. This effect was

maintained when PFS2 was considered, from the date of iCDK initiation to subsequent treatment progression, with an HR of 2.74 (1.39-5.38) P= 0.024.
Conclusions: HER2low was associated with longer PFS and PFS2 in patients receiving treatment subsequent to iCDKs progression. Contrary to reports from other series the prevalence of low HER2 was 23%, much lower than expected (around 60-80%). These findings should be considered with caution and regarded as exploratory for designing future studies. Further follow-up is required to explore the prognostic impact of low HER2 in this setting.

P5-01-12: Subsequent Treatments After Progression On Cyclin-Dependent Kinase 4/6 Inhibitors - A Multicentric Portuguese Real-world Data Study

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Background: Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) (Ribociclib, Palbociclib or Abemaciclib) plus endocrine therapy (ET) is the standard first line treatment in hormone receptor-positive (HR+) human epidermal growth factor receptor two-negative (HER2-) metastatic breast cancer (MBC) patients (pts). There are no established guidelines on subsequent treatment after disease progression with CDK4/6i, especially in later lines.
Methods: We conducted a multicentric retrospective observational study including HR+/HER2- MBC pts who experienced disease progression with a CDK4/6i for metastatic disease, between January 2016 and July 2023. Data were collected from medical records. The primary objectives were to compare Progression-Free Survival (PFS) and Overall Survival (OS) across different treatment options. Survival curves were estimated with Kaplan-Meier method and compared with the pairwise log-rank test.
Results: We identified 222 pts (220 women, 2 men) with a mean age of 57.6±13.4 years at the time of metastatic disease diagnosis; 131 (59.5%) were postmenopausal. The majority had visceral disease (172 pts, 77.5%). CDK4/6i were used as a first-line treatment in 182 pts (82.0%), as second-line in 30 (13.5%) and as third-line in 10 (4.5%). After progression with CDK4/6i, the next line of treatment included ET (68 pts, 30.8%), capecitabine (62 pts, 28.1%), paclitaxel (30 pts, 13.5%), rechallenge with a different CDK4/6i (14 pts, 7.7%), other chemotherapies (12 pts, 5.4%) and other treatments (17 pts, 7.6%). After progression with CDK4/6i, PFS was higher for capecitabine treated pts (16.5 months (mo), 95% CI [7.6, 32.1]) and the lowest with paclitaxel (5.6 mo, 95% CI [3.5, 8.2]). However, the OS was higher for pts who were rechallenged with a different CDK4/6i (24.6 mo, 95% CI [18.2, not reached - NR]), followed by those on ET (14.0 mo, 95% CI [12.2, NR]) and capecitabine (15.6 mo, 95% CI [10.4, 20.8]), and it was the lowest for those treated with paclitaxel (9.0 mo, 95% CI [4.8, 15.0], $p<0.05$).
Conclusions: In our cohort, PFS was longer for pts treated with capecitabine, while OS was the highest for those treated with a different CDK4/6i or ET. The main limitation of this

study is its retrospective nature and the non-random assignment of treatments. Thus, our findings support emerging data suggesting a benefit in switching ET while maintaining CDK4/6i after progression.

P5-01-13: Efficacy of abemaciclib and endocrine therapy on the overall survival for advanced ER-positive/HER2-negative breast cancer according to the treatment of lines

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Backgrounds: Combination therapy of CDK4/6 inhibitors and endocrine therapy (ET) is standard care for ER-positive/HER2-negative (ER+/HER2-) advanced breast cancer. Progression free survival of each treatment is important, however, overall survival (OS) form the diagnosis of advanced breast cancer is also important for the patients. Instead of the high efficacy, CDK4/6 inhibitors also have high cost and toxicity. Not all patients will want to use CDK4/6 inhibitors from first-line therapy, depending on their circumstances and preferences.
Objective:This study was conducted to investigate the efficacy of the combination therapy of abemaciclib and ET according to the treatment line in terms of time to treatment failure (TTF) and OS.
Patients and Methods: A total of 114 patients who started combination therapy with abemaciclib and ET between 2018/12 and 2024/1 and were followed for at least three months were included in the study. Clinicopathological characteristics and TTF, OS from the start of abemaciclib treatment, OS from the diagnosis of the advanced breast cancer were analyzed. according to the treatment lines.
Results: Median age of the patients was 61 (range 25-90) years. Twenty-nine (25.4%) were pre-menopausal and 85 (74.6%) were postmenopausal women. Abemaciclib was used as the firtst line treatment in 50(43.9%), second line 23 (20.2%) and third line or later 41 (35.9%) patients. ET for combination was aromatase inhibitor (AI) 44 (38.6%), AI +LH-RHa 8 (7.0%), fulvestrant (FUL) 44 (38.6%) and FUL+LH-RHa 17 (14.9%) patients. De novo stage IV 31 (27.2%), relapse while on the first 2 years of adjuvant endocrine therapy (adjET) 13 (11.4%), relapse while on adjET but after the first 2 years, or relapse within 12 months of completing adjET 28 (24.5%), relapse after 12 months of completing adjET 32 (28.0%), and no adjET was 10 (8.7%). Abemaciclib use from the 1st line treatment was associated with the presence of visceral metastasis, liver metastasis. Abemaciclib use from the 3rd line or later was associated with relapse after 12 months of completing adjET. Median TTF of the abemaciclib treatment was 19.6 months as the first line, 12.7 months as the second line, 14.9 month as the third or later line. Median OS from the start of abemaciclib 43.3, 40.7 and 57.8 months in which abemaciclib was used as the first, second and third or later line. Thus, there were no statistical differences in TTF of the abemaciclib treatment and OS from the start of abemaciclib according to the treatment lines of abemaciclib. Median OS from the diagnosis of advanced breast cancer was 43.7, 58.8, 135.4

months in which abemaciclib was used as the first, second and third or later line ($p<0.0001$).
Conclusion:In routine practice, abemaciclib was used in different lines of treatment, depending on physician's choice and patient preference. The TTF of the abemaciclib treatment or OS from the start of abemaciclib was not different among the treatment lines. Median OS from diagnosis of advanced breast cancer was significantly longer in patients who used abemaciclib as a third or later line treatment compared to those who used abemaciclib as a first or second line. Although there is a selection bias, the results of this study suggest that abemaciclib does not necessarily have to be used as the first line treatment for advanced ER-positive/HER2-negative breast cancer.

P5-01-14: Real world evidence of continued CDK4/CDK6 inhibition and endocrine therapy beyond progression on a prior CDK4/6i in women with hormone receptor positive (HR+), Her2 negative advanced breast cancer (MBC).

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Introduction: The use of endocrine therapy (ET) in combination with CDK4/6i is considered standard of care for patients with HR+, Her2 negative ABC. However, there remains an unmet need to determine the optimal sequencing after failure of CDK4/6i therapy. Endocrine monotherapy after progression to CDK4/6i is considered suboptimal treatment and access to other target therapies is limited in several countries. Two randomized trials (MANTAIN and postMONARCH) have supported the benefit of switching a different CDK4/6i and ET after progression to first line therapy with CDK4/6i. Evidence in a real-world setting of the efficacy of this approach is limited.
Methods: In a retrospective multicenter study, we analyzed 30 Hispanic patients with HR+ Her2 negative advanced breast cancer treated with endocrine therapy and a CDK4/6i, who were switched to another ET with a CDK4/6i at progression. Primary outcome was to assess median progression free survival-1 (mPFS-1) for HR+ Her2- MBC to the first line with CDK4/6i and mPFS-2 after switching CDK4/6i and ET at progression. Secondary outcomes: To estimate mPFS-2 in relevant clinical subgroups as well as safety and tolerability.
Results: From 2021-2024, 30 women with HR+Her2 negative MBC treated with a CDK4/6i in combination with ET were included. Median age at diagnosis was 56.5 years (SD 15.18), 67% were postmenopausal, 50% presented with metastatic de novo and 50% had recurrent disease. Regarding the site of metastatic disease, 53.3% had visceral and 40% only bone metastasis. 93.3% (n=28) received a CDK4/6i + ET as first line of therapy for MBC, 24 (80%) as a second line and 6 (20%) as a third line or beyond. Among those that received a CDK4/6i in the first line, 53.5% received palbociclib in combination with an AI (93%) as backbone ET. All premenopausal women (n=10) received GnRH agonist in combination

with ET. At the second line, most patients received ribociclib (72.2%) combined with fulvestrant (87.5%). mPFS-1 to the first line with CDK4/6i was 19.7 (SD 14.1) months and 15.5 (32.0) months in the second line with the CDK4/6i continuation. In the subgroup analysis patients with only bone metastasis and a previous iCDK4/CDK6 treatment duration of more than 12 months showed a clinically meaningful benefit with a PFS of 26.8 (SD 47.7) months and 18.2 (SD 35.1) months respectively. Only 20% required dose reductions due to side effects and none had to interrupt CDK4/6i for toxicity.
Conclusions: In this international real-world data cohort of Hispanic women with HR+Her2 negative ABC, the switch of CDK4/6i and endocrine therapy after first line therapy showed a clinically significant benefit in terms of PFS, specially in patients who received palbociclib in the first line setting and then switch to another CDK4/CDK6i and fulvestrant at progression, with a favorable toxicity profile. This strategy represents an appealing option in the current treatment landscape of HR+, Her2 negative MBC, specially in well selected patients and limited access to other target therapies.

P5-01-15: Clinical verification of HR-positive/HER2-0 and HER2-low metastatic breast cancer patients treated with CDK4/6 inhibitors

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Background: Cyclin-dependent kinase 4/6 (CDK4/6) inhibitors in combination with endocrine therapy (ET) are now the recommended first-line treatment for hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative metastatic breast cancer (MBC). On the other hand, recently, the concept of HER2-low has been drawing attention as a new biomarker. HER2-low breast cancer has different molecular characteristics compared to HER2-0 or HER2-enriched type, and we evaluated the effect of CDK4/6 inhibitors for MBC patients with HR-positive/HER2-low.
Materials and Methods: A total of 152 patients with HR-positive MBC treated with CDK4/6 inhibitors between December 2017 and August 2023 were selected. We classified these patients according to HER2 expression with available immunohistochemistry (IHC) and/or in situ hybridization (ISH) results, and HER2-low-positive status was defined by IHC 1+ or 2+ with negative ISH, and HER2-zero by IHC 0. We evaluated the relationships between HER2 expression and clinicopathological features and prognosis, progression free survival (PFS) and overall survival (OS).
Results: We excluded one patient without HER2 data and 10 patients with HER2-enriched, and 141 patients were divided into two groups; HER2-0 (47 cases) and -low (94 cases). HER2-low patients were significantly associated with de novo breast cancer ($p = 0.003$) and high ER expression ($p = 0.021$), and tended to have higher ki-67 expression at diagnosis ($p = 0.093$). The median follow-up time for the assessment of prognosis was 22.4 months (2.6 – 66.8m), and there was no significant difference between HER2-0 and -low groups for PFS and OS ($p = 0.553$ and $p = 0.266$, respectively, log-rank). In univariate analysis, late treatment of CDK4/6 inhibitors (HRs = 2.265, 95%CI: 1.333–3.829, $p = 0.003$), low

expression of progesterone receptor (PgR) (HRs = 2.174, 95%CI: 1.188–4.303, p = 0.011) and low absolute lymphocyte count (ALC) at base line (HRs = 2.133, 95%CI: 1.201–3.840, p = 0.010) were independent prognostic factors for progression. However, multivariate analyses showed that high Ki-67 expression (HRs = 3.156, 95%CI: 1.338–7.855, p = 0.008) and low PgR expression (HRs = 3.368, 95%CI: 1.056–12.152, p = 0.040) were found to be independent poor prognostic factors.
Conclusions: HER2-low may not be a predictive marker of CDK4/6 inhibitor efficacy in patients with HR-positive MBC. Further work is needed to validate these findings.

P5-01-16: m6A reader YTHDF3 promotes epithelial-mesenchymal transition and metastasis through facilitating Notch2 translation in breast cancer

Hong-Yu Chen, Jing-Wen Bai, Guo-Jun Zhang

Breast cancer is a mammary epithelial tissues source tumor, the primary cause of death for breast cancer patients is distant metastasis. Epithelial-mesenchymal transition (EMT) is a crucial first step in the metastasis of the tumor. m6A methylation plays an important role in EMT of many tumors, but its role in breast cancer EMT is not completely clear. This study will reveal the new mechanism of m6A methylation-modified recognition protein YTHDF3 in regulating breast cancer EMT and explore its feasibility as a therapeutic target. Methods: First, database analysis and immunohistochemistry were used to examine the degree of YTHDF3 expression in breast cancer and related paracancer tissues, as well as its impact on patients' prognoses. The YTHDF3 gene was then stable overexpressed and knocked down in various human breast cancer cell lines. YTHDF3's impact on the EMT of breast cancer cells was then investigated in a variety of functional experiments. Then, lung metastasis and subcutaneous tumor transplantation animal models were used to study the impact of YTHDF3 on the growth and metastasis of breast cancer. By RNA immunoprecipitation, the target gene of YTHDF3 in breast cancer was examined, and function experiments were used to confirm the effect of YTHDF3 and its target gene on EMT of breast cancer cells. The precise method by which YTHDF3 regulates its target was examined using RNA stability tests, protein stability tests and so on. Finally, lipid nanoparticles (LNPs) were designed to encapsulate small interfering RNA (siRNA) targeting YTHDF3 (siYTHDF3) for the treatment of breast cancer. Results: It was found that m6A recognition protein YTHDF3 was highly expressed in breast cancer, and the high expression was associated with a shorter relapse-free survival time. Cell experiments confirmed that the EMT of breast cancer cells was significantly promoted after the overexpression of YTHDF3 in T-47D cells, while the knockdown of YTHDF3 in MDA-MB-231 cells was significantly inhibited. It was observed that the tumor volume and weight after YTHDF3 knockdown were significantly smaller than those in the control group. In the lung metastasis mouse model, the lung metastasis was significantly lower than that in the control group after YTHDF3 knockdown. RNA immunoprecipitation was conducted to determine Notch2 as the target of YTHDF3, and the mRNA site of Notch2 was identified by YTHDF3 to promote its translation, then the EMT

and metastasis of breast cancer were promoted by upregulation of Notch2. Additionally, the LNPs encapsulating siYTHDF3 exhibited tumor-specific fluorescence and effectively suppressed tumor growth and lung metastasis in vivo. Conclusions: This project reveals a novel mechanism in breast cancer EMT, that is, YTHDF3 accelerates breast cancer EMT and metastasis by promoting Notch2 translation. More importantly, the LNP/siYTHDF3 complex validates the therapeutic potential of targeting YTHDF3 in breast cancer.

P5-01-17: Unveiling epithelial cell heterogeneity in lobular breast carcinomas through single cell RNA sequencing

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Background: Invasive lobular carcinoma (ILC) is characterized by unique clinicopathological features, distinct patterns of infiltration, specific molecular alterations such as E-cadherin loss, FOXA1, and GATA3 mutations, and distinct outcomes compared to invasive ductal carcinoma (IDC). Despite these differences, hormone receptor-positive breast cancers (BC) are often treated uniformly in clinical trials. The emergence of single-cell RNA sequencing (scRNA-seq) has revolutionized our comprehension of tumor heterogeneity. Our study first aims to evaluate scRNA-seq on formalin-fixed paraffin-embedded tissue (FFPE), compared with matched fixed fresh tissue, enabling retrospective analysis of archival BC samples. Subsequently, this pioneering study encompasses FFPE cases of ILC and control IDC, seeking to unveil detailed transcriptomic profiles of ILC epithelial cells, thus advancing diagnostics and tailored therapeutic strategies.
Methods: We performed a comparative scRNA-seq analysis with 2 fixed fresh BC tissue samples and 2 corresponding matched FFPE samples, encompassing one ILC and one IDC. Following the evaluation of these initial results, we expanded our study to include a series of 15 FFPE samples from ILC cases (11 luminal, 2 luminal Her2+, 2 triple-negative) and 4 IDC cases (luminal). Our analysis focused on exploring the heterogeneity of epithelial cells, specifically contrasting luminal subtypes between ILC and IDC and across different molecular types in ILC. We utilized 10X Genomics technology for single-cell analysis and performed bioinformatic analysis primarily using Seurat in R software. Additionally, we conducted immunohistochemistry and fluorescence in situ hybridization for result validation.
Results: The comparative scRNA-seq analysis revealed equivalent quality and representation of cellular populations between FFPE and fixed fresh samples, validating the retrospective use of FFPE samples for scRNA-seq studies. When contrasting luminal subtypes between ILC and IDC, significant epithelial cell heterogeneity was observed among the 4 IDC cases, each displaying distinct specific population with unique expression profile marked by high expression of some genes like CEACAM5, HOXC10, DCD and PROM1. In contrast, among the 11 ILC cases, heterogeneity was less pronounced, with a predominant population present in most cases and additional populations recurring in a few others, marked by genes like LTF, MYBPC1 and IGHG1. Only two ILC cases exhibited unique and

specific epithelial populations characterized by genes such as TMEM176B, TRIM9 and SCGB1D2, SCUBE3, respectively. Across different molecular types in ILC, we identified 5 distinct epithelial populations among the luminal ILC cases, as well as specific epithelial populations unique to luminal Her2+ (CRYBG3, MSMB) and triple-negative (CEACAM6) tumors, which were not present in other samples and clearly differentiated in the UMAP plot.
Conclusions: This study demonstrates for the first time that both fixed fresh tissue and FFPE routine blocks can be utilized effectively for the robust detection of clinically relevant traits at the single-cell level. Secondly, it highlights significant epithelial cell heterogeneity in luminal IDC and comparatively less heterogeneity in ILC. In addition, a shared population was identified among many ILC cases and substantial differences in epithelial cells across molecular subtypes within ILC, with each population marked by specific genes related to different biological processes, varying differentiation states, and distinct behaviors.

P5-01-18: ESRα and RASSF1A promoter methylation changed significantly in benign tumors and in the early event of breast cancer progression

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Methylation in the estrogen receptor alpha (ESRa) promoter is an epigenetic abnormality in breast cancer (BCa) and hypermethylation-mediated loss of ESRa expression could provide the cell with growth-promoting characteristics such as insensitivity to antigrowth signals. To investigate if there is a direct link between P0/P1 promoters of ESRa aberrant methylation and risk of progression of the fibroadenoma and fibrocystic diseasee to BCa.
Methodology. We used pyrosequencing to assess the DNA methylation in a panel of genes (ESRa, RASSF1A, and HIN1) in benign and cancerous human breast tissues and BCa cell lines.
Results. There was a significant elevated level of DNA methylation in ESRa P1 promoter (P=0.0001) in fibroadenoma tissues than ER-negative BCa, and 2-fold increase ESRa expression in fibrocystic, and fibroadenoma. HIN1 and RASSF1A methylation were elevated in ER-positive when compared to ER-negative BCa (P-value<0.5). To check if DNA methylation influence multiple gene networks rather than a single gene, we assessed the interaction between DNA methylation and the 3 genes within several types of breast tissues (fibroadenoma, and ER-positive and ER-negative BCa). Two-way ANOVA Univariante analysis of variance revealed a significant interaction between gene and breast types. ANOVA mixed model was further performed and revealed a significant interaction between the RASSF1A gene with fibroadenoma and ER-positive BCa (P-value=0.004). ESRa prmoter methylation in ER-positive BCa is associated with molecular subtypes (P-value=0.014) and grade (P-value=0.022). Tumors with unclassified molecular subtypes (ER-positive, PR-negative, HER2-negative) had elevated levels of methylation (P-value=0.046) in the P0 promoter compared with luminal B (ER-positive, PR-positive, HER2-positive) tumors.

Tumors with grade 3 showed a bordeline association with ESRa P1 promoter methylation when compared with grade 2 tumors (P-value=0.056). These results showed a highly methylated ESRa P0 promoter in the initial stages of breast carcinogenesis while the methylation in the P1 promoter occurs at later stages of BCa with poor prognosis. Conclusion. These findings suggest that methylation of ESRa promoter and tumor-related genes occured in pre-invasive lesions as an early event in BCa progresson. Furthermore, methylation of ESRa promoters could be consider a biomarker for the development of a diagnostic test that could predict benign breast disease that are at elevated risk of progressing to BCa.

P5-01-20: Elucidating novel pro-apoptotic consequences of an altered sphingolipidome in endocrine therapy-resistance.

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Endocrine Therapy (ET)-resistant breast cancer remains a clinical problem, with approximately 40% of patients experiencing disease relapse. In our previous study, we demonstrated that preclinical models of ET-resistant breast cancer have an altered sphingolipid profile. This is characterized by decreased levels of endogenous ceramides across multiple models of ET resistance. The ET-resistant models are also more sensitive to ceramide-induced cell death compared to their ET-sensitive counterparts, suggesting a functional significance of the low levels of ceramides. Therefore, the current study is designed to identify mechanisms by which ceramides preferentially induce cell death in ET-resistant breast cancer models in order to identify novel therapeutic strategies to improve ET-resistant breast cancer patient outcomes. To understand how ceramides differentially affect ET-resistant breast cancer cells, we have performed total RNA-seq of multiple ET-resistant breast cancer cells with or without adding back ceramides. Our findings reveal that ceramide addback exclusively induces genes associated with endoplasmic reticulum stress (EnRS), particularly in the PERK/peIF2α/ATF4 pathway, in multiple models of resistance. Inhibitors of EnRS, PERK, and peIF2α partially reverse ceramide-induced cell death suggesting that ceramide-induced EnRS has lethal consequences for ET-resistant cells. We further investigated how ceramides preferentially cause a lethal EnRS in ET-resistant models. Since ceramides are well-known bioactive lipids, we hypothesized that ceramides interact with specific proteins that are instrumental in ET-resistant cell survival. We used a photoactivable-and-clickable ceramide probe and quantitative proteomics to identify differentially expressed ceramide-interacting proteins (CIPs) in ET-resistant breast cancer cells. Proteomic analyses suggest that ceramide interacts with a group of clinically relevant CIPs which are located in the endoplasmic reticulum (EnR) membrane. Higher expression of these CIPs is associated with more Luminal B disease as well as worse relapse-free patient survival. While most of these CIPs have yet to be implicated in ET resistance, a subset of these CIPs is known to be involved in attenuating EnRS. To test whether ceramide-induced EnRS is mediated by CIPs, we performed siRNA-mediated knockdown of one of the top CIPs in the EnR membrane, TRAM1. We found that TRAM1

knockdown phenocopies ceramide treatment by significantly increasing cell death and a higher level of EnRS specifically in ET-resistant but not ET-sensitive cells. These findings suggest that ceramide interaction with EnR membrane proteins, such as TRAM1, may disrupt their function to induce a lethal level of EnRS in ET-resistant cells. Together, we find that ceramide induces preferential cell death in ET-resistant cells through interactions with a novel array of proteins leading to the inhibition of key survival proteins and a consequential activation of lethal levels of EnRS. Understanding how ceramides interact with and affect these proteins could lead to the development of novel strategies to selectively treat ET-resistant breast cancers and improve patient outcomes.

P5-01-21: Epigenetic changes induced by combinatorial pterostilbene and resveratrol in Her2-positive and triple-negative mammary cancer via gut microbiome-metabolome axis modulation.

Sebanti Ganguly, Trygve O. Tollefsbol

With evolution of chemotherapy, the mortalities associated with the two most aggressive breast cancer subtypes, Her2 overexpressing and triple-negative breast cancers (TNBC), have significantly declined. The natural phytoalexins pterostilbene and resveratrol, extracted from grapes and berries, showed promising effects in inducing apoptosis and cell cycle arrest in human TNBC cell lines when administered singly and in combination, with minimal toxicity towards non-malignant cells. The primary goal of this study was to evaluate the effectivity of the phytochemicals in vivo considering the interference by tumor microenvironment, immune cells, bioavailability of the phytochemical, differential metabolism, and varied gut microbial composition. After administering a long regime of dietary intervention of the phytoalexins in transgenic mice models, we observed a delayed occurrence of mammary neoplasms with a significant decrease in progression of tumor volume and weight in treatment groups vs. the control group. The group of subjects treated with combination of 3:1 (w/w) pterostilbene and resveratrol demonstrated synergistic effects in impeding the mammary cancer phenotype. Concurrently, we discovered that these treatments increase the abundance of specifically Firmicutes phyla of bacteria which indicates recuperation from diseased condition. Simultaneous evaluation of plasma short chain fatty acid indicates an increase in butyric acid levels in pterostilbene high group and decrease in caproic acid. Our findings also reveal notable differences in expression of tumor suppressor genes associated with Her2 (Erbb2) signaling pathway such as p53, PTEN, p21 and oncogenes such as Her2/neu, Ras, Bcl2 and Myc. We also found significant changes in the expression of epigenetic modulators such as histone deacetylases (HDACs), histone demethylases (KDMs) and DNA methyltransferases (DNMTs). Overall, our results demonstrate efficacious in vivo application of combinatorial pterostilbene and resveratrol in prevention of aggressive hormone response-negative breast cancer via the modulation of gut microbiome and metabolome and suggests possible epigenetic mechanisms associated with anticancer gene regulation.

P5-01-22: Establishing an Ex-Vivo Model for Postpartum Involution and Breast Cancer Susceptibility

Samaneh Karami, Tasneem Bawa-Khalfe

Postpartum breast cancer (PPBC) increases the risk of breast cancer (BCa) for up to 10 years after childbirth, in younger women (< 45 years old), and exhibits poor prognosis and elevated risk of incurable metastasis. Epidemiological factors such as higher number of pregnancies and older maternal age increase the risk of PPBC. Postpartum mammary gland (MG) restructuring and involution are intricately linked with cancer development. Yet, how epidemiological risk factors differentially impact postpartum involution to drive PPBC still remains largely undefined. The objective of the current project is to establish a low-cost, highly reproducible postpartum involution model (PIM) to test the link with PPBC susceptibility factors and MG restructuring. Specifically, we have established a "gland-on-a-plate" model to evaluate different stages of postpartum window of susceptibility. Isolated mouse MEC on this model platform respond to hormonal cue with changes in morphology and gene expression. Like the postpartum mammary gland, PIM undergoes lactation-associated gland remodeling with the formation of bifurcating epithelial lined ducts and milk accumulation in the central lumen; hence, it recapitulates both the morphology and function of the MG in anin vitro environment. Additional studies with MECs isolated from (24-week-old) female nulliparous (no pregnancy), age-matched parous (2-3 pregnancies) mice revealed quantifiable and statistically significant differences in the remodeling potential and functional properties between the groups when cultured on the PIM. Hormone-treated nulliparous organoids, undergo a notable morphological transformation characterized by distinct secretory branching patterns. However, when subjected to hormone withdrawal, nulliparous organoids exhibit postpartum morphology characterized by a reduction in size and branching. In contrast, organoids derived from parous MECs, respond to lactation hormones but exhibit impaired involution and gland remodeling. Ongoing studies will test whether involution defect observed in multiparity organoids favor cancer development, specifically we will evaluate involution disruption in our PIM models, by assessing cell proliferation, apoptosis, and epithelial-mesenchymal transition (EMT) markers. In addition, bulk and single-cell transcriptome analysis will be conducted to identify aberrant pathways that contribute to the parity-association involution defect and subsequent support cancer transformation. These studies will enhance our understanding of possible correlation between parity hormones, involution, and the postpartum windows of BCa susceptibility. Future studies will focus on evaluating molecular mechanisms and exploring both existing and novel therapies targeting PPBC using our mice post-weaning MEC and human postpartum MEC-derived organoid models. Consistently, we expect the established model will be an excellent tool to test preventative and therapeutic approaches on the biological mechanism of PPBC.

P5-01-23: Linking Spatial Distribution of Core Fucosylated N-Glycans to Triple Negative Breast Cancer Outcomes

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Triple Negative Breast Cancer (TNBC) is an aggressive breast cancer subtype with poor prognosis, partly due to its heterogenous molecular profile. Unlike other breast cancer subtypes, targeted therapies for TNBC are lacking, highlighting an urgent need for a better understanding of the underlying biology of the disease that can be used to develop more effective, tailored therapies. In this study, changes in glycosylation of TNBC primary tumors were analyzed in order to gain better insight into the biology of the disease with the goal of pinpointing specific glycan structures that might be useful as diagnostic and prognostic biomarkers that could assist in early detection, risk assessment and creating personalized treatments. Our past work studying glycosylation in breast cancer has linked specific glycan structural classes to different tissue regions, including stromal, necrotic and tumor areas. Fucosylation, a specific form of glycosylation, influences various immune and hormonal physiological processes and is frequently expressed by tumors. Previous studies by others have linked core fucosylation to increased proliferation, metastatic potential and therapeutic resistance in a variety of cancers, including breast cancer. Further, we have reported that tumor-associated core-fucosylated polylactosamine glycans are significantly more prevalent in metastatic breast cancers compared to non-metastatic ones.

The present study utilizes MALDI Imaging Mass Spectrometry to elucidate the spatial distribution of core-fucosylated N-glycans in TNBC tissues and assess their correlation with clinical outcome, tumor stage and patient survival status. Tissue samples were from female TNBC patients that self-identified as African ancestry (n=79). Tissues were previously banked for all research purposes and not specifically for this study; MUSC IRB approval as exemption #4. Clinical data includes tumor stage and grade, tumor nuclear grade, receptor status, metastatic sites, histology, surgical plan, chemotherapy status, BMI, age, and family history of cancer. Data showed that core fucosylation associated with stage of TNBC, with increases in stage III (ANOVA ≤ 0.001). By random forest machine learning, a set of three core fucosylated glycans were the primary differentiating factors in discriminating between stages of TNBC. We further found 9 specific core-fucosylated N-glycans that associated with survival status in the cohort (5-year survival n=39 alive; n=41 deceased; Kaplan-Meier (Log-Rank Mantel Cox test) p-value ≤ 0.01, Hazard ratio ≥ 2.5.

In summary, our analysis revealed that TNBC tumors with high levels of core fucosylation were frequently associated with advanced cancer stage and poor patient outcomes, including reduced overall survival. Further studies are warranted to explore the mechanistic role of these glycans in TNBC progression. The association between increased core fucosylation and adverse clinical outcomes underscores the potential utility for targeting core fucosylation, either alone or in combination with immune-stimulating therapies, to improve outcomes for TNBC patients.

P5-01-24: PPFIA4 mediates glucose metabolism reprogramming to alter the biological characteristics of breast cancer

Haochen Yu, Lingfeng Tang, Jihan Qiu, Shengchun Liu

Objective: Tumor growth requires a large amount of energy supply. If the energy supply can be blocked, the progress of the tumor can be slowed down or even killed. Warburg effect, which is highly dependent on glycolysis, in a variety of malignant tumors, including breast cancer. The Warburg effect limits the treatment targeting oxidative phosphorylation (OXPHOS) in breast cancer. There is an urgent need to explore new pathways and inhibitors for energy metabolism and clarify their specific mechanisms. Our team has previously found that glycolysis and OXPHOS pathways are higher in breast cancer than in normal tissues, and the antibiotic Tigecycline (Tige) may inhibit both pathways. However, whether the decreased energy metabolism changes the proliferation, invasion, migration, and other phenotypes of breast cancer needs to be further verified, and whether there are clear specific targets involved in the energy regulation process also needs to be further explored.
Methods: Bioinformatics analysis, immunohistochemistry (IHC), Western Blot (WB), glucose and lactate assays, and Seahorse energy metabolism assays were used to identify OXPHOS and glycolysis in breast cancer clinical samples and multiple cell energy knockdown models. Electron microscopy, JC1, and reactive oxygen species (ROS) fluorescence detection were used to determine the mitochondrial morphology, membrane potential, and ROS in the cells after energy metabolism down-regulation. Triple-negative breast cancer MB231 and MB468 cells and estrogen receptor-positive breast cancer MCF7 and T47D cells were treated with Tige at different concentrations for different times. CCK8 assay, colony formation assay, Edu fluorescence detection, and sphere formation assay were used to evaluate the effects of Tige on proliferation and tumor stemness in vitro. Nude mice xenograft tumor model and organoid culture were used to verify the phenotypes in vitro. Wound healing and Transwell assay were used to detect cell migration and invasion. RT-PCR and Western blot were used to detect the expression of multiple rate-limiting enzymes and other pathway markers in glucose metabolism pathways. RNA sequencing was used to explore the differentially expressed genes, analyze the related pathways, and identify the targets. Computer drug structure simulation was used to explore the binding domain between Tige and target genes. Lentiviral transfection was used to knock down and over-express the target gene, and the above phenotype and pathway exploration were repeated to prove the role of the target gene.
Results: TCGA database, IHC, WB, and Seahorse analysis of clinical specimens all showed that OXPHOS and glycolysis were more active in breast cancer than in normal tissues. Electron microscopic observations revealed alterations in the structure of mitochondrial cristae. Respiratory chain complex proteins and membrane potential of mitochondria were affected, and intracellular ROS production was increased after the energy decreased. After the down-regulation of energy metabolism, the proliferation, invasion, and migration of breast cancer cells were affected, which was mainly due to the arrest of the S phase of cell cycle, which requires a large amount of energy supply. In vivo experiments using organoids

have also obtained consistent results. The key enzymes of glucose metabolism in four breast cancer cell lines showed inconsistent decreases after Tige treatment. WB showed that in addition to the glucose metabolism pathway, the PI3K/AKT/mTOR pathway was also significantly inhibited. Bioinformatics analysis showed that the differentially expressed genes were mainly related to the inhibition of cell cycle, and the clear target may be PPFIA4. After changing the expression of PPFIA4, the rescue experiments in vivo and in vitro confirmed that PPFIA4 was involved in the energy metabolism of breast tumors. Conclusion: Both glycolysis and OXPHOS pathways are more active in breast tumors than in normal tissues. Tige treatment can successfully establish the down-regulated energy metabolism model of breast cancer and successfully inhibit the progression of breast cancer in vitro and in vivo. PPFIA4 may be involved in the regulation of PI3K/AKT/mTOR pathway and energy metabolism, which is worthy of further attention. At the same time, it also provides more theoretical support for expanding the clinical application of Tige.

P5-01-25: Romidepsin Targets HDAC1/2 to Induce Ferroptosis in TNBC Enhancing Chemosensitivity to Eribulin

Weipeng Zhao, Xichuan Li, Xing Yang, Yulong Wang, Shuling Wang, Qian Liu

Triple-negative breast cancer (TNBC) is a highly aggressive solid tumor with limited clinical treatment options, necessitating the urgent development of new drugs or therapeutic strategies. Romidepsin is a selective inhibitor of HDAC1/2 with notable antitumor activity in lymphomas, but its therapeutic efficacy in solid tumors like TNBC remains unclear. Our research reveals that romidepsin significantly induces ferroptosis in TNBC cells. Through RNA-seq analysis, we identified that romidepsin markedly alters the expression of several ferroptosis-related genes. Romidepsin regulates GSH metabolic reprogramming via the SLC7A11-CHAC1-GLS2 axis and causes iron overload in TNBC cells by modulating the Keap1-Nrf2-Hmox1 axis. Additionally, it promotes lipid peroxidation by upregulating the LPCAT3-POR-CYB5R1 axis. These biological functions of romidepsin are achieved through epigenetic modifications of target genes by HDAC1/2. Knockdown of HDAC1 or HDAC2 exacerbated romidepsin-induced ferroptosis in TNBC cells. To explore the clinical potential of romidepsin in TNBC treatment, we investigated its effects in combination with several first-line chemotherapeutic agents, including PTX and DDP, through cell viability assays. We found that romidepsin significantly enhanced the antitumor activity of multiple chemotherapy agents, with the most pronounced synergistic effect observed in combination with the anti-microtubule agent eribulin. The combination of romidepsin and eribulin effectively inhibited TNBC proliferation and promoted apoptosis and ferroptosis, demonstrating potent antitumor effects both in vitro and in vivo. Our study provides novel insights into the potential application of romidepsin in the clinical treatment of TNBC. The combination of romidepsin and eribulin holds promise for future clinical application in TNBC therapy.

P5-01-26: Selective Targeting of CDK2 Using Molecular Glue Degraders for the Treatment of HR-Positive/HER2-Negative Breast Cancer

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Cyclin dependent kinases 4 and 6 (CDK4 and CDK6) and cyclin dependent kinase 2 (CDK2) act sequentially to coordinate cell cycle progression through the G1/S phases and effectively drive cell proliferation via RB phosphorylation and repression. CDK4/6 inhibitors in combination with endocrine therapy are approved agents for the treatment of hormone-receptor (HR)-positive/HER2-negative breast cancer. While these agents offer substantial benefit, patients eventually relapse. It has been reported that approximately 30% of resistant tumors following CDK4/6 inhibitor treatment exhibit upregulation of CCNE1 expression, and others are thought to adapt to chronic CDK4/6-inhibition by increased tumor reliance on the CDK2 pathway to sustain downstream signaling along the RB-E2F axis. Hence, targeting CDK2 in conjunction with CDK4/6 inhibition is expected to provide more sustained responses in this difficult-to-treat patient population. We sought to identify molecular glue degraders (MGDs) that selectively target CDK2. Using our MGD discovery engine QuEENTM encompassing biochemical and cellular assays as well as in silico modelling, we identified and further optimized molecules that induce CRBN engagement and selective degradation of CDK2, while sparing other proteins such as closely related CDKs. Unlike CDK2 inhibitors, CDK2 MGD inhibits cell proliferation in an RB-dependent manner, attesting to its superior selectivity. Furthermore, this MGD induces robust downstream pathway suppression, as evidenced by downmodulation of RB phosphorylation and E2F-driven gene expression. When dosed orally in preclinical models of HR-positive/HER2-negative breast cancer, this compound drives deep tumor regression in combination with CDK4/6 inhibitor or triple combination with endocrine therapy (fulvestrant), resulting in enhanced downstream pathway suppression compared to CDK4/6 inhibitor alone. Owing to its superior selectivity, we expect that a CDK2 MGD will avoid dose-limiting toxicities associated with less selective CDK2 inhibitors. Hence, a CDK2 MGD provides novel means to target an inadequately drugged target, offering a unique angle for populations in desperate need of treatment options.

P5-01-27: The relation between local tumor infiltrated T cells (TILs) and neutrophil-to-lymphocyte ratio (NLR) of peripheral blood in patients with De novo Stage IV breast cancer

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Purpose: We investigated whether the relation of tumor infiltrated T cells and neutrophil-to-lymphocyte ratio (NLR) can be a novel predictive factor for the benefit of surgery for de novo stage IV breast cancer (DnIV BC) and if primary tumor resection (PTR)'s surgical advantage related to clinical outcomes, the surgery timing in responders to systemic therapy.
Patients and Methods: We reviewed the cases of the DnIV BC patients who received systemic therapies and/or underwent PTR at our institution (Jan. 2004–Dec. 2022). Blood tests and NLR measurement were performed before and after each systematic therapy and/or surgery. The kinetics of peripheral blood lymph cell counts, NLR etc. were analyzed simultaneously. The immunopathological stain was performed using surgical specimens or biopsy samples by anti-CD3, -CD8, -CD163 and -CD25(Foxp3) antibodies.
Results: Sixty patients had undergone PTR local surgery (Surgery group); 81 patients had not undergone surgical treatment (Non-surgery group). In both groups, systemic treatment was performed as chemotherapy (95%) and/or endocrine therapy (92.5%) ($p<0.0001$). The groups' respective median progression-free survival (PFS) durations were 88 and 30.3 months ($p=0.004$); their overall survival (OS) durations were 100.1 and 31.8 months ($p=0.0002$). The Surgery-group responders to systemic therapy lasting >8.1-months showed significantly longer OS ($p=0.044$). The PFS and OS were significantly associated with the use of postoperative systemic therapy ($p=0.0012$) and the NLR ($p=0.018$). A low NLR (≤3) was associated with significantly better prognoses (PFS and OS; $p<0.0001$). The immunopathological study of surgical specimens showed the CD3, CD8+ T lymphocyte infiltration has significant increase in patients with low NLR. In contrast, there was no significant difference of CD163+ macrophage and CD25+ lymphocytes.
Conclusions: A longer effective duration of systemic therapy (>8.1 months) and a low pre-surgery NLR (≤3.0) could predict PTR's surgical advantage for DnIV BC. These variables may help guide decisions regarding the timing of surgery for DnIV BC.

P5-01-28: A comprehensive analysis of NTRK3 expression in breast cancer

Jae-Ho Lee

The ETV6-NTRK3 gene fusion is found in various cancers and showed their clinical significances. We clarified clinical and prognostic values of NTRK3 expression in breast cancer (BC) by open gene expression databases, such as TIMER, UALCAN, KM, OSlihc, and LinkedOmics, and immunohistochemistry analysis. Then, we also analyzed cell invasion and migration in BC cell lines transfected with NTRK3-siRNA. NTRK3 expression was higher in BC tissues and associated with various clinicopathological characteristics. It was also correlated with immune cell infiltration of B cells, macrophages, myeloid dendritic cells, and neutrophils. Survival analysis showed that NTRK3 expression induced favorable prognosis in BC patients. The expression level of NTRK3 as significantly downregulated in both MCF-7 cell line transfected with NTRK3-siRNA compared to that in cells transfected with NC siRNA. NTRK3 significantly affected cell viability and wound healing. This result suggested that NTRK3 expression contribute to BC pathogenesis and prognosis.

P5-01-29: Development of a Novel Antigen Presentation Assay in Triple-negative Breast Cancer

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Triple-negative breast cancer (TNBC) is the most lethal form of breast cancer, partly due to its ability to significantly reduce antigen presentation, allowing the tumor to evade immune surveillance. As a result, accurately measuring tumor antigen presentation could provide critical insights into the immune response against TNBC. However, existing methods for studying antigen presentation are cumbersome and time-consuming. These methods typically rely on known peptide sequences and antibodies that recognize those peptides. A major concern with using specific peptide sequences is the potential for unstable presentation and antigen loss during the antigen editing process in endoplasmic reticulum/endosomes, thus leading to inconsistent results.
In this study, we present a novel and cost-effective approach to examine breast cancer antigen presentation using Click-it chemistry. This reaction specifically occurs between azides and alkynes/cyclooctenes. By pre-labeling tumor antigens with azides or alkynes, these antigens can be taken up by antigen-presenting cells, and their presentation can be detected through interactions with fluorophore-conjugated alkyne/cyclooctene or fluorophore-conjugated azide, respectively.
We employed two types of antigen-presenting cells—dendritic cells and macrophages—and used tumor antigens collected from two TNBC cell lines. Our results demonstrated successful and very stable antigen presentation on the surface of both macrophages and dendritic cells. Additionally, we found that breast cancer antigens remained stable within the phagolysosomes of these cells. Importantly, antigens labeled via click chemistry throughout the tumor antigen fragment showed better presentation via MHC class II compared to antigens labeled only at the terminus. This method also allows for the natural antigen editing process within the endoplasmic reticulum or endosomes to select high-affinity antigens for MHC presentation, resulting in a more stable presentation than conventional methods.
In conclusion, our novel antigen presentation assay offers several advantages over existing methods, including faster turnaround times, cost-effectiveness, stable antigen presentation, and reliable detection signals. When combined with mass spectrometry, our method has the potential to identify specific tumor peptides that are stably presented and could serve as valuable targets for the development of immunotherapies to treat TNBCs.

P5-01-30: Prolactin-Enhanced Iron Uptake via CD44 and Its Impact on Breast Cancer Metastasis

Reagan Farrell, Trevor Jones, Kuan-Hui Ethan Chen

Metastasis is the leading cause of cancer mortality, with the epithelial-to-mesenchymal transition (EMT) serving as an early event in tumor invasion and metastasis. Iron, abundant

in the tumor microenvironment (TME), plays a crucial role in cellular processes, including metastasis, and tumor cells are "ferrophilic," showing a high iron dependence. In our previous work, we found that breast cancer cells secrete the autocrine hormone prolactin into the TME, enhancing iron uptake via the cell-surface glycoprotein CD44, bound by hyaluronate. To date, there are more than 50 spliced transcripts of CD44, with at least 27 of them leading to protein translation. However, the role of CD44 spliced variants in iron regulation and EMT remains unclear. This study aims to investigate the correlation between CD44 splicing and iron-dependent EMT. Examining two human breast cancer cell lines, MCF7 (Luminal A) and MDAMB 468 (Triple Negative Breast Cancer, TNBC), we demonstrated a positive correlation between mesenchymal transition and intracellular iron content. To determine which specific CD44 isoform(s) are involved in iron uptake and drive EMT, we examined gene expression across a broad panel of CD44 spliced variants using quantitative PCR. In both cell lines, we observed consistent upregulation of the CD44 V3 and V5 isoforms following prolactin treatment. In the more aggressive TNBC cell line, MDAMB468, additional CD44 spliced isoforms of bulk cultures, including V6 and V9, were also upregulated. Given the critical role of cancer stem cells in metastasis, we extended these analyses to CD44 splicing in cancer stem cells. Prolactin stimulation consistently induced overexpression of CD44 V3 and V5 in stem cells, with an overall higher CD44 expression compared to bulk tumor cultures. We observed an overall upregulation of CD44 expression in MCF7 stem cell cultures compared to bulk cultures. Notably, neutralizing CD44 V5 reduced intracellular iron levels and reversed EMT, highlighting its specific role in these processes. Thus, our study establishes the prolactin-CD44 axis as a key regulator of EMT, specifically identifying CD44 V5 as a potential therapeutic target for disrupting iron uptake and metastasis in breast cancer.

P5-02-01: Does Community-Level Distress Correlate with the Risk of Developing Lymphedema Among Breast Cancer Patients?

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Background: Lymphedema is a debilitating condition that significantly affects the quality of life of breast cancer survivors. Breast cancer-related lymphedema (BCRL) occurs in 3-8% of patients undergoing sentinel lymph node biopsy (SLNB) and 13-60% of those undergoing axillary lymph node dissection (ALND), with rates being two-fold higher in low-income and ethnic minority groups. While the risk of lymphedema development from breast cancer treatments has been well documented, there is scarcity of literature focusing on the socioeconomic factors impacting lymphedema development socio-geographically. The Distressed Communities Index (DCI) is a validated socio-geographic measure of economic well-being that provides a comprehensive assessment of community-level distress. Results from a recent study, utilizing a similar index, found correlation between patients from deprived areas, risk for lymphedema, and odds of lymphedema diagnosis. Therefore, our study aims to investigate whether higher distressed communities, as measured by DCI

quintiles, is associated with an increased risk of developing lymphedema in breast cancer patients.
Methods: This retrospective study examined the association between DCI quintiles and the incidence of lymphedema among breast cancer patients aged 18 years and older who were treated or diagnosed at Montefiore Medical Center between 2011 and 2022. The study population was extracted from two databases: one with patients who had been referred to physical medicine for lymphedema therapy, where those who developed lymphedema in the arms were selected, and the other from the institutional tumor registry. Patients' zip codes were matched into the corresponding DCI score and DCI quintiles and stratified into low distressed (quintiles 1-3) and high distressed (quintiles 4-5) groups. A DCI quintile above 3 indicates a highly distressed area. Chi-square tests and logistic regression were performed to determine the association between DCI quintiles and lymphedema, when controlling for race, insurance status, and ethnicity.
Results: Our study included 650 breast cancer patients, with 80.6% from higher distressed areas (DCI quintiles 4-5) and 19.4% from less distressed areas (DCI quintiles 1-3). The mean BMI and age for patients from low distress areas were 28.0 ± 6.86 and 56.2 ± 12 years, respectively, while those from high distress areas had a mean BMI of 30.6 ± 5.72 and a mean age of 59.1 ± 11.8 years. The chi-square test showed a significant association between DCI quintile and lymphedema when grouped into high and low distress groups ($p = 0.005$). Controlling for race, ethnicity, and insurance status, patients in higher distressed areas had a 20% increased risk of developing BCRL compared to those in less distressed areas (OR 1.20, 95% CI [1.00, 1.43], $p = 0.049$). Ethnic minorities had about twice the risk of developing BCRL compared to white patients (OR 1.91, 95% CI [1.40, 2.62], $p < 0.0001$). Stratifying by race, patients who identified as black ($p = 0.001$), "Other" ($p = 0.009$), or "Unknown" ($p = 0.002$) had higher risk of lymphedema compared to white patients. Additionally, patients with Medicare (OR 0.478, 95% CI [0.326, 0.703], $p = 0.0002$) or Medicaid (OR 0.670, 95% CI [0.458, 0.980], $p = 0.048$) had lower risk of lymphedema compared to those with private insurance.
Conclusion: Patients in higher distressed communities (DCI quintiles 4-5) have a 20% increased risk of developing lymphedema compared to those in less distressed areas (DCI quintiles 1-3), when controlling for race, ethnicity and insurance. Furthermore, race and insurance status had a significant impact on the risk of BCRL.

P5-02-02: Gender Disparities in the National Institutes of Health Funding for Gastrointestinal Oncology.

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Background: Reportedly, there has been an under-representation of women in academic medicine, resulting in discriminatory distribution of research grants. This study examines the trend of funding allocation of R01 grants in Breast oncology by the National Institutes of Health with specific focus on the distribution of funding between gender.

Methods: The data were retrieved from the NIH RePORTER (Research Portfolio Online Reporting Tools Expenditure) using breast oncology-related search terms from 2018-2021. The gender was categorized using Genderize. The number of citations, publications, H-index, and seniority were obtained from Scopus and Web of Science in December 2022. Consumer Price Index was used to adjust funding amount to 2021 equivalent U.S. dollars. Linear regression was used for analysis.
Results: A total of 885 NIH-funded R01 grants amounting to $444.6 million were awarded for breast oncology research. Women (n=390; 44.1% [95% CI: 40.8%-47.3%]) received relatively fewer grants than men (n=495; 55.9% [95% CI: 52.7%-59.2%]). From 2018-2021, there was a significant increase in the number of grants awarded among both men (90 to 155, $p<0.01$) and women (71 to 115, $p=0.036$). Similarly, there was a significant increase in the grant amount (in millions) awarded among men (43.4 to 76.5, $p<0.01$) but not in women (36.7 to 56.1, $p=0.11$). Of the 212 co-PIs, 133 (62.7% [95% CI: 56.2%-69.2%]) were men and 76 (37.3% [95% CI: 30.8%-43.8%]) were women.
There was no significant difference in h-index (48 vs 44, $p=0.12$), number of publications (158 vs 138, $p=0.08$), and citations (13272 vs 8092, $p=0.06$) between the male and female PIs. Funding amount was significantly associated with number of publications ($\beta=0.35$, $p<0.01$), seniority ($\beta=0.25$, $p<0.01$) and institution ($p<0.01$).
Conclusion: Our analysis shows continued gender disparity as only 44% of total R01 grants were awarded towards females for breast oncology between the fiscal years 2018 to 2021. Though the proportion of discrepancy is lower as compared to other malignancies. A collaborative effort is still needed to bridge the gap and advance gender equality.

P5-02-03: Racial Disparity in the Genomic Landscape of Patients with Breast Cancer and Association with Clinical Outcomes

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Introduction: Several studies have shown that African Americans (AA) with breast cancer have inferior clinical outcomes. The underlying reasons for this racial disparity are multifactorial, including lack of access to healthcare, and several socioeconomic factors such as lower income and education. In addition to the social determinants of health, tumor biology may also play a crucial role in this disparity. However, there is limited data on the role of tumor biology in the survival outcomes of breast cancer among different races. We aim to analyze the prevalence of somatic mutations among different races and their association with survival outcomes in breast cancer.
Methods: Clinical and transcriptomic data were obtained from the TCGA Breast Invasive Carcinoma PanCancer Atlas database from cBioPortal. Patients with breast cancer were stratified into different racial groups, including Whites, AAs, Asians, and Others. We studied the incidence of commonly altered genes in breast cancer such as TP53, PIK3CA, BRCA1/2, ATM, CDH1, ESR1, and PTEN among different racial groups. Furthermore, we investigated the role of these mutations in the survival outcomes of patients across different racial

groups. Clinical and genomic characteristics were compared using Fisher's exact test for categorical variables. Cox proportional hazard ratio and log-rank test were employed for survival analysis.
Results: A total of 1,084 patients were included in the analysis. The majority of the patients self-identified as White (69.3%, n=751), followed by AAs (16.8%, n=182), Asians (5.5%, n=60), and Others (8.4%, n=91). Most of the tumors were luminal A (46%) and AJCC T2 stage (57.7%). PIK3CA and TP53 were the most commonly altered somatic mutations (32.6% each) in the analyzed samples. The frequency of CDH1, PTEN, BRCA2, BRCA1, ATM, and ESR1 mutations was 12.2%, 5.4%, 2.7%, 2.5%, 2.3%, and 0.8%, respectively, in the entire cohort. AAs had more TP53 (47% vs 31%, p= 0.001) and fewer PIK3CA (21.6% vs 36.7%, p= 0.001) and CDH1 (5.6% vs 14.6%, p= 0.001) mutations compared to Whites. There was no statistically significant difference observed in other somatic mutation distributions among AAs and Whites (PTEN: 5.6% vs 6.4%, p= 0.71; BRCA1: 5% vs 2.5%, p= 0.27; BRCA2: 1.9% vs 2.7%, p= 0.66; ATM: 2.5% vs 2.9%, p= 0.92; ESR1: 1.2% vs 1.6%, p= 0.97). AAs had significantly higher odds of TP53 (odds ratio (OR) 1.79, 95% CI 1.27-2.50, p= 0.0008) and lower odds of possessing PIK3CA (OR 0.45, 95% CI 0.30-0.67, p<0.001) and CDH1 (OR 0.35, 95% CI 0.17-0.70, p= 0.001) mutations compared to Whites. AAs also had higher odds of carrying BRCA1 (OR 2.14, 95% CI 0.90-5.07, p= 0.11) and lower odds of developing ESR1 (OR 0.76, 95% CI 0.16-3.43, p= 1), PTEN (OR 0.84, 95% CI 0.40-1.76, p= 0.86), ATM (OR 0.83, 95% CI 0.28-2.45, p= 1), BRCA2 (OR 0.65, 95% CI 0.19-2.22, p= 0.78) mutations; however these were not statistically significant. The 5-year disease-free survival (DFS) of AAs with PTEN (75% vs 97%, p=0.04) and PIK3CA (77% vs 88%, p=0.04) mutations was lower than that of Whites. The 5-year overall survival (OS) was also observed to be lower in AAs with PTEN (76% vs 87%, p=0.23) and PIK3CA mutations (85% vs 90%, p=0.46); however, it was not statistically significant. Survival analysis of other mutations among AAs vs Whites was not statistically significant.
Conclusion: In our study, we observed disparities in the prevalence of somatic mutations and associated survival outcomes among different races. TP53 mutations were observed more frequently in AAs, while PIK3CA and CDH1 mutations were observed more frequently in Whites, emphasizing the need to target these mutations in these races to address the disparity. Larger studies are necessary to identify biological and non-biological risk factors for acquiring these somatic mutations and their impact on clinical outcomes.

P5-02-04: Factors Predicting Transition to Independent Grants among NIH K awardees in Breast Oncology

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Introduction: The transition from NIH mentored career development (K) awards to independent funding (R01/R01 equivalent grants) is a crucial milestone in the career of biomedical scientists. There is evidence of gender disparity among K-awardees and K-to-R

transition rates, with female scientists less likely to receive K awards and convert them to R grants. Multiple factors such as family commitment and resulting career interruptions, limited institutional support, and societal factors represent barriers in academic success for women while cultural and language barriers, or reduced peer support present unique challenges for foreign graduates in career advancement. We evaluated the factors predicting success in obtaining independent grants in breast oncology and the K-award distribution and K-to-R transition by gender and education background between 1997 and 2021.

Methods: We queried the NIH rePORTER database to identify K awardees (K01, K07, K08, K22, K23, K99) in breast oncology from 1997 to 2021. Data on Principal Investigator (PI) name, year beginning the K award, organization and subsequent R01 or equivalent grants, if obtained, were abstracted from the database. PI gender was inferred by using their first name in genderize.io and confirmed with their LinkedIn or institutional profiles. Terminal degree and education details were collected from institutional, or LinkedIn profile and foreign graduates (medical or gradual school and/or doctoral programs outside US) were identified. Median time to first independent grant was calculated. Univariate and multivariate logistic regressions models identified predictive factors for attaining independent grants.

Results: We identified a total of 497 K awardees (PI) in breast oncology. Among them, 306 (61.6%) were females and 191 (38.4%) were males. Majority had a PhD degree (65%), followed by an MD (24%) and an MD/PhD degree (11%). A total of 35% of the PIs came from institutes ranked in the top 20 for NIH funding. About 25% of the PIs were foreign graduates, with fewer female foreign graduates compared to males (19% vs. 34%, respectively, P=0.0002). Overall, male PIs were more likely to receive subsequent R01 or R01 equivalent grants compared to females (62.6% vs. 54.6%, P=0.09) and more likely to receive a single PI grant for their first independent grant (86% vs. 80%, respectively, P=0.27), though not statistically significant. The median time from the K award starting date to the first independent grant was 4.6 years among male PIs compared with 5.4 years among females (P= 0.04). There was no significant difference in median K-to-R time between foreign and US graduates (4.8 vs. 5.02 years, P=0.65); even when stratified by gender. The distribution of female and male K awardees (60-64% female and 36-40% male, P= 0.97) and R01 or equivalent grant recipients (56-58% female and 42-44% male, P= 0.46) has remained stable from 1996-2020. The multivariate analysis did not identify a definitive association with K grant type, K award start time, NIH funding tier, or having a PhD degree in obtaining an R01 level grant in this cohort. Additionally, neither female gender (OR 0.67, CI 0.43-1.03, P= 0.07) nor foreign graduate status (OR 1.35, CI 0.82-2.21, P=0.24) significantly predicted the attainment of subsequent independent grant.

Conclusion: Female K-awardees in breast oncology took a longer time to obtain their first independent R01-level grant than males. Only 25% of the K awardees were foreign graduates, with fewer female foreign graduates compared to males. Neither gender nor foreign education status of K awardees was predictive of subsequent independent grant achievement. This study underscores the need to further study the underrepresentation of

female foreign graduates among K awardees and the need for greater support and mentorship for women in academia to reduce the K-to-R transition time.

P5-02-05: OncotypeDx Molecular Assay Association with Breast Cancer Outcomes in Different Racial Groups

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Background: The OncotypeDx (ODX) score is widely used to guide adjuvant treatment decisions in hormone receptor-positive (HR+) breast cancer. However, racial disparities in breast cancer outcomes raise questions about the prognostic utility of ODX across different populations. This study investigates the differences in ODX scores and associated outcomes among women of diverse racial backgrounds treated at University Hospitals Seidman Cancer Center in Cleveland, OH.
Methods: We conducted a retrospective analysis of 1,122 women diagnosed with early-stage breast cancer who had clinical ODX testing performed from 2013 to 2023. Patients were categorized by self-reported race: African American (AA), White, Asian, and other racial groups. ODX scores were classified into low (0-18), intermediate (19-30), and high (31-100) risk categories. Clinical outcomes, including overall survival (OS) and recurrence-free survival (RFS), were analyzed using Kaplan-Meier survival analysis and Cox proportional hazards models.
Results: The cohort included 1,122 women: 142 (12.7%) AA, 967 (86.2%) White, 10 (0.9%) Asian and 3 (0.3%) patients from other racial groups. Median follow up time was 5.5 years. AA women had higher mean ODX scores compared to White women (18.6 vs. 17.9). AA women had a higher proportion of intermediate-risk ODX scores (34.5% vs. 31.3%) and high-risk ODX scores compared to White women (12.7% vs. 9.0%). The 5-year OS rates were similar between AA and White women (94.0% vs. 93.2%), but the 10-year OS rates showed a slight advantage for AA women (78.2% vs. 74.8%). Similarly, the 5-year RFS rates were also comparable (90.7% for AA vs. 90.4% for White), with a 10-year RFS of 76.8% for AA and 74.6% for White. Multivariable analysis revealed no significant differences in OS (HR 1.12, 95% CI 0.57-2.21, p=0.735) or RFS (HR 1.25, 95% CI 0.72-2.19, p=0.426) between AA and White women after adjusting for ODX risk groups and other clinical factors, though on interaction testing there was a trend towards worse RFS for AA women than White women with intermediate-risk scores. Higher risk level (intermediate and high vs. low, larger T stage, nodal involvement, and older age were all significantly associated with RFS and OS, with diabetes also associated with worse OS but not RFS in this cohort ($p<0.01$). Race was not associated with differences in RFS or OS at any time point. 5-year RFS was similar for low-risk AA and White patients (97.4% vs. 92.1%) and high-risk AA vs. White patients (92.9 vs. 86.9%). There was, however, a trend toward worse RFS for women with intermediate-risk scores between AA and White patients (88.1 vs 80.5%) interaction models indicating that AA women with intermediate-risk scores had a higher hazard ratio

for RFS compared to White women (HR 7.32, 95% CI 2.58-65.43, p=0.07).
Conclusions: Our study found that AA women with early-stage breast cancer had higher proportions of intermediate- and high-risk ODX scores compared to White women. Despite this, the long-term RFS and OS outcomes were generally similar between the two groups, with some suggestion of worse outcomes for AA with intermediate-risk scores. ODX remained prognostic for RFS across all racial groups. These findings suggest that while ODX scores indicate higher risk, they do not fully account for the observed survival outcomes across different racial groups. Further research is needed to understand the underlying factors contributing to these disparities and to optimize the use of genomic tests like ODX in diverse populations.

P5-02-07: Racial Disparities in Breast Cancer Outcomes Based on MammaPrint Scores

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Background: MammaPrint (MP), a 70-gene expression profile, is used to guide treatment decisions in early-stage breast cancer by classifying patients into high or low risk of recurrence categories. However, racial disparities in breast cancer outcomes necessitate an evaluation of MP's prognostic utility across diverse populations. This study explores differences in MP scores and associated outcomes among women of various racial backgrounds treated at University Hospitals Seidman Cancer Center in Cleveland, OH.
Methods: This retrospective cohort study included women diagnosed with early-stage breast cancer from 2013 to 2023 who underwent MP testing at University Hospitals Seidman Cancer Center. Patients were stratified by self-reported race: African American (AA), White, Asian, and other racial groups. MP scores were categorized into high- and low-risk. Clinical outcomes, including recurrence rates and overall survival (OS), were analyzed, and compared across racial groups using Kaplan-Meier survival analysis and Cox proportional hazards models.
Results: The cohort comprised 1349 women with MP testing performed: 212 (15.7%) AA, 1125 (83.4%) White, 8 (0.6%) Asian and 4 (0.3%) patients from other racial groups. In the overall cohort, 873 patients (64.7%) had low-risk MP and 476 (35.3%) had high-risk scores. AA women had a significantly higher proportion of high-risk MP scores compared to White women (49.1% vs. 32.7%). Although the 5-year RFS rates were comparable between AA and White women (76.5% vs. 77.2%), the 5-year OS rates were slightly lower for AA women compared to White women (77.8% vs. 78.2%). MP remained prognostic for RFS at 3-, 5-, and 10 years regardless of race. Multivariable analysis revealed no significant differences in OS (HR 0.94, 95% CI 0.47-1.89, p=0.866) or RFS (HR 0.83, 95% CI 0.43-1.59, p=0.572) between AA and White women after adjusting for MP risk groups and other clinical factors. High-risk MP scores were associated with worse OS (HR 3.06, 95% CI 1.64-

5.70, p<0.001) and RFS (HR 2.68, 95% CI 1.55-4.62, p<0.001) compared to low-risk scores. Other factors associated with worse RFS and OS on MVA were larger tumor size, nodal involvement, and age. Interaction models indicated no difference is OS or RFS in AA women and White women with either low-risk or high-risk MP, respectively.
Conclusions: AA women with early-stage breast cancer were more likely to have high-risk MP scores compared to White women. Despite the higher risk classification, the long-term RFS and OS outcomes were similar between AA and White women. MP remained prognostic for RFS at 3-, 5-, and 10 years regardless of race. These findings suggest that MP scores alone do not fully capture the survival differences across racial groups. Further research is needed to understand the underlying factors contributing to these disparities and to refine the use of genomic tests like MP in diverse populations.

P5-02-08: Assessing Medical Mistrust among Black Adults with and without a Family History of Breast Cancer

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Background: Medical mistrust is a significant barrier to healthcare utilization and outcomes, particularly in Black communities where historical and systemic inequities persist. Understanding factors influencing medical mistrust is crucial for developing strategies to improve healthcare engagement. This study aims to assess differences in medical mistrust among Black individuals with and without a family history of breast cancer, hypothesizing that a familial history might influence mistrust levels.
Methods: Using community-based recruitment methods in partnership with national Black-led organizations, we conducted an online survey on family history of cancer in Black communities. Individuals could participate if they self-identified as Black or African-American, were 18 or older, resided in the US, and could complete a survey in English. In the survey, participants were asked if members of their immediate families had ever been diagnosed with cancer, and if so, which type of cancers. Participants were then dichotomized into having a family history of breast cancer or not. Medical mistrust was measured using a 5-item validated patient trust in the medical profession scale. Participants were asked to rate their level of agreement with the following statements: Sometimes doctors care more about what is convenient for them than about their patients' medical needs; Doctors are extremely thorough and careful; You completely trust doctors' decisions about which medical treatments are best; A doctor would never mislead you about anything; All in all, you trust doctors completely. Scores on the scale could range from 5 to 25 with higher scores indicating more medical mistrust. The primary outcome was the median mistrust score, and differences between groups were assessed using Wilcoxon rank sum tests.
Results: A total of 95 participants were included, with 39% (n=35) reporting a family history of breast cancer and 61% (n=60) without. The overall population exhibited high mistrust, with a median score of 18. The median mistrust score for those with a family history of breast cancer was 18 (IQR: 16-21), compared to 18 (IQR: 16.5-21) for those

without. The Wilcoxon rank sum test showed no statistically significant difference in median mistrust scores between the groups (p = 0.87).
Conclusion: In this community-based survey of Black adults, medical mistrust was uniformly high, regardless of a family history of breast cancer. These findings underscore the need for interventions that promote trustworthiness in medical institutions to mitigate high levels of mistrust in Black communities.

P5-02-09: Evaluating the Clinical Utility of Bioimpedance Spectroscopy for Early Detect of Breast Cancer-Related Lymphedema in Ethnic Minority Populations

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Background: Advances in breast cancer treatments have significantly improved long-term survival rates. With increased survivorship, addressing the long-term outcomes of breast cancer treatments is crucial. Breast cancer-related lymphedema (BCRL) is a chronic complication that significantly impacts the quality of life of affected patients. Historically, BCRL occurs in 3-8% of patients undergoing sentinel lymph node biopsy (SLNB) and 13-60% of those undergoing axillary lymph node dissection (ALND). These rates are two-fold higher in low-income and ethnic minority populations, with Black women showing a greater susceptibility to BCRL. Early detection is crucial, and SOZO®, a device utilizing bioimpedance spectroscopy (BIS), has proven to be an accurate tool in detecting lymphedema. However, there is a paucity of literature examining the utility and applicability of BIS in diverse ethnic populations. Therefore, the aim of our study was to evaluate the clinical utility and applicability of SOZO® in diagnosing BCRL within ethnic minority groups.
Methods: A retrospective chart review of patients who underwent axillary surgery between January 2019 to December 2023 was conducted. Demographic and clinical data were collected for all patients. Patients undergoing axillary surgery were assessed for their preoperative L-Dex score and then followed up postoperatively with measurements at 3-month intervals. Clinical lymphedema was defined as having an absolute L-Dex score of ≥ 10 and an increase of 6.5 or more from the baseline. Descriptive and Chi-square analyses were performed on the data.
Results: Of the 427 patients, 80% were from ethnic minority backgrounds (Black race and Hispanic ethnicity). SLNB was performed in 83.6% of cases, ALND in 16.4%, and 67.2% underwent radiation therapy. In total, 40 patients developed BCRL, with 33 cases being transient lymphedema and 7 persistent. No significant difference in BCRL incidence was observed across different racial groups. The overall lymphedema rate was 1.64%. Compared to a historical BCRL incidence of 40.4% following ALND in ethnic minorities, our study reported a significantly lower rate of 12% ($p < 0.001$).
Conclusion: SOZO® can accurately detect BCRL, aiding in its early detection and management. Despite historical data indicating higher BCRL rates in low-income and

minority groups, our study found significantly lower rates among our Black race cohort compared to historical controls. Of the 40 patients who developed BCRL in our study, 33 experienced transient lymphedema, while 7 had persistent lymphedema. Early recognition through SOZO® led to the resolution of lymphedema in 33 patients, preventing progression and reduced quality of life. However, future studies with larger sample sizes across multiple institutions are needed to bolster our findings.

P5-02-10: Factors and Trends in Place of Death Among Breast Cancer Patients: A Population-Based Study in Brazil

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Background: The quality of death serves as an indicator of the quality of care, shaped by factors such as the place of death, access to palliative care, and symptom management. In the context of Brazil, a diverse landscape with regional social disparities can lead to unequal access to healthcare, particularly in breast cancer (BC), which is the leading cause of cancer-related deaths among Brazilian women. Unlike in developed countries, dying at home in Brazil may suggest inadequate end-of-life care. Understanding the patterns of place of death and their association with social inequalities is crucial for developing effective end-of-life care policies for women with breast cancer in Brazil.
Methods: This cross-sectional study in Brazil used data from the Mortality Information System (2001-2021) to analyze breast cancer deaths (ICD-10 C50) based on place and year of death. Deaths were categorized as hospital or home deaths, and evaluated variables included age, race, education, marital status, and regional Human Development Index (HDI). Trends in death rates were assessed by using Joinpoint Regression, and the relationship between home deaths and regional HDI was assessed by Pearson correlation. Univariate logistic regressions calculated odds ratios to determine factors influencing home deaths, with statistical significance set at $p<0.05$.
Results: Between 2001 and 2022, there were 303,279 BC deaths in Brazil, with 299,775 cases included in the study after excluding men (3,329) and patients with missing data on sex (17) or place of death (158). Of these, 253,581 (84.6%) occurred in hospitals, while 46,194 (15.4%) occurred at home. Home deaths were more frequent in the Northeastern (15,438, 25.1%) and Northern (2,255, 20.4%) compared to the Southern (8,367, 15.6%), Central-Western (2,647, 14.4%), and Southeastern (17,487, 11.3%) regions. Patients from the lower HDI regions of the North, Northeast, and Central-West had higher rates of home deaths (OR = 2.04, 95% CI = 2.00-2.08) compared to those in the higher HDI Southeastern and Southern regions, showing a strong inverse correlation between home death proportion and regional HDI (Pearson's Correlation Coefficient = −0.932, p = 0.021). Temporal trend analysis indicated a significant continuous reduction in home deaths from 2001 to 2017, decreasing annually by 2.6% from 19.1% to 12.5% (APC = −2.6%, 95% CI = −3.0 to −2.3, $p < 0.001$). This was followed by an increase to 17.7% in 2020 (APC = 12.4%,

95% CI = 4.3 to 21.2, p = 0.005) and a return to a non-significant decrease to 16.2% by 2022 (APC = −4.3%, 95% CI = -10.4 to 2.2, p = 0.172). Higher rates of BC home deaths were associated with older age (≥ 60) (OR = 1.79, 95% CI = 1.75–1.83), lack of formal education (OR = 3.02, 95% CI = 2.92-3.13), absence of a partner (OR = 1.28, 95% CI = 1.25-1.30), and self-reported ethnicity, with black and mixed-race (OR = 1.14, 95% CI = 1.11-1.16) and indigenous women (OR = 1.92, 95% CI = 1.45-2.56) having higher home death rates compared to white women.
Conclusions: The study underscores a high rate of home deaths among BC patients in Brazil, highlighting the absence of national end-of-life care programs and palliative care structures, especially in underdeveloped regions. Home death rates were inversely correlated with regional HDI. Temporal trends showed a continuous reduction in home deaths, with a temporary increase likely linked to the COVID-19 pandemic. Factors such as ethnicity, educational level, and marital status also influenced the place of death. These findings underscore the need for policies addressing the impact of social factors like race, education, and HDI on the location of end-of-life care for BC patients in Brazil.
Keywords: Breast cancer; endo-of-life, social disparities, place of death.

P5-02-11: Health-care disparities between ethnically and religiously diverse population with advanced breast cancer

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Background: In Israel all residents are covered by universal health care and yet disparities in health care access and utilization exist. Jerusalem is the largest and poorest city in Israel, with significant Jewish ultra-orthodox and Arab communities. These two groups have shared reproductive factors, similar patriarchal community structures and often live in poverty. Under-utilization of health care services exist and yet no research has been performed on these populations in the advanced breast cancer (ABC) setting.Methods: Women with ABC were prospectively recruited after consenting and self-identified their religion & level of religiosity. Clinical and demographic data were collected from the electronic medical record. Statistical comparisons were performed of demographic, clinical data and patient outcomes. Comparison of categorial variables was conducted by means of Chi Square calculations. Survival analyses were conducted by Kaplan-Meier analysis and Cox regression analysis. Focus groups and cross-sectional surveys of health care professionals (HCPs) were performed to assess perceived barriers to care. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) (version 28.0).
Results: The study included 179 patients, of whom 139 (77.7%) identified as Jewish Israeli and 39 (21.8%) as Arab. Amongst the Jewish patients, 31.4% identified as ultra-orthodox and 88% of the Arab patients identified as Muslim. 57% of patients were under the age of 50 with no difference between ethno-religious groups. Ultra-orthodox patients had a median of 6 children (range 0-16), the Muslim 4.5 (range 0-8) and the Jewish-other 3 (range 0-10), p<0.001. Menopausal status differed between groups - 55.5% of Jewish-other

patients were post-menopausal, as compared to 20.9% of ultra-orthodox and 39.4% of Muslim patients, p=0.032. At diagnosis, 75% of ultra-orthodox patients and 63.6% of Muslim patients had stage IV disease as compared to 34.3% of secular, 26.9% of traditional and 59.4% of orthodox Jewish patients, p<0.001. 87.9% of Muslim patients had HR+/HER2-neg subtype compared to 68.2% of the ultra-orthodox, 67.7% of orthodox, 68% of traditional and 57.1% of secular patients, p=0.006. Once diagnosed with metastatic disease, there were no significant differences in standard of care management as measured by performance of biopsy at recurrence, administration of standard of care therapies and performance of genetic and genomic testing when indicated.
Overall survival (from initial diagnosis until death) adjusting for age & stage at diagnosis & subtype was significantly worse for the Muslim patients, followed by the ultra-orthodox as compared to the Jewish-other population (p<0.001). Hazard ratio (HR) for death for an ultra-orthodox patient compared to Jewish-other was HR 3.61 [95% CI 1-13] and for a Muslim patient compared to Jewish-other was HR 4.64 [95% CI 1.2-17.]
Key themes amongst the HCPs with regards to both minority groups was the perception that both receive sub-optimal care and that many barriers exist including poor communication, poor understanding of culture and norms of these groups as well as a sense of judgment towards these groups. This led to strong feelings of helplessness and frustration amongst the HCPs. The findings were similarly expressed towards both minority groups. However, regarding the Arab patients the main barrier identified was language.
Conclusions: Significant health care disparities exist amongst women with ABC in terms of disease characteristics and patient outcome, with less favorable characteristics and outcomes amongst Muslim Arab and Ultra-orthodox Jewish patients compared to the general Jewish population in Jerusalem. Further research is needed to understand the cause of these disparities and to create effective interventions to bridge these gaps.

P5-02-12: Increasing clinical trial enrollment rates of black women with breast cancer through Patient Navigation and Community Education programs

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Background: While Black women represent 12% of breast cancer cases in the US, they comprise only 3% of participants in clinical trials. At the Vanderbilt Ingram Cancer Center (VICC), Black patients represent 9% of all breast cancer patients, but only 4% of those are enrolled in clinical trials.
Objective: This pilot study evaluated the role of Patient Navigators (PNs) to increase knowledge of clinical trials among Black women with breast cancer at the Breast Cancer Clinic at VICC. Concurrently, we provided community-based education focused on breast cancer and clinical trials.
Methods: All Black women in the Breast Clinic at VICC were offered PN services. Those who agreed were asked to complete a needs assessment and provided with PN resources as

needed throughout the duration of their treatment. As part of the PN program, patients were provided with educational material about clinical trials as well as assistance with identified needs. Patients were surveyed one-month following the start of the navigation experience and at the end of their navigation experience. For the community education component, a trained educator presented information on breast cancer and the importance of clinical trials at established community events and participants were asked to complete a post-education survey.
Results: From February through May 2024, 29 of 33 (88%) of eligible patients joined the PN program. Of 14 patients who completed the first post-navigation survey, all found meetings with PNs to be valuable. Additionally, 11 (79%) reported that they knew very little to nothing about clinical trials prior to PN intervention with 8 of 11 (73%) reported having increased knowledge following PN. Of 12 respondents, 6 (50%) reported that they would be more likely to enroll on a clinical trial as a result of PN, and 9/14 (64%) indicated that they would share information about clinical trials with others. We have held 4 community-based education activities since February 2024, with an average of about 21 attendees per event. Of 56 patients who completed the post-event survey, 54 (96%) indicated they were satisfied with the information presented, 45 (80%) stated that they are willing to advance new medicine and treatments by participating in clinical trials, and 53 (95%) agreed that mistrust, being uninformed, and being uninsured are reasons racial and ethnic groups do not participate in research.
Conclusions: Implementation of patient navigation services for Black patients with breast cancer coupled with community-based education is an effective approach to increase knowledge of and identifying barriers to enrollment on clinical trials. As accrual continues in our pilot study, we will examine the impact of patient navigation on the patient care experience and enrollment in clinical trials and the knowledge about clinical trials gained from community-based education.

P5-02-14: The Value of RNASeq in Addressing Breast Cancer Disparities Among Minority Populations: A Focus on African American Women

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Background: Breast cancer is a heterogenous disease with diverse behavior, the basis of which is unclear. Breast cancer (BC) is a significant health concern for women globally, but it manifests with notable disparities across different ethnic groups. African American (AA) women experience a paradox in breast cancer—they have a lower incidence rate of breast cancer compared to their white counterparts, yet they suffer from a higher mortality rate. This disparity spotlights a gap in understanding the underlying biological factors contributing to these outcomes. Genetic factors play a crucial role in breast cancer risk and prognosis. In complement to DNA, RNA sequencing (RNASeq) is able to demystify these pathways. Unlike DNA sequencing, which provides static information about genetic mutations and variants, RNASeq offers dynamic insights into gene expression levels and patterns, revealing how genes are regulated and expressed in different tissues and

conditions. There is a significant lack of research exploring gene expression and patterns prevalent in African American women and other minority groups. Methods: Differential expression was run on a pilot study of 10 subjects via RNAseq data. An AA BC group (n=5) and a White (W) BC group (n=5). When comparing W tumor to W normal, 2921 genes were significantly different; comparing AA tumor to AA normal, 4913 genes were significantly different. These lists were filtered to only contain log2fold changes of the absolute value of 3 or greater, which filtered W to 108 genes and AA to 149 genes. These lists were uploaded to NIH's DAVID Bioinformatics, which provides functional annotation clustering. Results: Using NIH's DAVID Bioinformatics, a general overview of the two racial groups was assessed. The W BC subgroup was characterized to have significant enrichment in oxidoreductase pathways. Genes highlighted are STEAP4, ACADL, ADH1A, ADH1C, AOX1, AKR1C1, CDO1, CYP3A5, MAOA, RRM2. This cluster is statistically significant demonstrated by a P-value of 2.6E-5 and a Benjamini value of 7.6E-3. The AA BC subgroup was characterized to have significant enrichment in genes involved with mitosis and cell proliferation. Genes identified under this subgroup are NEK2, NUF2, TPX2, ASPM, AURKA, AURKB, CDC20, CDCA8, CENPA, CENPF, and KLHL13. This cluster is statistically significant demonstrated by a P-value of 3.8E-7 and a Benjamini value of 2.1E-5. Outside of DAVID, significant genes were assessed for each racial group. The white group had significant mutations in EGFR, ESR1, FGF10/12/13, and IDH1. While the African American group had significant mutations in CDH1, CDK4, FGF7, FGF9, FGFR4, HRAS, KDM6A, MSH3, MSH6, and PTEN. Both groups were noted to have unique fusion patterns. Conclusion: Each subgroup exhibited unique key genes that were significantly upregulated or downregulated. While mutations in genes such as EGFR and ESR1 in the W group are extensively studied, several genes identified in the AA group, except for CDK4, require further research to elucidate their roles in breast cancer. According to the DAVID analysis, the AA BC group exhibited significant enrichment clusters under mitosis. CDK4/6 inhibitors, a popular BC treatment that targets cell proliferation, might be beneficial for AA women. However, since African American women also have a higher incidence of triple-negative BC, where CDK4/6i (HR+/HER2− monotherapy) is not indicated, the BC treatment guidelines for AA women can be unclear. Understanding unique gene signatures in African American women with breast cancer is essential. Incorporating novel technologies like RNASeq into treatment plans can help address disparities between populations. This approach can lead to more comprehensive BC prevention, diagnosis, and treatment strategies. Ultimately, this will not only improve outcomes for African American women but also enhance our overall understanding of breast cancer across diverse populations.

P5-02-15: Impact of socioeconomic factors on stage of diagnosis and mortality among breast cancer patients

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Background: Breast cancer (BC) is the most common cancer and the second leading cause of cancer deaths among US women. From 1989 to 2017, mortality rates dropped by 40%, largely attributed to the widespread adoption of mammogram screening and advancements in treatment. However, disparities persist with Black women experiencing 40% higher mortality than White women. Delays in diagnosis due to limited access to screening and treatment contribute to this disparity. Social determinants of health further impact the accessibility and quality of healthcare received. This study examines socioeconomic influences on BC stage at diagnosis and mortality.

Methods: We conducted a retrospective analysis of data from the National Cancer Database (NCDB), a joint project of the Commission on Cancer of the American College of Surgeons and the American Cancer Society. All female patients (pts) ≥18 years with a diagnosis of stage 0-IV breast cancer (BC) treated from 2004 to 2020 were included. Race/ethnicity included White (W), Black (B), Hispanic (H), and Other (O). Income and education were categorized into quartile (Q) I, II, III, and IV from lowest to highest per NCDB definitions. Kaplan Meier analysis with log rank test was done to compare the overall survival from diagnosis and Cox proportional analysis was done to estimate hazard ratio.

Results: A total of 1,826,818 pts were included in the analysis. Of these, 79.5% were White (W), 11% Black (B), 4.9% Hispanic (H), and 4.6% Other (O) that includes (Asian, American Indian, and other). Overall, most pts were in income and education Quartile 4 (38.1% and 42.4%). W pts had a higher proportion of stage I disease at diagnosis (47%) when compared to B (35.6 %) and H (38.2%) pts ($p<0.001$). In contrast, W had a lower proportion of stage III (7.9%) when compared to B (11.2%) and H (10.7%) ($p<0.001$). B pts had a higher proportion of stage IV (5.2%) compared to W (3%) and H (3.4%) pts ($p<0.001$). Income Q4 had a higher proportion of stage I (46.6%) compared to Q1 (41.2%). Income Q4 had a lower proportion of stage III (7.3%) and IV (2.7%) compared to income Q1 (10.3% and 4.4% respectively). Similarly, education Q4 had a higher proportion of stage 1 (47.1%) compared to Q1 (40.3%). Education Q4 had a lower proportion of stage III (7.3%) and IV (2.7%) compared to Q1 (10.5% and 4.4% respectively). All these differences were statistically significant $p<0.001$. Income distributions among race/ethnicity showed that W had a lower proportion in Q1 (10.9%) compared to B (38.4%) and H (22.4%). In contrast, W had a higher proportion in Q4 (40.5%) compared to B (18.2% and H (26.9%). We found similar disparities when we evaluated education distributions among race/ethnicity. Kaplan Meier analysis showed that mortality was significantly higher for B, lowest income quartile, and lowest education quartile for all stages (log rank $P<0.001$). Cox proportional analysis showed that mortality was highest for B (HR: 1.12; 95% CI, 1.11-1.13, $P<0.001$), lowest income quartile (HR: 1.22; 95% CI, 1.21-1.24, $P<0.001$), and lowest education quartile (HR: 1.09; 95% CI, 1.08-1.10, $P<0.001$).

Conclusion: Results of this study show that race and ethnicity as well as pt income and education level were associated with stage of diagnosis and mortality among BC pts. Identifying barriers to accessing screening imaging and adequate BC treatment can help us develop systems that will give extra support to pts in need. Future studies should focus on developing interventions to decrease these disparities and improve outcomes.

Acknowledgement:

The data used in the study are derived from a de-identified NCDB file. The American College of Surgeons and the Commission on Cancer have not verified and are not responsible for the analytic or statistical methodology employed, or the conclusions drawn from these data by the investigator.

P5-02-16: Thymidine kinase activity as a prognostic biomarker for first line CDK4/6 inhibitor efficacy in the Personalised Disease Monitoring in Metastatic Breast Cancer study

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Background: There is an unmet need for biomarkers that improve prediction of treatment efficacy in metastatic breast cancer (MBC). Thymidine kinase (TK) is an enzyme with a critical role in DNA synthesis downstream of the CDK4/6 pathway. We assessed serum TK activity (TKa) as a prognostic biomarker in the Personalised Disease Monitoring in Metastatic Breast Cancer (PDM-MBC) study.
Method: The PDM-MBC study (NCT04597580) is a biomarker development study that enrolled 97 patients with estrogen receptor-positive, HER2-negative MBC treated with an aromatase inhibitor (AI) plus a CDK4/6 inhibitor as 1st line endocrine therapy. Follow-up is ongoing until May 2026. Computed tomography (CT) assessed by RECIST 1.1 (and magnetic resonance imaging (MRI) in case of bone-predominant disease) was performed every 3 months year 1 and every 3-4 months thereafter or earlier if clinically indicated. Serum samples were collected at baseline (BL), day 15 of cycle 1 (C1D15), day 1 of cycle 2 (C2D1), thereafter at each imaging timepoint and subsequent doctor's visit until disease progression. TKa was analyzed with the FDA cleared DiviTumÒ TKa assay (Biovica). The association between TKa levels and early TKa dynamic patterns and progression-free survival (PFS) and overall survival (OS) was evaluated using the Kaplan–Meier method, logrank test and univariable Cox regression analysis.
Results: In total, 86 patients had TKa results available at BL and 77 had results for all three timepoints. The median PFS and OS were 20.8 and 51.8 months, respectively. High TKa (above the FDA stipulated cut-off 250 DuA) at BL was associated with shorter PFS (median 13.5 months vs not reached [NR], p=0.002), shorter OS (median 30.3 months vs 51.8, p=0.017), higher risk of progression (hazard ratio [HR] 2.46, 95% confidence interval [CI] 1.37 to 4.44, p=0.003) and higher risk of death (HR 2.53, 95% CI 1.15 to 5.58, p=0.022). Using ≥100 DuA to define high TKa, four patterns of early TKa dynamics were observed during the first treatment cycle (BL, C1D15 and C2D1) based on BL TKa and the level of subsequent suppression. PFS (p=0.003) and OS (p=0.03) differed significantly by logrank test between the four groups:
Low-Low-Low (n=14) median PFS and OS not reached;
High-Low-Low (n=26) PFS 23.8m (95%CI can't be calculated) and OS 51.8m (95%CI 15.4 to 88.1);

High-Low-High (n=24) PFS 17.5m (95%CI 10.2 to 24.8) and OS 34.3m (95%CI15.1 to 53,7)
High-High-High PFS 10.3m (95%CI 1.73 to 18.9) and OS 30.3m (95%CI 15.1 to 45.6)
In exploratory analyses no significant differences between median TKa at C1D15 or C2D1, or early TKa dynamic patterns were observed between patients treated with palbociclib (n=48) vs ribociclib (n=29), p=0.33 to 1.0.
Conclusions: In the PDM-MBC study, we confirm that TKa is a valuable prognostic biomarker in patients treated with AI and CDK4/6 inhibitor as first-line therapy for MBC. TKa dynamics during the first cycle identifies patients at risk of early progression and those with more prolonged response. TKa analysis at later time points is ongoing. TKa is an encouraging biomarker for personalized disease monitoring in MBC.

P5-02-17: Concordance Analysis of Non-Invasive determination techniques of PIK3CA and ESR1 mutations in patients with advanced luminal breast cancer. Study CANIPE

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Approximatly 70% of breast cancer (BC) patients are Hormone Receptors (HR) positive, the most common subtype with the best prognosis. The combination of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) with Endocrine Therapy (ET) is tje standard firs-line therapy for HR+/HER2- metastatic BC. However, 20% of patients are intrinsically resistant, and those who initially respond often develop acquired resistance, making the management of resistant HR+/HER2- metastatic BC a significant clinical challenge. Clinical guidelines emphasize the need of comprehensive real-time monitoring of the dynamic mutational landscape during and after treatment to improve resistance prediction. Early detection of mutations in ESR1 and the PIK3 pathway usinf circulating tumor DNA (ctDNA) may allow the ending of ineffective endocrine therapies and initiation of alternative treatments for these patients, without performing tissue biopsies before radiological progression occurs. To achieve this, it is crucial to implement non-onvasive genotyping that allows better-adapted pharmacologiical interventions. However, clinical trials have been predominantly carried out with selected populations and single drugs (Palbociclib, Ribociclib or Abemaciclib). There is a lack of studies of optimal agreemnt between diagnostic methods, especially in liquid biopsy. There is no relieble data abaout PIK3CA or ESR1 status in ctDNA from real-world cohorts that could help to define the best testing strategy for the clinica routine.
Therefore, it is imperative to establish evidence that the use of ddPCR is equally or more sensitive for ctDNA analysis than qPCR or NGS, the current gold standard methods.

Aim: This study aims to perform a concordance analysis between NGS and ddPCR technologies for detecting mutations in PIK3CA and ESR1 by testing the ctDNA from HR+/HER2- metastatic breast cancer patients.

M&M: CANIPE is a randomised, open-label study performed al 14 Spanish hospitals in 6 autonomous communities. Patients aged 18 years or older, with confirmed HR+/HER2- breast cancer, stage IV, Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0 to 2, who iniciate first-line tratment with CDK4/6i plus aromatase inhibitors. All procedures followed the Helsinki Declaration guidelines and were approved by the Ethics Commitee of Santiago-Lugo under approval reference number 2022/386. All patients provide written informed consent. A total of 60 women were recruited at baseline (before therapy initiation) while 21 patients at 10 months after treatment. 40 mL of blood was obteined from each patient at both time points. For ddPCR, cfDNA was isolated from 5 mL of plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Venlo, The Netherlands). Multiplex ddPCR was performed with de Bio-Rad QX-200 system to identify PIK3CA mutations, including the most frequent variants (E542K, E545K, H1047L, H1047R, R88Q, N345K, C420R, E545A, E545G,Q546K), and ESR1 mutations (L536R, D538G, E380Q, Y537C, /537S, Y537N,S463P). A mutation was considered present if at least 6 mutated events were detected by ddPCR. Additionally, for NGS, cfDNA was isolated from 4 mL of plasma and analysed with the AVENIO ctDNA Expanded kit (Roche Diagnostics), which includes 77 genes, including PIK3CA and ESR1. An allele fraction od 0.5 % or higher was consideredindicative of a mutation.

Results: At baseline, and considering the pre-defined criteria, ctDNA analysis detected PIK3CA mutations in 32.25% and 44.44% of patients by AVENIO and ddPCR, respectively. ESR1 mutations were detected in 3.22% and 9.37% by AVENIO and ddPCR, respectively. For PIK3CA mutations, the Kappa value was 0.62 (p-value: 0.0004) and 0.47 for ESR1 mutations (p-value: 0.0039). However, when NGS data was included for mutations with allele fraction less than 0.5%, the Kappa value was 0.92 for PIK3CA and 1 for ESR1, indicating almost perfect agreement bvetween the two technologies (p-value < 0.0001).

Conclusion: The analysis of ctDNA bu Multiplex ddPCR of HR+/HER2- metastatic breas cancer patients represent a sensitive tool to identify PIK3CA and ESR1 mutations with a high concordance compared with the NGS, which is currently the reference technology.

P5-02-18: mRNA Expression of ER, PR, Her2 and Ki67 compared to immunohistochemistry in 374 breast cancer core needle biopsies to validate and benchmark routine diagnosis using the APIS Breast Cancer Subtyping Kit

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Determination of the Estrogen- as well as Progesteronreceptor (ER and PR), Her2 (ERBB2) and Ki67 are fundamental in the first line diagnostic of breast cancer diagnosed on core needle biopsy (CNB) with important implication on further individualized clinical management. With the intention to first benchmark routine diagnostic results and furthermore validate the APIS Breast Cancer Subtyping Kit in a large cohort of breast cancer cases (n=374) immunohistochemistry (IHC) as well as mRNA expression ER; PR, Her2 and Ki67 were compared. The APIS Breast Cancer Subtyping Kit included an additional set of proliferation-relevant genes that could be compared to Ki67.
Case characteristics were the diagnosis of invasive carcinoma on CNB with tumour Nottingham grade “1” (n=58/16.1%), “2” (226/62.8%), “3” (76/21.1%) and undetermined (14/na). The APIS Breast Cancer Subtyping Kit is a multiplex RT-PCR based test that has previously been developed by our groups and is described in detail with training and test results (Diagnostics 2024, 14, 2411). Here we report the prospective application of this test. Using IHC as a gold standard the overall performance for the different biomarkers is shown in the sequence of sensitivity, specificity and accuracy as well as the kappa value with 95% CI in brackets:
ER: 83.7% (79.1-87.3) / 100% (94.1-100) / 86.3% (82.4-89.6) / 0,626; PR: 89.9% (85.9-92.9) / 82.1% (72.6-88.9) / 88.2% (84.4-91.3) / 0.761; ERBB2: 80.0% (68.2-88.2) / 98.7% (86.7-99.5) / 86.8% (82.9-90.1) / 0.831; MKI67: 88.6% (82.8-92.5) / 70.3% (63.7-76.2) / 78.5% (73.9-82.6) / 0.575; Proliferation (V KI67 IHC): 86.7% (80.7-91.1) / 70.3% (63.7-76.2) / 77.7% (73.1-81.8) / 0.559
Discordance could be largely explained by tumour-heterogeneity and threshold values which were predetermined, for both methods, and not adopted within this study. However, re-evaluation with an alternate technique might well be suitable to redefine these threshold values which can be especially important in general determination of KI67 or other proliferation relevant genes/proteins and in addition to better define the so far not sufficiently described “Her2-low”-group. Since the ground truth is finally unknown there will also be further studies to investigate the functional and clinical relevance of these respective approaches using endpoints like outcome and chemo-endocrine responsiveness.
In conclusion this approach is suitable to determine ER, PR, Her2 and Ki67 in cases where 1) IHC is not available, 2) where IHC should be benchmarked as a quality control measure and 3) in critical cases where IHC-results are inconclusive.

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P5-02-19: Why Are We Failing to Cure So Many Cases of Lobular Breast Cancer?

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Invasive lobular cancer of the breast (ILC) is responsible for more than its share of treatment failures. Long-term survival of women with classic ILC has not improved significantly over the past half century, despite major improvements in breast cancer therapy and diagnosis. Foote and Stewart considered lobular carcinoma in situ (LCIS) to be the precursor of ILC, and that the "the mass eruption of tumor cells" occurred through "some lytic action of the tumor cells, naturally not to be detected by anatomic study." Ackerman and Del Regato accepted this proposed mechanism, concluding that ILC arises from the acinar epithelium of the breast lobule. Despite the absence of clear evidence, these speculations were accepted as established fact more than 70 years ago. An inconvenient observation, the lack of E-cadherin staining, was assumed to result from a "loss" of that protein during tumor development. Our research group, the Swedish Organized Service Screening Evaluation Group, has examined all histologically proven ILC cases from Dalana County Sweden diagnosed from 1996-2019 with follow-up to the end of 2021. Histopathologic study of large section (8x10 cm) pathology slides, imaging and molecular biomarkers of 329 consecutive diffuse form of ILC showed a macroscopic structure unlike breast cancers of epithelial origin, a 19-year survival (56 %), poorer than expected from the histochemical biomarkers, and a growth pattern closely resembling that of normal breast tissue, hindering mammographic detection. Our group considered that ILC may originate from mesenchymal hybrid cells through the process of mesenchymal-epithelial transition (MET). Our cell culture studies from typical ILC cases progressed through more than 10 cell cycles in one year's time and produced cells with the properties of mesenchymal hybrid cells. Histopathology-breast imaging correlation indicated two ILC subgroups with separate sites of origin. The classic, diffuse type of ILC appears to evolve from the extralobular mesenchyme of the breast. A second subgroup appears to evolve from and generally remain within the intralobular mesenchyme of the breast, appearing as multiple small colonies, each surrounding the acini and terminal ducts of the lobule, which invariably have normal, non-malignant epithelium. This intralobular subgroup has distinctly different imaging biomarkers, appearing as a distinct tumor mass easily detected at mammography. The 231 consecutive intralobular cases had 84% survival at 19-year follow-up. Differentiating the two is difficult based on the limited field of view offered by the conventional 1x3 inch glass slides. However, low-power histopathology of large sections correlates well with imaging findings and assists in differentiating these two subtypes. This translational research is consistent with new directions in precision medicine. Conclusions: The accepted terminology and assumptions of the nature of "ILC" must be reconsidered if we are to improve the poor survival rate of this misunderstood malignancy. Therapies, such as radiation and chemotherapy, are effective in treating epithelial breast cancers but are less effective in treating classic ILC, possibly due to a stem

cell origin. Likewise, the IHC biomarkers are less predictive of prognosis in classic ILC for the same reason. Further, surgical removal of this diffuse malignancy is often incomplete as the full disease extent is difficult to evaluate by any imaging technique. So long as this unusual breast malignancy is termed "classic diffusely infiltrating lobular carcinoma," implying that it has its origin in the acinar cells of the terminal ductal lobular units (TDLUs), we are unlikely to achieve any real progress in our efforts to control it. Appreciation of its mesenchymal stem cell origin offers a radically new approach to research and treatment.

P5-02-20: Clinicogenomic landscape and function of PIK3CA, AKT1, and PTEN mutations in breast cancer

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Background: The AKT inhibitor capivasertib was recently approved in the 2L+ setting for patients (pts) with hormone receptor-positive metastatic breast cancer with alterations in PIK3CA, AKT1, and/or PTEN. There is a need to further characterize the landscape of PIK3CA, AKT1, and PTEN mutations in breast cancer and their functions, to improve our understanding and therapeutic targeting of PI3K-driven breast cancer.

Methods: 51,767 pts with breast cancer who underwent tumor sequencing using FoundationOne®CDx or FoundationOne® were included in this analysis. We assessed the frequency, type, and pathogenicity of genomic alterations found in PIK3CA, PTEN, and AKT1 overall and stratified by receptor subtype, patient ancestry, and sample type. Co-occurrence with other pathogenic variants was explored using a Fisher's exact test with a FDR correction. We correlated PTEN mutations with functional data from deep mutational scanning.

Results: The most frequently altered PI3K-pathway gene was PIK3CA (37.4%, 19,384/51,767), followed by PTEN (13.5%) then AKT1 (5.4%), including both pathogenic and VUS alterations in all genes. Among 1,024 distinct PIK3CA alterations identified, 690 (67.4%) were missense mutations. The most common missense mutations were H1047R (35.6% of PIK3CA-altered cases), E545K (19.7%), and E542K (11.7%). Among 288 AKT1 alterations identified, 205 (71.2%) were missense mutations. The most common missense mutation was E17K (69.7% of AKT1-altered cases) followed by gene amplification (14.0%). The most common alteration among patients bearing PTEN alterations was gene deletion (37.3% of PTEN-altered cases). Among 1,723 distinct PTEN alterations identified, 721 (41.9%) were frameshift mutations, 494 (28.7%) were missense mutations, and 246 (14.3%) were splice site mutations. To determine function, PTEN mutations were correlated with existing PTEN deep mutational scanning datasets; these data will be presented.

PIK3CA pathogenic alterations were more prevalent in estrogen receptor (ER)+/HER2- and HER2+ cases (40.3% and 37.6% of cases) versus triple negative breast cancer (TNBC;

20.9%), consistent with previously described data. Among all pathogenic PIK3CA alterations, those outside the 542, 545, and 1047 codons were more common in TNBC (42%) compared to ER+/HER2- or HER2+ (33%, p=0.002 and 33%, p=0.002). AKT1 mutations were more prevalent in ER+/HER2- disease (6.0%) than HER2+ or TNBC (1.7%, p=4.7x10^-20 and 3.0%, p=3.5x10^-5). In contrast, PTEN alterations were more commonly found in TNBC (17.9%) than in ER+/HER2- and HER2+ cases (11.3%, p=1.8x10^-8 and 3.9%, p=2x10^-57). PIK3CA alterations were enriched in metastases compared to local tumors (39.6% vs 33.2%, p=6x10^-41) and were less prevalent in African American pts than other groups (27.1% v 38.6% in Europeans, p=9.6x10^-81), while PTEN and AKT alterations were similar across ancestries.

PIK3CA, AKT1, and PTEN pathogenic alterations are all mutually exclusive to each other. Statistically significant co-occurring mutations included PIK3CA with CDH1, MAP3K1, SOX2, TBX3, and PRKCI; PTEN with FAS, TP53, and RB1; and AKT1 with NF1.

Conclusions: Here we describe the clinicogenomic landscape of PIK3CA, AKT1, and PTEN alterations in a large breast cancer cohort. Our results corroborate previously reported findings that PIK3CA alterations are present in around 40% of ER+/HER2- breast cancer and concentrated in 3 hotspots. However, one-third of patients have variants beyond E542, E545, and H1047 with less certainty about targetability. The preponderance of rare PIK3CA mutations and co-occurrences between PIK3CA, AKT1, and PTEN with genes outside the PI3K pathway merits functional investigation.

P5-02-21: Tumorspheres derived from circulating cancer stem cells in breast cancer patients exhibit elevated levels of immune regulatory molecules

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Background: Circulating tumor cells, particularly a rare subpopulation of them known as circulating cancer stem cells (cCSCs), play a significant role in cancer recurrence and metastasis. While the exact mechanism of cCSCs evasion by the immune system remains unclear, recent studies suggest that increased expression of certain immune checkpoint molecules in cancer stem cells may contribute to immune evasion and promote cancer stem cells self-renewal. Our study was designed to assess the number of tumorspheres and the expression levels of PDL-1, PDL-2 and CTLA-4 on tumorspheres derived from cCSCs in breast cancer patients.
Methods: The study included 110 patients with breast cancer at various stages of disease. Determination of circulating cancer stem cells was performed using the sphere formation assay. Staining with anti-PDL-1, anti-PDL-2 and anti-CTLA-4 antibodies was used to examine the expression of PDL-1, PDL-2 and CTLA-4 on breast tumorspheres.
Results:
We have developed an innovative in vitro platform for detection of cCSCs from peripheral

blood of cancer patients. The number of tumorspheres increased significantly with tumor progression and aggressiveness of primary tumor. Patients with metastatic disease had statistically more tumorspheres as compared to patients without metastasis (30 vs 10/100µl blood, $p<0.05$). Patients with multiple metastasis had more tumorspheres compared to patients with single metastases (60 vs 30/100µl blood, $p<0.05$). The number of tumorspheres was positively correlated with triple-negative histopathology. We observed elevated expression of PDL-1, PDL-2 and CTLA-4 and their considerable heterogeneity in enriched tumorspheres.
Conclusion: The number of tumorspheres cultured from peripheral blood directly reflects the aggressiveness and proliferative capacity of the primary tumor. The presence of tumorspheres with enhanced expression of PDL-1, PDL-2 and CTLA-4 may indicate their immunoregulatory potential. A better understanding of the interaction between cCSCs and tumor immunology could help to identify strategies to target the small subpopulation that escapes conventional therapy.

P5-02-22: Urinalysis of breast cancer patients identifies disease-specific miRNA patterns.

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Background: One in eight women will suffer from breast cancer. Markers, ideally non-invasive, for early detection, diagnosis and progression monitoring are key in future therapy. Small RNA molecules, like miRNAs, are very stable in almost all body fluids and could serve as these sought-after markers,
Methods: Using an isolation of total miRNA from 4 milliliters of urine samples (slightly less than a teaspoon), 82 women (50 healthy individuals and 32 breast cancer patients) were studied using miRNA-sequencing to determine whether consistent patterns of miRNA regulation could be found despite this small amount.
Result: From a still small group of patients we generated the first data using miRNA chip analysis, but these are already very promising for the possibility of valid testing of the disease by urinalysis. The primary goal was to validate the patterns found in a larger group of patients. Therefore, we subsequently validated and evaluated the recently tested method of miRNA sequencing in our clinic on the larger (82) sample cohort and investigated to what extent the predictive power for breast cancer subtype patterns of miRNAs holds up or can even be improved in this larger cohort. The sequencing analysis reliably detected over 4000 individual miRNAs in each patient. Analysis revealed subcategory specific miRNA patterns that allowed us to stratify patients according to urinalysis.
Discussion: Our data indicate that urinalysis could be a valuable tool in the non-invasive detection and monitoring of breast cancer patients. Clearly this first patient cohort is still too small to make predictions for the disease as a whole but the distinct patterns of breast cancer subclasses with only very few overlapping miRNAs between them makes a prognostic value for these analyses highly likely.

Conclusion: We identified miRNA patterns in small urine samples from breast cancer patients by using miRNA sequencing with Random forest analysis allowing us to distinguish healthy individuals from cancer patients.

P5-02-23: Unveiling the Antitumor Mechanism of abemaciclib in Human Breast Cancer Through Circulating Tumor Chromatin Analysis

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Background: The study of circulating tumor DNA (ctDNA) and cell-free chromatin (cf-Chr) has become instrumental in advancing our understanding of cancer biology. This research delves into the antitumor mechanism of abemaciclib in ER-positive, HER2-negative metastatic breast cancer patients by examining cf-Chr fragmentation patterns.

Methods: In this investigation, 72 ER-positive, HER2-negative metastatic breast cancer patients, as defined by ASCO/CAP guidelines and treated with abemaciclib at Chiba University Hospital in Japan from 2020 to 2022, were enrolled. Pre- and post-treatment serum samples were analyzed, with nucleic acids extracted for whole-genome sequencing (WGS) and differential enrichment analysis. Given the observed correspondence of cf-Chr fragment patterns with open chromatin regions (OCRs), indicative of gene activation zones, differential enrichment analysis was specifically focused on cell type-specific regulatory regions—namely enhancers and promoters—in luminal breast cancer cells and T cells, to elucidate the transcriptionally active regions within the genome.

Results: Our investigations have revealed a significant decrease in cf-Chr concentrations, showing a 50% reduction following treatment with abemaciclib. The analysis of cf-Chr in breast cancer patients, both pre- and post-abemaciclib treatment, unveiled differential chromatin enrichment within regions tied to antitumor pathways that are modulated by the drug. Notably, the cf-Chr analysis preceding treatment disclosed disturbances in the Notch signaling pathway, thereby revealing an aspect of abemaciclib's antitumor mechanism that has not been previously established in human clinical specimens.

Conclusions: Our study suggests for the first time alterations in cf-Chr in human patient specimens that may reflect the antitumor activity of abemaciclib that had previously been confirmed only in vitro. Our study presents a pivotal step in understanding the impact of abemaciclib on the tumor chromatin landscape, providing a direct observation of the drug's mechanism of action in patients. These insights pave the way for future research into the mechanisms of existing therapies and their optimization for treating metastatic breast cancer.

P5-02-24: Targeting FOXK2 signaling in breast cancers with FOXK2 amplification/overexpression and PIK3CA oncogenic mutations: a promising therapeutic strategy?

Hong Zhang, Yang Yu, Weng-Ming Cao, Feng Cheng, Edaise da silva, Higinio Dopeso, Hui Chen, Xiaosong Wang, Chunchao Zhang

Oncogene activation through DNA amplification or overexpression plays a critical role in cancer initiation and progression. Chromosome 17 harbors numerous cancer-associated genetic anomalies, some of which are strongly linked to poor prognosis in breast cancer patients. The FOXK2 gene, located on 17q25, encodes a transcription factor with a forkhead DNA binding domain. Integrative analyses of publicly available genomic datasets reveal that FOXK2 is frequently amplified and overexpressed in breast cancer, correlating with poor overall survival. FOXK2 knockdown via shRNA significantly inhibited the proliferation, migration, and anchorage-independent growth of breast cancer cells, causing G0/G1 cell cycle arrest. The C-terminal nuclear localization signal (NLS) is crucial for FOXK2's oncological function. Additionally, FOXK2 knockdown in MCF7 breast cancer cells delayed tumor growth in a xenograft mouse model. Inhibiting FOXK2 expression also sensitized breast cancer cells to frontline chemotherapy agents, including doxorubicin, 5-fluorouracil, and etoposide in vitro. Co-overexpression of FOXK2 and PI3KCA with oncogenic mutations (E545K or H1047R) induced the transformation of non-tumorigenic MCF-10A cells, suggesting that FOXK2 acts as an oncogene in breast cancer and contributes to PI3KCA-driven tumorigenesis. CCNE2, PDK1, and ESR1 were identified as direct transcriptional targets of FOXK2 in MCF-7 cells. Moreover, co-inhibition of FOXK2 function, either through knockdown or inhibition of its transcriptional targets (CCNE2/CDK2 by Dinaciclib or PDK1 by DCA) and PI3KCA by alpelisib, had synergistic anti-tumor effects in breast cancer cells with FOXK2 overexpression and PI3KCA oncogenic mutations. FOXK2 functional inhibition by the CDK2 inhibitor Dinaciclib enhanced the tumor-suppressive effect of alpelisib in a MCF7 breast cancer xenograft mouse model. These findings provide compelling evidence that FOXK2 plays an oncogenic role in breast tumorigenesis. The combination of PIK3CA and CCNE2/CDK2 inhibition by alpelisib and Dinaciclib offers a potential therapeutic strategy for breast cancer with FOXK2 overexpression and PIK3CA oncogenic mutation.

P5-02-25: Deciphering the transcriptomic landscape of early HR+/HER2- breast cancer in very young women

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Introduction: Breast cancer (BC) in very young women (VYBC), specifically those under 35 years old, presents biological and clinical differences compared to older patients with breast cancer (OBC). Previous studies have shown discordance in tumor characteristics and treatment responses between younger and older patients, notably within HR+/HER2-

subtype. This study aims to elucidate the transcriptomic landscape of HR+/HER2- in VYBC compared to OBC, focusing on identifying age-associated molecular differences that could decipher personalized treatment strategies for these patients.
Methods: This study included 49 BC patients treated at Hospital Clínico Universitario de Valencia. RNA sequencing was performed on formalin-fixed samples from 22 VYBC (age ≤35 years) and 27 OBC (≥50 years) patients. Differential expression analysis and gene set enrichment analysis (GSEA) were performed to identify differentially expressed genes. Deconvolution analysis using MCP-counter and quanTIseq algorithms was employed to evaluate tumor cellular composition. Histological examination described tumor infiltrating lymphocytes (TILs) and tertiary lymphoid structures (TLS). Statistical analyses were assessed using R (version 4.0.2).
Results: The BCVY cohort included 12 (55%) luminal, and 10 (45%) non-luminal BCs while the OBC cohort were 17 (63%) luminal and 10 (37%) non-luminal patients. Transcriptomic analysis revealed significant age-associated differences particularly in HR+/HER2- subtype (133 and 106 genes down- and up-regulated (log2 fold change < ± 2, adjusted p value < 0.05). HR+/HER2- VYBC tumors exhibited greater proliferation signatures (p = 5.17e-05) and ki67 (IHC, p = 0.021), increased chromosomal instability (CIN70, p = 1e-06), and higher expression of immune-related gene signatures compared to OBC tumors. Interestingly, despite no differences in estrogen and progesterone receptor by IHC, lower expression was found at RNA level in HR+/HER2- VYBC patients (ESR1: p= 0.0027, PGR: p= 0.0476). Deconvolution analysis confirmed an enhanced infiltration of immune cells in HR+/HER2- VYBC tumors, identifying them as immunoreactive tumors, characterized by high infiltration of cytotoxic T lymphocytes and overexpression of immune checkpoint molecules such as PD-1 and its ligand PD-L1. Histological validation confirmed increased TILs and TLS in VYBC tumors. Finally, PAM50-based chemoendocrine score (CES) was also determined for HR+/HER2- samples showing higher levels of CES, which are predictive of better response to chemotherapy in HR+/HER2- VYBC patients.
Conclusions: Our study provides a comprehensive understanding of the molecular landscape of HR+/HER2- BC in very young women. We found age-related gene expression changes not only in cancer cells but also in the tumor microenvironment. These data suggest that HR+/HER2- tumors from VYBC patients could be more endocrine-resistant and immunoreactive than those from OBC patients. Accordingly, personalized therapeutic strategies in this subgroup are needed.

P5-02-26: Trial in progress: Imatinib to convert triple negative breast cancer into estrogen receptor (ER) positive breast cancer - a window of opportunity trial

Sophie Lehn, Barbro K. Linderholm, Elisabeth Kapocs, Anikó Kovács, Elisabeth Werner Rönnerman, Kristian Pietras

Background: A large component of the tumor microenvironment in breast cancer consists of cancer-associated fibroblasts (CAFs), a cell type which can influence tumor progression,

angiogenesis and therapy resistance. Receptors on CAFs are activated by ligands in the tumor microenvironment, resulting in establishment of a malignant paracrine crosstalk supporting the tumor as a whole. Preclinical data suggest a specific role for tumor cell-secreted Platelet-derived growth factor-CC (PDGF-CC) in maintaining the triple-negative breast cancer (TNBC) phenotype through paracrine activation of PDGFR (Platelet-derived growth factor receptor) on CAFs. PDGF-CC expression in tumor cells has been shown to be significantly associated with the basal-like PAM50 molecular subtype. We have previously reported that inhibition of PDGF-CC, and thereby the paracrine signaling between tumor cells and CAFs, in preclinical TNBC models results in tumors converting to a luminal Estrogen Receptor (ER) positive subtype sensitive to endocrine treatments. Imatinib, a PDGFR inhibitor, was also tested in the preclinical setting yielding similar results. Based on these results, a window-of-opportunity trial was launched to test if ER expression can be induced in TNBC by inhibiting CAFs.
Methods: The study is a window-of-opportunity, single center clinical trial investigating the efficacy, safety and feasibility of short term imatinib (400 mg per day for 10 days) for TNBC patients planned for surgery, who are not eligible for neoadjuvant chemotherapy (Clinical Trial Identifier NCT05722795, EudraCT 2020-005200-19). The primary endpoint is to determine changes in Estrogen Receptor (ER) expression before and after imatinib treatment by analyzing the diagnostic biopsy and the surgical specimen. If ER expression is changed from 0% to 2% or more, or ER is changed from 1-9% to either ≥10% or at least 2% increase combined with a significant increase in luminal gene transcripts, the primary endpoint is met. Secondary endpoints include determining safety of short-term imatinib and assessing blood and tissue markers such as ctDNA (circulating tumorDNA), immune cell markers and analyses of activity in luminal gene expression programs, and PAM50 gene signatures before and after imatinib treatment. Thirty-five patients will be recruited with an interim analysis planned after recruitment of 20 patients. The study is open for inclusion and 4 patients have been included as of 10th of June 2024.

P5-02-27: Investigating the Prognostic Significance of Circulating Tumor Cells in Ductal Carcinoma In Situ Patients

Neha Nagpal, Brittany Rupp, Yan Hong, Fariba Behbod, Max Wicha, Sunitha Nagrath

Raised awareness of the importance of early diagnostics in breast cancer is leading to an increased incidence of ductal carcinoma in situ (DCIS) being detected through mammographic screening. DCIS is widely defined as a ‘Stage 0’ carcinoma with neoplastic cells still confined to the basement membrane. However, while most DCIS patients will receive widespread overtreatment for their carcinoma, the 20-year breast cancer mortality rate following a DCIS diagnosis, with or without treatment, remains at 3.3%. To better understand breast cancer carcinogenesis, recent studies have drawn attention to the clonal diversity within the DCIS tumor microenvironment and the presence of disseminated tumor cells in DCIS patients. However, there still exist gaps in understanding of characteristics at the time of diagnosis that make certain DCIS patients more likely to progress or recur.

Ultimately, the goal of the project is to better inform DCIS patient risk stratification via the high throughput isolation and analysis of circulating biomarkers.
In this study, we establish a workflow that targets the gaps in traditional tissue-based diagnostics by pivoting towards liquid biopsies. Our study not only builds on emerging literature on the early dissemination of breast cancer cells during carcinogenesis, but also investigates the value of circulating tumor cells (CTCs) as a biomarker in DCIS. To achieve a more holistic understanding of a patient's carcinoma, we supplement our study with the Mouse INtraDuctal (MIND) models - an animal model that recapitulates the tumor microenvironment of DCIS in the mammary ducts of mice.
We employed a high throughput, label-free microfluidic platform to enrich CTCs from DCIS patient samples. CTCs isolated from the unbiased platform were processed for single cell RNA sequencing along with white blood cells, red blood cells, and platelets that enabled further investigation of the circulating microenvironment and potential inter-cellular interactions. Further, to correlate findings in patient samples to an animal model wherein the timeline of progression to invasive disease can be shortened to 9 to 12 months (vs. 10+ years in patients), we analyzed blood samples from three different MIND models in a time course study over 12 months.
Applying our workflow to 26 patient samples and 10 healthy controls, we identified CTCs in 96% of the patient samples. The average concentration of CTCs per mL of blood across all DCIS patients was significantly greater than that in the healthy controls ($p < 0.0001$, Mann-Whitney U Test). Through single cell RNA sequencing, inferred CNVs were found in two cell clusters that also encapsulated potential CTCs based on presence of canonical epithelial cell markers but absence of white blood cell genes. An epithelial to mesenchymal cell phenotype score was calculated (scale 0 to 100, respectively) for each potential CTC, and it was found that the median score tended around 50 to 60, supporting expanding literature on the hybrid epithelial-to-mesenchymal state of CTCs. In addition, during the MIND model time course study, CTCs were found in circulation as early as 3 months after formation of the models and while the models were still pre-invasive.
Overall, our study enables the assessment of CTCs as a prognostic biomarker for DCIS patients. By studying CTCs in patient samples and their derived MIND models in parallel, we aim to continue to build an understanding of the drivers behind early dissemination. Further experimentation is also being planned to study the original tumor at a single cell level in comparison to the blood sequencing that has already been performed. The overarching goal is to compose a more holistic understanding of the origin of these malignant cells in circulation. Ultimately, we hope to investigate the value of CTCs as a clinical biomarker to inform personalized risk stratification for DCIS patients.

P5-02-28: Genomic markers of sacituzumab govitecan (SG) response in metastatic triple-negative breast cancer and hormone receptor-positive breast cancer

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Background: Sacituzumab govitecan (SG), an antibody-drug conjugate comprising an anti-Trop-2 antibody coupled to the irinotecan analog, SN-38, is currently approved for use in metastatic triple-negative breast cancer (TNBC) and hormone receptor-positive (HR+) breast cancer. Preclinical studies demonstrate that SG results in increased activity in tumors with homologous recombination deficiency, which may be attributed to the SN-38 payload increasing double-strand DNA breaks. However, biomarker analyses of SG show that patients benefit regardless of germline BRCA 1/2 mutation status and that patients with TNBC expressing higher Trop-2 expression benefit more from SG. Utilizing next-generation sequencing (NGS) data, we sought to identify whether genomic biomarkers were also associated with response to SG in patients with metastatic breast cancer (MBC).
Methods: NGS of tissue and circulating tumor DNA from patients with MBC treated with SG were obtained by chart review. Genomic data was obtained through routine clinical practice. Progression-free survival (PFS) was determined from the first day of treatment until disease progression or death. Oncogenic alterations from NGS were analyzed according to PFS with univariate cox proportional hazards analysis.
Results: 128 patients treated with SG between April 2020 and November 2023 were included in the analysis. 95 (74%) patients had TNBC, while 33 (26%) had HR+ breast cancer. 32 (25%) patients received SG in the 1st or 2nd line, while 96 (75%) received SG in later lines. The median PFS was 2.7 months (95% CI, 2.4-4.1). 115 (90%) patients had NGS tests available, with a total of 211 NGS tests included. Of these, 160 (76%) tests were performed prior to SG, 46 (22%) after SG, and 5 (2%) at unknown date. 78 different oncogenic alterations were identified in 2 or more patients. Of the 128 patients, the most common oncogenic alterations were TP53 (69%), PIK3CA (20%), PTEN (14%), ERBB2 (12%), and MYC (10%). Five alterations were significantly associated with shorter PFS, including KIT (HR 20.9, p = 0.0001, n=2), MAP3K1 (HR 5.0, p = 0.002, n=4), RAD21 (HR 8.3, p = 0.004, n=2), MTAP (HR 4.5, p=0.01, n=3), and TSC1 (HR 5.0, p = 0.03, n=2). In TNBC, the alterations MTAP (HR 5.8, p=0.004), MAP3K1 (HR 4.8, p=0.01) and CDKN2A (HR 2.5, p=0.02) were associated with shorter PFS. In HR+ disease, the alterations KIT (HR 14.9, p=0.004), FGF19 (HR 6.3, p=0.02), and FGF3 (HR 6.3, p=0.02) were associated with shorter PFS.
Conclusions: Five oncogenic alterations were associated with a poor response to SG in MBC, including KIT, MAP3K1, RAD21, MTAP, and TSC1. In TNBC, MTAP, MAP3K1, and CDKN2A were associated with a poor response, whereas in HR+ disease, KIT, FGF19, and FGF3 were associated with a poor response. These results suggest that alterations in these genes may be potential resistance mechanisms to SG which merits further exploration.

P5-02-29: Redefining PARP inhibitor paradigms to target DNA repair dysfunction in lobular breast cancer

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PARP inhibitors (PARPi) transformed the treatment of BRCA1/2-associated cancer by leveraging that tumors with homologous recombination DNA repair (HR) deficiency are vulnerable to PARPi. However, PARPi use is uncommon for patients with invasive lobular carcinoma (ILC), as ILC are rare among BRCA-mutation carriers, and ILC have very low 'BRCA-like' genomic scarring of HR deficiency. Importantly, though most ILC appear "low risk" (e.g. ~95% are estrogen receptor α/ER-positive), ILC have poorer long-term outcomes than other breast cancers, are associated with treatment resistance, and lack ILC-targeted therapies. Novel treatments for ILC are needed, and our work suggests PARPi target a unique vulnerability in ILC and provide an opportunity for precision treatment.

We identified a novel crosstalk between ER and DNA repair in ILC – via mediator of DNA damage checkpoint 1 (MDC1), a key double-strand break repair protein – that facilitates endocrine response and anti-estrogen resistance, but compromises DNA repair downstream of MDC1. We find homologous recombination (HR) induction and resolution is limited and inefficient in ILC; e.g., in I-SceI assays for HR versus non-homologous end-joining activity, ILC cells (MDA MB 134VI, SUM44PE) showed ≤10% of repair events via HR, while ≥40% of repair events were via HR in non-ILC breast cancer cells (MCF7, T47D). Importantly, these cell line data are paralleled by ILC tumor data, wherein subsets of ILC are defined by elevated DNA repair protein levels and increased tumor mutational burden. These ILC have the poorest disease-free and overall survival, supporting that high-risk ILC tumors also present with a novel DNA repair dysfunction.

Based on this putative HR dysfunction, we tested PARPi talazoparib against ILC models. In vitro, talazoparib was equipotent against proliferation in ILC and non-ILC breast cancer cell lines (IC50 ~25nM). However, non-ILC cells recovered after talazoparib washout, but ILC cells remained growth suppressed 7d post-washout with outgrowth reduced by 50-80%. In vivo, we tested talazoparib (0.33mg/kg p.o. 5d/w) alone ± estrogen withdrawal (to mimic aromatase inhibition/AI), with 6 weeks treatment against MDA MB 134VI or SUM44PE xenografts. In MM134, talazoparib suppressed tumor growth vs control (ANOVA $p=0.0016$) and durably suppressed growth as seen in vitro. Combining talazoparib with AI was superior to single agents (ANOVA $p=0.0006$ vs talazoparib, $p= 0.0037$ vs AI) and best prolonged host survival (cohort survival: ctrl = 158d; AI = 175d, log rank vs ctrl $p=0.054$; talazoparib = 182d, $p=0.069$; AI + talazoparib = >209d, $p=0.0021$). Combined AI + talazoparib also best suppressed SUM44PE xenograft growth (ANOVA AI vs combination, $p<0.0001$), and median time to tumor progression (doubling in size: ctrl = 104d; AI = 130d, log rank vs ctrl $p=0.11$; talazoparib = 121d, $p=0.68$; AI + talazoparib = >153d, $p=0.0018$; survival study ongoing).

Our work suggests that despite the absence of the BRCA-like phenotype, ILC have a previously unappreciated form of DNA repair dysfunction that makes cells sensitive to PARPi. PARPi represent potential first-in-class precision treatments specifically targeting ILC biology. Understanding HR dysfunction in ILC can broaden PARPi use paradigms and build new conceptual frameworks for understanding DNA repair capacity and associated therapeutic vulnerability.

P5-03-01: Open-label, randomized, multicenter, phase 3, ELAINE 3 study of the efficacy and safety of lasofoxifene plus abemaciclib for treating ER+/HER2-, locally advanced or metastatic breast cancer with an ESR1 mutation

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Background: Most patients with estrogen receptor-positive (ER+), metastatic breast cancer (mBC) treated with endocrine therapy (ET) will ultimately develop resistance to treatment. A large unmet medical need exists especially when resistance occurs following a cyclin-dependent kinase 4/6 inhibitor (CDK4/6i), potentially driven by a mutation in the ERa-coding gene, ESR1. Lasofoxifene (LAS), an oral, next-generation ET and ER breast antagonist, was evaluated in two phase 2 studies of women with ER+/HER2- mBC and an ESR1 mutation who had disease progression on previous ET and CDK4/6i. In the ELAINE 1 trial, LAS monotherapy provided numerically greater progression-free survival ([PFS] median, 5.6 mos vs 3.7 mos; HR 0.669 [95% CI, 0.445-1.125]; P=0.138), objective response rate (ORR, 13% vs 3%; P=0.124), and clinical benefit rate (CBR, 37% vs 22%; P=0.117) compared with the ER degrader fulvestrant (fulv), and a favorable safety profile (Goetz MP, et al. Ann Oncol. 2023;34:1141-1151). The single-arm, ELAINE 2 trial showed that LAS combined with abemaciclib (Abema) was well tolerated with a median PFS of ~13 mos, ORR of 56%, and CBR of 66% (Damodaran S, et al. Ann Oncol. 2023;34:1131-1140). Based on these promising earlier-phase data, the phase 3, registrational, ELAINE 3 trial was initiated. Other recent results from postMONARCH confirmed superior efficacy of fulv/Abema versus fulv/placebo (median PFS, 6.0 mos vs 5.3 mos; HR 0.73 [95% CI, 0.57-0.95], nominal P=0.02) in non-biomarker–selected mBC that progressed on a prior CDK4/6i and aromatase inhibitor (Kalinsky K, et al. J Clin Oncol. 2024;42[suppl 17]: abstract LBA1001 [slides]). The ELAINE 3, global trial will compare LAS/Abema with fulv/Abema in a post-CDK4/6i, ESR1-mutation–selected mBC population.
Methods: ELAINE 3 (NCT05696626) is an open-label, phase 3, multicenter study evaluating the efficacy, safety, and tolerability of LAS plus Abema versus fulv plus Abema. Study enrollment is currently underway at sites in the United States, Canada, France, Italy, Israel, and Spain, and is planned for expansion into Australia, Belgium, China, Germany, Hungary, Poland, Romania, Singapore, South Korea, Taiwan, and the United Kingdom. Key inclusion criteria are pre- and postmenopausal women and men aged ≥18 yrs; ER+/HER2-, locally advanced and/or mBC (measurable and/or non-measurable disease); ≥1 acquired ESR1 mutation; progression on an aromatase inhibitor plus palbociclib or ribociclib as their first hormonal treatment for advanced/mBC; and ≤1 line of chemotherapy in the advanced/metastatic setting. Patients will be randomized 1:1 to receive LAS 5 mg/day plus Abema 150 mg BID, or fulv 500 mg IM on days 1, 15, and 29, then once monthly plus Abema 150 mg BID. Treatment will continue until progression, death, unacceptable toxicity, or withdrawal from the study. The primary endpoint is PFS by blinded independent central

review (BICR); key secondary endpoints are ORR, overall survival, CBR, duration of response, and time to response. Time to cytotoxic chemotherapy, quality of life, and safety will also be evaluated. Blood samples for circulating tumor DNA (ctDNA) will be collected for genomic analyses at screening, at weeks 4 and 8 and every 8 weeks thereafter, and at the final visit. Outcomes with LAS/Abema and fulv/Abema will be compared using a stratified Cox proportional hazards model and stratified logrank test with an expected PFS hazard ratio of 0.68 at final analysis. To achieve 90% power with a one-sided type I error rate of 0.025, the target sample size is 400 patients. Full recruitment is expected to occur over 18 mos.

P5-03-03: Pertuzumab and Trastuzumab Biosimilars in Real-Life Association for the First-Line Treatment of HER2-Positive Metastatic Breast Cancer: the prospective, observational PETRA study

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Background: Trastuzumab biosimilars (TBs) have been available in Europe since 2018 and are widely prescribed for HER2-positive breast cancer. However, reported data on their efficacy and tolerance, especially when used in combination with pertuzumab and chemotherapy in a first-line metastatic setting, are lacking.
Methods: The observational, multicenter Pertuzumab – Trastuzumab (PETRA) study prospectively enrolled French patients with HER2-positive metastatic breast cancer (MBC) receiving chemotherapy (CT) plus pertuzumab (P) plus TB, regardless of the EMA-approved product. Eligible patients may have received adjuvant or neoadjuvant TB and/or P, with an interval of at least 12 months between the completion of the adjuvant or neoadjuvant anti-HER2 therapy and the diagnosis of metastatic breast cancer. The primary endpoint was the progression-free survival at 6 months (PFS-6), defined as patients with stable, partially responsive, or completely responsive disease at that time. Secondary endpoints included safety, patient exposure to the treatment combination (defined as the number of cycles of CT, P, and TB received, and the relative dose intensity of CT in milligrams per day [RDI]), and the percentage of treatment discontinuation due to side effects.
Result: Between February 3, 2021, and July 31, 2023, 112 patients from 30 centers were included: median age 59.0 years [50.5 – 71.5]; PS 0: 38.9%, PS 1: 53.7%, PS 2: 7.4%; median baseline left ventricular ejection fraction (LVEF): 65.5% [60.0 – 69.0]; HR+: 63.4%; de novo metastatic: 58.9%; visceral metastases: 55.4%; previous (neo)adjuvant trastuzumab: 23.2%. Median follow-up was 5.7 months [4.5-6.3]. TBs used were Trazimera® in 39 patients (34.8%), Ontruzant® in 27 (24.1%), Herzuma® in 23 (20.5%), Ogivri® in 19 (17.0%), and Kanjinti® in 4 (3.6%). The majority of chemotherapy regimens were paclitaxel in 90 patients (80.4%), followed by docetaxel in 18 (16.1%) and vinorelbine in 4 (3.6%). The PFS-6 was 80.36% [95% CI: 71.78, 87.26]. The objective response rate (ORR) was 69.64% [95% CI: 60.24, 77.98]. There were 3 deaths unrelated to treatment (2.7%) and

18 tumor progressions (16.4%). The median number of TB cycles was 7.0 [5.5 – 9.0], with 41 patients (36.6%) starting maintenance treatment with trastuzumab and P before 6 months. The median number of CT cycles was 6.0 [4.0-6.0], with 54 patients (48.2%) receiving fewer than 6 cycles. The median RDI was 14.6 mg/day [10.8-17.0]. Grade 1-2 side effects were observed in 61.6% of patients, grade ≥ 3 in 36.6%, primarily peripheral neuropathy in 22.6% (including 5.4% grade 3); diarrhea in 20.3% (including 0.9% grade 3); and asthenia in 15.7% (including 13.8% grade 3). Fifteen patients had infusion reactions, including 3 related to TB (1 grade 3). Three patients had adverse effects leading to the permanent discontinuation of the biosimilar: 1 due to interstitial lung disease (attributable to BS) and 2 due to heart failure. Twelve patients experienced cardiac dysfunction at 3 or 6 months: among them 3 had previous exposition to anthracycline in adjuvant setting, 11 had an absolute decrease in LVEF ≥ 10%, 5 had LVEF < 50%, and 3 presented symptomatic grade 3 cardiac dysfunction (2 had received anthracyclines). All were alive by the time of final analysis.
Conclusions: Trastuzumab biosimilars appear to be similar to Herceptin when combined with pertuzumab and chemotherapy in the first-line treatment of metastatic HER2-positive breast cancer. However, 13% of patients experienced a decrease in LVEF during follow-up, which is significant. Systematic and regular monitoring of cardiac toxicity is mandatory.

P5-03-04: The effectiveness of post-T-DXd treatments in patients with HER2-positive metastatic breast cancer: A nationwide Japanese cohort study (EN-SEMBLE)

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Background: Trastuzumab deruxtecan (T-DXd) is a new standard of care for human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer after treatment with anti-HER2 antibody and taxane. In clinical practice, some patients (pts) discontinue T-DXd treatment due to progressive disease (PD) or adverse events (AEs). Thus, an unmet medical need remains to determine optimal post-T-DXd treatment regimens. The EN-SEMBLE study investigated post-T-DXd treatments in terms of effectiveness and incidence/exacerbation of interstitial lung disease (ILD) in the Japanese real-world (rw) setting.
Methods: This study was a multicenter cohort study (jRCT1030220506). Among pts who received T-DXd from May 25, 2020, to November 30, 2021, and had been registered for the Japanese post-marketing all-patient surveillance, this study enrolled pts who discontinued T-DXd and received subsequent anti-tumor therapy for metastatic breast cancer from May 25, 2020, to May 31, 2023, and were followed up until November 30, 2023. The endpoints were the distribution of post-T-DXd treatment regimens, effectiveness (rw progression-free survival [rwPFS], rw time to next treatment [rwTTNT], overall survival [OS], etc.) and ILD

incidence/exacerbation rate by subsequent anti-tumor therapies. Reasons for T-DXd treatment discontinuation were also analyzed.
Results: A total of 664 pts were registered from 222 sites nationwide in Japan: median age, 60.0 (range 30–89) years; female, 99.5%; ECOG-PS ≥2, 8.6%; visceral and brain metastasis, 79.7% and 23.0%; de novo stage IV and recurrent breast cancer, 37.7% and 59.3%. The median treatment line for T-DXd was 4th regimens (range 2–43). The most common reason for T-DXd discontinuation was PD, 67.5% (448/664), followed by ILD, 22.0% (146/664), and AEs other than ILD, 5.1% (34/664). The subsequent regimens (1st post-T-DXd treatment) after T-DXd discontinuation were anti-HER2 antibody (trastuzumab, pertuzumab)–containing regimens, 54.4% (361/664); anti-HER2 TKI–containing regimens (lapatinib, other anti-HER2 TKIs), 17.0% (113/664); ADC (trastuzumab emtansine), 1.8% (12/664); and others, 26.8% (178/664). In overall population, anti-HER2 antibody, anti-HER2 TKI, and ADC regimens, rwPFS were 4.1 (3.9–4.5), 4.1 (3.8–4.6), 4.3 (3.8–6.2), 2.6 (1.0–4.7); rwTTNT, 5.0 (4.6–5.5), 5.1 (4.5–5.7), 5.3 (4.4–6.8), 3.0 (1.0–5.9); and OS, 16.2 (13.8–17.2), 17.2 (15.1–19.8), 16.3 (12.2–18.7), 9.3 (4.3–not reached) (respectively; all shown as median [95% CI] months). The HR (95% CI) for anti-HER2 TKI to anti-HER2 antibody was 1.06 (0.84–1.34) for rwPFS, 1.07 (0.85–1.35) for rwTTNT, and 1.17 (0.88–1.54) for OS. Among the anti-HER2 antibody–containing regimens, the HR (95% CI) for a trastuzumab + pertuzumab–containing regimen (n=203) to a trastuzumab-containing regimen (n=157) was 0.94 (0.74–1.20) for rwPFS, 1.00 (0.79–1.26) for rwTTNT, and 0.98 (0.73–1.30) for OS. For pts who discontinued prior T-DXd treatment due to PD or AEs including ILD, rwPFS were 3.5 (3.0–3.8), 7.3 (5.7–10.3), rwTTNT, 4.2 (3.8–4.6), 8.3 (6.2–11.3), and OS 12.0 (10.9–13.6), 32.4 (22.3–not reached), respectively (median [95% CI] months). The incidence of ILD was observed in 10 pts during the first post-T-DXd treatment: 4 pts were new onset, 5 pts were recurrent or exacerbation (3.4%, 5/146), and 1 pts was unknown.
Conclusion: About half of the pts received anti-HER2 antibody regimens in the 1st treatment after T-DXd. No differences were observed between anti-HER2 antibody and TKI-based regimens in terms of rwPFS, rwTTNT, or OS. Post-T-DXd treatments were generally safe in pts who had discontinued T-DXd due to ILD, with 3.4% of exacerbation.

P5-03-05: AI Based Quantitative Estimation of HER2 Protein Expression in Low and Ultra Low Ranges

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The anti-HER2 monoclonal antibody drug trastuzumab (Herceptin®) was approved for treatment of HER2 positive breast cancer by the FDA in 1998. Concurrently, the HER2 IHC assay HercepTest™ was approved as an aid in the assessment of breast cancer patients where trastuzumab treatment was considered (review by Jørgensen et al. 2021). Recently, FDA approved antibody drug conjugate (ADC) trastuzumab deruxtecan (T-DXd; Enhertu®)

for the treatment of metastatic HER2-positive and HER2-low breast cancer. Results from the Destiny Breast-06 clinical trial showed that T-Dxd provides statistically significant and clinically meaningful benefits to HER2-ultralow breast cancer patients as well.
However, sensitive, accurate and quantitative evaluation of HER2 expression based on currently approved IHC assays remains challenging, especially in low and ultra-low ranges of HER2. Advanced computational approaches could improve the interpretation of such IHC assays and could be of high benefit for identifying treatment options for current and future HER2 targeted therapies. More importantly, it could provide a basis for objectively assessing the lower level of HER2 protein expression that maximizes therapeutic benefits, while minimizing drug exposure risks for patients.
Here we demonstrate a novel AI-based method that quantifies HER2 protein expression following staining using the HercepTest™ mAB pharmDx (Dako Omnis, GE001) assay (HercepTest™ (mAB)) for breast cancer cases characterized as HER2 IHC 0 or 1+. We inferred HER2 expression spatially across the entire breast cancer tissue section and quantified its heterogeneity. We identified low levels of HER2 expression that are challenging to detect by the human eye and provided a graphical overlay of stained tissues for visualization and quantification of the heterogeneity of HER2 expression.
We used the quantitative IHC (qIHC) method described by Jensen et al. (2017) to quantitively measure the HER2 expression in invasive breast carcinomas. These measurements were used to train an AI model to predict the expression based on the HercepTest™ (mAb) stain which was previously demonstrated to detect HER2 expression with higher sensitivity in the lower ranges of HER2 compared to PATHWAY 4B5 (Ruschoff et al, 2022). Note that quantitative analysis of the qIHC assay could also be used to directly quantify HER2 expression.
82 formalin fixed, paraffin embedded (FFPE) tissue blocks of invasive breast carcinoma with HER2 IHC scores 0 or 1+ and with areas of solid tumor tissue were selected for this study. Serial sections from each tissue block were stained with H&E, HercepTest™ (mAB), qIHC and p63 (GA662, Dako Omnis). Stained tissues were scanned on the Philips Ultra Fast Scanner and digitally aligned. Tumor areas were manually selected and reviewed by expert pathologists in HercepTest™ (mAB) using aligned H&E and p63 IHC staining information on consecutive tissue sections to help identify tumor areas and separate invasive tumor areas from carcinoma in situ. HER2 expression was evaluated based on the qIHC assay in each 128μm2 area within tumor regions. The differences in average qIHC expressions across tumor areas were found to be statistically significant between IHC 0, IHC>0 and IHC<1+, and IHC 1+ groups. We observed high level of spatial heterogeneity of the HER2 expression levels within the same tissue, up to five-fold in some cases. Using these qIHC based estimates of HER2 expression as ground truth, we trained an AI based interpretation of HercepTest™ (mAB) assay. K-fold cross validation scheme was used to separate train and test slides, with test folds aggregated for model evaluation. We demonstrated high slide-level agreement of the AI-based estimate of HER2 expression in tumor regions and the ground-truth with Pearson correlation of 0.94, and R2 of 0.87.
In the future we expect AI-assisted quantification and visualization of HER2 expression to enable fast and safe treatment decisions.

P5-03-06: Correlation of Mutational Changes in Circulating Tumor DNA with Clinical Outcomes in HER2 Positive Metastatic Breast Cancer

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Background: The presence of certain genetic mutations can be correlated with a poor response to HER2 directed therapies. Circulating tumor DNA (ctDNA) is a noninvasive tool to assess genomic alterations in progressive metastatic breast cancer (MBC). Understanding mutational changes in patients' ctDNA at disease progression can aid prognostication and identify potentially targetable mutations associated with resistance to specific therapies in HER2 positive MBC.
Materials/Methods: Patients with HER2 positive breast cancer either at diagnosis on pathology or by ctDNA were included in the analysis. Patients were prospectively enrolled from 2016-2024 under an IRB approved clinical trial (NU16B06) at Northwestern University. Plasma ctDNA was analyzed by Guardant 360 and tissue NGS was performed using commercially available tests such as FoundationOne® or TempusX. Specific mutations that have known clinical relevance (e.g. PIK3CA, ERBB2, ESR1, TP53, MYC, and FGFR) were identified via ctDNA and/or tissue NGS. Additional clinical, pathologic, treatment, and response data was retrospectively collected as well. Statistical analyses were completed via a multivariate regression model.
Results: One hundred and twenty patients met inclusion criteria for the analysis. Seventy-four percent of patients were HER2 positive at initial diagnosis while twenty six percent developed HER2 positivity at time of metastatic spread. Hormone Receptor positivity (ER or PR) was seen in 77% of HER2 positive patients. Visceral involvement of metastatic disease was identified in 52% of patients and bony involvement in 45% of patients. NGS and ctDNA findings showed PIK3CA mutation in 26% of patients, ERBB2 in 30%, ESR1 in 8%, TP53 in 53%, MYC in 15%, FGFR in 15% of patients. Mortality rate of the cohort was 41%. There were 86% of patients who received HER2 directed therapies. The median progression free survival (PFS) was 3.5 months (95% CI 2.6, 5.3). Patients with PIK3CA mutations (p=0.028) and TP53 mutations (p=0.042) had a worse 10-year survival rate than those without. Patients with PIK3CA and TP53 co-mutations had a worse 10-year survival rate (p=0.21) than those with neither mutation or having either PIK3CA or TP53 alone. Patients with TP53 and PIK3CA co-mutations had worse PFS HR 1.90 (1.02-3.55, p=0.044). Patients with TP53 and PIK3CA co-mutations also had worse PFS when being treated with Trastuzumab (HR 0.07, p=0.003) compared to patients with other mutational changes. Patients with PIK3CA and TP53 co-mutations were more likely to have evidence of progression to visceral metastatic disease on Trastuzumab, OR 0.16 (0.02-0.70, p=0.028).
Conclusion: Molecular alterations identified in ctDNA at progression of disease in HER2 positive MBC can aid in understanding genomic evolution and drift as well as pathways to treatment resistance. This analysis of ctDNA in HER2+ MBC shows that patients with TP53 and PIK3CA co-mutations are shown to have poorer 10-year survival rates, shorter PFS, worse response to Trastuzumab, and overall evidence of more aggressive cancers

progressing into visceral metastatic disease. Understanding how the presence of these mutations correlate with resistance to specific therapies can guide individualized treatment decisions on which line of therapies will be most effective for patients with progressive HER2 positive MBC.

P5-03-07: 64Cu-DOTA Trastuzumab -PET to predict CNS response to Trastuzumab-deruxtecan (TDXd) in patients with metastatic HER2 low and HER2 positive breast cancer

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Background: We have utilized 64Cu-DOTA-Trastuzumab-PET to image patients with advanced HER2+ breast cancer. In our experience, uptake on 64Cu-DOTA-Trastuzumab-PET correlated with the qualitative assessment of HER2 by IHC. In women treated with the antibody-drug conjugate (ADC), ado-Trastuzumab emtansine (TDM1), we identified a threshold level of 64Cu-DOTA-Trastuzumab-PET uptake that predicted for lack of response to TDM1. The ADC, Trastuzumab-deruxtecan (TDXd), has demonstrated activity in patients with metastatic HER2 positive breast cancer with brain metastases. The DESTINY 04 trial included patients with HER2 1+ and 2+ disease and demonstrated superior survival compared to standard of care. Only 5% of patients enrolled had CNS metastases making efficacy of TDXd difficult to assess. As it is not always feasible to biopsy brain metastases to determine tumor markers, we initiated a pilot study to determine if functional imaging could predict response or lack of response to TDXd in patients with HER2 low and HER2 positive breast cancers that have metastasized to the brain. We hypothesize that uptake on pretreatment 64Cu-DOTA-Trastuzumab PET/MRI would predict response of brain metastases in patients treated with TDXd.
Primary Endpoint:
64Cu-DOTA-Trastuzumab PET/MRI will identify tumor heterogeneity in patients with HER2 low and HER2+ brain metastases.
1. The level of uptake of 64Cu-DOTA-Trastuzumab on PET/MRI will predict for CNS response to TDXd.
Secondary Endpoint:
1. Uptake of 64Cu-DOTA-Trastuzumab on PET/MRI will be predictive of response duration to TDXd.
Eligibility:
Patients with recurrent HER2 1+, 2+ or 3+ breast cancer that has metastasized to the brain as detected by CT or MRI, and are to undergo treatment with TDXd.
Methods: Pretreatment staging workup included the following: pathologic review of HER2 status, disease staging with 18FDG-PET or CT of the chest/abdomen/pelvis and bone scan, and 64Cu-DOTA-Trastuzumab PET/MRI of the brain. TDXd was administered every 3 weeks per standard of care. After initiation of TDXd, MRI of the brain and systemic staging were repeated every 6 weeks for the first 24 weeks and every 9 weeks thereafter. Post-

contrast MRI scans were used to delineate tumors for analysis of 64Cu-DOTA-Trastuzumab uptake and tumor perfusion. SUVmax on the PET, as well as the Ktrans (volume transfer constant) and vp (plasma volume fraction) on the brain MRI were analyzed.
Progress: We have enrolled 5 patients, with 4 having HER2+ cancer by IHC and 1 HER2 1+; 3 having cancer confined to the brain, and 2 having additional systemic disease. Three patients demonstrating tumor regression on post-treatment MRI had higher uptake of 64Cu-DOTA-Trastuzumab (SUVmax: 6.1 +/- 1.5, Ktrans: 0.12 +/- 0.049, vp: 0.016 +/- 0.0038). Two patients with lower 64Cu-DOTA-Trastuzumab (SUVmax: 1.8 +/- 0.1, Ktrans: 0.13 +/- 0.028, vp: 0.020 +/- 0.0041) did not respond to therapy. An SUVmax greater than 2 was predictive of tumor response to TDXd in the initial cohort. To date, the responding patients continue therapy for 8-10 months. Non-responding patients experienced disease progression within 2 to 3 months. On average, responding tumors had lower plasma volume fraction as measured by DCE-MRI (n=5, not significant). The goal is to accrue 10 patients to this pilot study, to investigate the combined value of brain MRI perfusion parameters and 64Cu-DOTA-Trastuzumab uptake values.

P5-03-08: Characteristics of ESR1-Mutant HER2 Positive Metastatic Breast Cancer (MBC)

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Background: It is well established that ESR1 mutations encode resistance to endocrine therapies in Estrogen Receptor positive (ER+) metastatic breast cancer and portend a worse prognosis. However, studies on ESR1 mutations have excluded patients with HER2 positive (HER2+) disease to date. We report a first-of-its kind finding of ESR1 mutations in HER2+ mBC.
Methods: This study included 120 patients with HER2+ MBC who received systemic treatment under an IRB-approved clinical trial (NU16B06) at Northwestern University Robert H. Lurie Cancer Center (2016–2024). Patients were considered HER2+ if, at time of ESR1 mutation detection, they had either biopsy-proven HER2+ disease or HER2+ circulating tumor cells (CTCs). Plasma ctDNA was analyzed by Guardant360 NGS. CTC enumerations were performed via CELLTRACKS (Menarini). ER, PR, HER2 and Ki67 in each patient's biopsy tumor tissue before surgery, surgical tumor tissue and metastatic tumor tissue were evaluated by NU PathCore. Additional clinical, pathologic, therapy, and response data were retrospectively collected and analyzed.
Results: Twelve patients with ER+/HER2+ mBC developed ESR1 mutations. One patient had HER2+ disease since primary diagnosis, one patient developed HER2+ at time of metastatic spread, and all twelve patients shed HER2+ CTCs at time of ESR1 detection with a median 16 HER2+ CTCs (range 2–114). Median age of diagnosis was 57 (range 43–80). Patients were 66% White, 25% Asian, and 8% African American. Patients received a median of three hormonal therapies including an aromatase inhibitor prior to ESR1 detection. Most patients (92%) were treated with a CDK4/6 inhibitor prior to ESR1 detection. Median time from

metastatic diagnosis to detection of ESR1 mutation was 40.2 months (range 24.6–105.9). ESR1 point mutations in codons L536 (4 patients), E380 (3 patients), D538 (3 patients), Y537 (2 patients), P353 (1 patient), and K362 (1 patient) were identified. Co-mutations included PIK3CA (58%), TP53 (42%), PTEN (33%), and ERBB2 (17%). Median PFS after detection of ESR1 mutation was 3.2 months (range 1.9 –30.7), with all patients receiving HER2-directed therapy and four patients receiving Fulvestrant. No patients received Elecestrant. Patients most often developed metastasis to bone (92%), followed by liver (67%), CNS (50%), lymph nodes (42%), and lungs (33%). All patients died, with median time to death after ESR1 detection of 15.1 months (range 1.9–62.8).
Conclusions: Patients who develop ESR1 mutations in the setting of HER2+ mBC most often had point mutations in L536, E380 and D538, and had high rates of co-mutation with PIK3CA, which may have prognostic implications. To date, clinical trials regarding ESR1 mutations in breast cancer have excluded patients with HER2+ disease. These patients should be included in future studies of ESR1-targeted therapies.

P5-03-09: Evaluating Post-T-DXd Treatment Strategies in HER2-positive Metastatic Breast Cancer

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Background: Trastuzumab deruxtecan (T-DXd) is an antibody-drug conjugate (ADC) that has proven highly effective in the treatment of HER2-positive metastatic breast cancer (MBC). However, drug resistance and disease progression eventually occur in the majority of patients. A critical examination of treatment strategies in the post-T-DXd setting is imperative in order to appropriately care for patients with TDXd-resistant breast cancer.
Methods: We retrospectively examined baseline characteristics and clinical outcomes for patients after they had received T-DXd for HER2-positive metastatic breast cancer at MSKCC. We delineated six categories of treatment in the post-T-DXd setting: monoclonal antibodies (mABs), ADC, chemotherapy-based treatment, hormone therapy, tyrosine kinase inhibitor (TKI) based therapy, and clinical trials/novel combinations. Median overall survival time (OS) and progression free survival (mPFS) were estimated, as well as the mPFS per treatment line, further stratified by treatment type, using the Kaplan-Meier method. Progression free survival (PFS) analyses were performed using Cox proportional hazards regression, clustering by patient and in multivariate analysis, adjusting for treatment line, T-DXd stop reason, hormone receptor (HR) status, and the presence of brain metastases.
Results: We identified 81 patients with HER2-positive MBC who continued treatment after T-DXd. The median age was 52 years, 60% had ER+ disease, and 54% had brain metastases. T–DXd was given as the 4th line of therapy, on average, in this population. Median OS from the time of T-DXd completion was 19 months; 52% of patients had died at the time of data cutoff. Of the patients included in this study, 78% stopped T-DXd due to disease progression, 11% stopped due to pneumonitis/ILD, and 11% stopped due to other toxicity

complications. After T-DXd, patients received a median of 2 lines of further therapy (range 1-9) with a mPFS of 3.7 months per treatment line; a total of 199 lines of treatment were included in this analysis. Chemotherapy (including eribulin or gemcitabine plus trastuzumab) was the most frequently given treatment, received by 50 patients (62%), with mPFS of 3.4 months. Forty-two (52%) patients received TKI-based treatments (with trastuzumab, tucatinib, and capecitabine being the most common), and exhibited a mPFS of 3.9 months. Twenty-one (26%) patients were enrolled in clinical trials or received novel drug combinations, demonstrating a mPFS of 4.2 months. ADCs (most commonly T-DM1) were given to 16 (20%) patients, with a mPFS of 2.4 months. Eleven (14%) patients received hormone therapy, and 7 (8.6%) received mAB, with a mPFS of 9.1 months and 14 months, respectively. In multivariate analysis, patients who stopped T-DXd due to toxicity (rather than disease progression) had a dramatically improved mPFS on subsequent lines of treatment (HR 0.35; p <0.001). Later treatment line and the presence of brain metastases were numerically associated with shorter mPFS (HR 1.06; p=0.06 and HR 1.23; p=0.2 respectively), and HR status had no effect.
Conclusion: We observe that prognosis is generally poor in the post-T-DXd setting. No therapeutic approach could be identified as clearly superior in our cohort, however, a subset of patients seemed to benefit from hormone therapy or TKI-based therapy, which warrants further investigation. Of special note, the reason for ending T-DXd treatment was a strong predictor of response to subsequent therapies, such that patients who stopped treatment due to toxicity had a 65% reduction in risk of disease progression or death. Overall, our study indicates that patients emerge from T-DXd treatment with highly refractory disease. Characterizing T-DXd resistance and finding new therapeutic options for this population represents an urgent clinical need.

P5-03-10: Safety and Preliminary Efficacy of Tucatinib and Alpelisib in Patients with HER2-positive PIK3CA-Mutated Metastatic Breast Cancer

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Background: Activating mutations in the gene of phosphotydylinisotol-3 kinase catalytic subunit alpha (PIK3CA) are present in up to 40% of breast cancers overexpressing human epidermal growth factor receptor 2 (HER2). These mutations are linked to resistance to HER2-targeted agents and poorer patient outcomes. Based on preclinical efficacy, we are conducting a study to evaluate the combination of HER2-inhibitor tucatinib and PI3Kα-inhibitor alpelisib in patients with HER2-positive (HER2+) PIK3CA-mutated metastatic breast cancer (MBC) (NCT05230810).
Methods: This phase IB study aims to determine the maximal tolerated doses of tucatinib and alpelisib using the Time-to-Event Bayesian Optimal Interval Design, with dose limiting toxicity (DLT) window of 28 days. Safety is evaluated by CTCAE v.5.0 with standard definition for DLTs. Eligible participants include post-menopausal women or pre-menopausal women undergoing ovarian suppression, diagnosed with HER2+ PIK3CA-

mutated MBC, and previously treated with at least two HER2-targeted agents. Patients are allowed 1 prior line of HER2 tyrosine kinase inhibitor for MBC, including prior tucatinib. Treatment consists of twice-daily tucatinib and daily alpelisib at prespecified dose level (DL) with concurrent fulvestrant for hormone receptor-positive cases.
Results: As of July 1st, 2024, 8 patients have been treated. The median age was 53 years (range: 45-66), with a median of 2 prior lines of therapy for MBC (range: 1-4). Prior HER2-targeted therapies included trastuzumab and pertuzumab (8 patients), T-DM1 (5 patients), tucatinib (4 patients), T-DXd (4 patients), and margetuximab (1 patient). Six patients had visceral metastases and 4 had CNS metastases.
No DLTs were observed in 4 patients treated at DL1 (tucatinib 300 mg BID, alpelisib 250 mg daily), with one additional patient in the DLT window at the time of data cut-off. At DL2 (tucatinib 300 mg BID, alpelisib 300 mg daily), all 3 patients experienced DLT. Two patients had grade 3 (G3) diarrhea despite maximal anti-diarrheal regimen that resolved with treatment hold and dose reduction. One patient developed G3 rash with mucositis, eosinophilia and G4 elevated creatinine and came off study, all symptoms resolved without sequelae with hydration and steroid treatment. Other notable adverse events, primarily G1-2, included hyperglycemia (6 patients), decreased appetite (5 patients), weight loss (5 patients), fatigue (5 patients) and elevated liver enzymes (4 patients). These side effects were managed with supportive medications, nutritionist consultations and avoidance of hepatotoxic agents. No new safety signals were observed from the study drugs. One heavily pretreated patient developed progressive CNS metastases after 6 months on study and died on hospice within 30 days of follow up. At the time of data cutoff, 5 patients were evaluable for tumor response: 2 had stable disease, and 3 had partial response (PR). All patients who experienced PR had PIK3CA H1047R mutation. All PRs occurred after 2 cycles of therapy and included significant reduction of breast tumors, liver and lung metastases and resolution of cancer lymphangitic spread in the lungs. Four out of 8 patients remained on the regimen for more than 6 months. The longest treatment duration is 15 months and ongoing.
Conclusions: The combination of tucatinib and alpelisib is tolerable at DL1 and shows remarkable antitumor activity with partial responses in 3 out of 5 evaluable patients (60% overall response rate), including responses in patients treated with prior tucatinib and T-DXd. Enrollment continues at DL1. Updated safety and efficacy findings will be presented at SABCS 2024 conference.

P5-03-11:DEMETHER: A single-arm phase II trial to evaluate the efficacy & safety of subcutaneous pertuzumab & trastuzumab maintenance after induction treatment w/ trastuzumab deruxtecan (T-DXd) for previously untreated HER2-positive advanced breast cancer

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Background: HER2 (human epidermal growth factor receptor 2) is overexpressed in 15-20% of all breast cancers. Pertuzumab combined with trastuzumab and taxanes is the standard first-line therapy for HER2-positive advanced breast cancer (ABC) based on the results from CLEOPATRA trial. T-DXd has demonstrated unprecedented antitumor activity in patients (pts) with previously treated HER2-positive ABC and has the potential to move into the first-line treatment of this patient population (DESTINY-Breast09). Despite a manageable toxicity profile, prior studies have raised safety concerns about potential serious adverse events associated with its use and a potential negative impact on quality of life (QoL). Taking this into consideration, the rationale of the DEMETHER study is to find the right balance between T-DXd shorter treatment duration and subcutaneous fixed-dose combination of pertuzumab and trastuzumab maintenance therapy to ensure efficacy and tolerance in previously untreated HER2-positive ABC pts. Trial Design DEMETHER (NCT06172127) is an international, multicenter, open-label, single-arm phase II trial. Key inclusion criteria include: (a) Pts aged ≥18 years with centrally-confirmed HER2-positive ABC; (b) No prior chemotherapy and/or HER2-targeted therapy for advanced disease. Participants who have received chemotherapy and/or HER2-targeted therapy in the neo(adjuvant) setting are eligible if ≥ 12 months from completion of systemic treatment to metastatic diagnosis; (c) ECOG performance status of 0-1; (d) Evaluable disease according to RECIST v.1.1. Pts will first receive an induction treatment consisting of 6 cycles of T-DXd (5.4 mg/kg, intravenously, on day 1 of each 21-day cycle). If any T-DXd unacceptable toxicity occurs during the induction phase, participants may receive taxane-based chemotherapy concomitantly with subcutaneous fixed-dose combination of pertuzumab and trastuzumab treatment at the discretion of the Investigator. In the maintenance phase, all participants will be treated with a subcutaneous fixed-dose combination of pertuzumab and trastuzumab each 21-day cycle until disease progression, unacceptable toxicity, patient withdrawal, or up to 3 years after T-DXd initiation. During the maintenance phase, hormone receptor-positive pts will also receive concomitant endocrine therapy. The primary objectives are: 1-year (1-y) progression-free survival (PFS) rate, as locally determined by the Investigator according to RECIST v.1.1; and 3-year (3-y) overall survival (OS) rate. Secondary objectives are median PFS and OS, objective response rate, clinical benefit rate, time to response, duration of response, best percentage of change in tumor burden as per RECIST v.1.1, changes in health-related QoL assessments from baseline (EORTC QLQ-C30, QLQ-BR45, and EQ-5D-5L), and safety and toxicity profiles according to the NCI-CTCAE v.5.0. The primary analyses will be performed using the maximum likelihood method for exponential distributions, with a one-sided significance level of 2.5% for both endpoints. Applying sequentially rejective test, the type I error will be controlled at 5% level. A sample size of 165 patients will allow to achieve 80% power. Date of registration: May (Europe), July-August (FDA-IRB, US), December (Brazil), 2024. First patient enrolled: June (Europe), September (US), December (Brazil), 2024.

P5-03-12: Liquid Cytopathology for HER2 Status in Breast Cancer: Beyond Tissue Biopsy

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Background: Around 15% of breast cancers (BC) are considered human epidermal growth factor receptor 2 positive (HER2+). HER2 status is assessed on tissue biopsies which, despite being the gold standard for tumors' molecular characterization, present limitations. These limitations could be overcome by using liquid biopsy. In particular, circulating tumor cells (CTCs) allow an in-depth characterization of the disease at various levels (DNA, RNA, protein) and can be used as a surrogate of tissue biopsy, also for HER2 evaluation. In this work, we investigated the potential of CTCs to assess HER2 status in BC patients, in comparison to HER2 evaluation on tissue.
Methods: Blood samples were collected from 318 patients (pts) with metastatic BC treated at Northwestern University (Chicago, IL) between 2016 and 2021. CTCs were enumerated and stained for HER2 using the CellSearch® platform. Only patients with ≥5 CTCs were considered for correlation between tissue and CTCs HER2 status. HER2 expression on CTCs was quantified using the ACCEPT software and each CTC was categorized as HER2-negative, HER2 2+ and HER2 3+ (moderate and strong expression, respectively). For each sample a CTC-HER2ratio was calculated as the number of HER2 2+ and 3+ CTCs divided by the total number of CTCs. Tissue HER2 status (evaluated on the available biopsy collected nearest in time to the blood draw) was compared to the HER2 expression on CTCs. Receiver operating characteristic (ROC) curves were used to define the optimal CTC-HER2ratio cutoff associated with HER2 tissue status.
Results: The study cohort included 143 patients with metastatic BC and ≥5 CTCs. Of these, 15% (N=22) had HER2+ and 85% (N=121) had HER2 negative (HER2-) BC. Among the pts with HER2- BC, 36 were further categorized as HER2zero and 46 as HER2low (for 39 patients the information was not available). HER2+ CTCs (including HER2 2+ and 3+) were detected in 91% and 96% of patients with HER2+ and HER2- BC, respectively. Despite a similar incidence, the median CTC-HER2ratio was significantly different among the 2 subgroups: 0.30 (interquartile range [IQR] 0.10-0.73) for the HER2+ cohort vs 0.09 (IQR 0.03-0.18) for the HER2- one, p-value = 0.00028, indicating a higher proportion of HER2+ CTCs in HER2+ BC pts. When comparing HER2zero and HER2low BC cohorts, we could not observe a difference in the proportion of HER2+ CTCs. The median CTC-HER2ratio was 0.08 (IQR 0.03-0.16) and 0.12 (IQR 0.03-0.21) for HER2zero and HER2low, respectively (p-value = 0.32708). Both were significantly lower than the CTC-HER2ratio of the HER2+ BC cohort (p-value = 0.00076 and 0.00614 for HER2zero and HER2low, respectively). The ROC curve analysis for HER2+ and CTC-HER2ratio resulted in an area under the curve (AUC) of 0.7417, with an optimal cutoff at 0.22. At this threshold, sensitivity was 63.64% and specificity reached 82.64%. The positive predictive value was 40.00% while the negative predictive value was 92.59%. The ROC curve analysis for HER2-low and CTC-HER2ratio resulted in an AUC of 0.5646, with an optimal cutoff at 0.13. At this optimal cutoff, sensitivity was 50% and

specificity was 72.22%. The positive predictive value was 69.70% while the negative predictive value was 53.06%.
Conclusion: It is possible to identify and quantify HER2 expression on CTCs. HER2+ CTCs were detected in a high percentage of patients regardless of their HER2 status on tissue, indicating high intra-patient heterogeneity. HER2+ BC cohort exhibited a significantly higher proportion of HER2+ CTCs compared to the HER2- BC cohort. No difference was instead observed when comparing HER2zero with HER2 low subgroups. Moreover, we showed that the CTC-HER2ratio is a good predictor of HER2+ status on tissue suggesting a potential application of liquid cytopathology for molecular markers.

P5-03-13: Trastuzumab emtansine versus disitamab vedotin for HER2-positive metastatic breast cancer: a multicenter retrospective real-world study

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Background: An estimated 15–25% of breast cancer patients exhibit overexpression or amplification of human epidermal growth factor receptor 2 (HER2), characterized by high invasiveness and poor prognosis. The antibody drug conjugate (ADC) drugs, especially trastuzumab deruxtecan (T-DXd), have tremendously improved efficacy in HER2-positive metastatic breast cancer (MBC). However, there is as yet no consensus on the superiority-inferiority differentiation of other anti-HER2 ADC drugs, especially trastuzumab emtansine (T-DM1) and disitamab vedotin (RC48).
Methods: This multicenter retrospective study included 180 HER2-positive MBC patients initially treated with T-DM1 (n=105) or RC48 (n=75) from July 2017 to March 2023. Enrolled patients had received T-DM1 (3.6 mg/kg iv q3w) or RC48 (2.0 mg/kg iv q2w) until disease progression, intolerable toxicity, or death. Baseline characteristics were balanced using inverse probability of treatment weighting (IPTW). The primary endpoint was progression-free survival (PFS) and secondary endpoints were objective response rate (ORR), disease control rate (DCR), overall survival (OS), and adverse events (AEs). The study was approved by the Institutional Review Board of Shandong Cancer Hospital and Institute (No. SDTHEC2023004017).
Results: The clinical data was retrospectively collected up to 31 October 2023. All baseline characteristics were comparable between the two groups after IPTW. In the unmatched cohort, the median PFS of T-DM1 was significantly superior compared to the RC48 group (7.8 months vs. 5.5 months; p = 0.002); and the patients in T-DM1 group had a longer trend of OS (26.8 months vs. 18.0 months; p = 0.240). The ORR (40.0% vs. 29.3%, P=0.141) and DCR (74.3% vs. 61.3%, P = 0.064) of patients with T-DM1 were better than that of patients with RC48. After IPTW adjustment, T-DM1 was still associated with improved survival, with median PFS of 6.2 versus 5.5 months (p = 0.002) and median OS of 25.5 months versus 17.8 months (p = 0.240). In the subgroup analyses of PFS, T-DM1 groups were favored over

RC48 groups in all pre-specified subcohorts. Moreover, T-DM1 treatment improved ORR (odds ratio [OR] 1.08, 95% CI 0.91-1.27, p = 0.380) and DCR (OR 1.10 95% CI 0.93-1.30, p = 0.286) compared with RC48 group. Grade 3 AEs occurred in 28.6% and 33.3% of patients in the T-DM1 and RC48 groups, respectively (p = 0.494). The most predominant grade 3 AE in the T-DM1 group and the RC48 group were reduced platelet count (13.3%) and neutropenia (13.3%), respectively. No grade 4 and 5 AEs occurred.
Conclusions: T-DM1 treatment showed a significant improvement in progression-free survival versus RC48 in patients with HER2-positive metastatic breast cancer. Moreover, both drugs demonstrated good tolerability. And prospective randomized studies are needed for further verification.

P5-03-14: Efficacy and safety of inetetamab-containing regimens in patients with HER2-positive metastatic breast cancer in first-line/second-line setting

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Inetetamab is a novel recombinant humanized anti-Human epidermal growth factor receptor 2 (HER2) monoclonal antibody. This real-world retrospective study assessed the efficacy and safety of inetetamab-containing regimens in first-line/second-line treatment of HER2-positive metastatic breast cancer (MBC). This study retrospectively recruited HER2-positive MBC patients who received inetetamab- containing regimens from June 2020 to May 2023. The outcomes included progression-free survival (PFS), objective response rate (ORR), and disease control rate (DCR). A total of 329 patients were enrolled and included in the efficacy analysis. The most frequently used treatment strategy was contained inetetamab plus pyrotinib (205/329, 62.3%). Of 329 patients for efficacy evaluation, the median PFS was 14.5 months. Patients treated with first-line regimens benefited the most, with a median PFS of 15.0 versus (vs.) 10.0 months (first-line- vs. second-line inetetamab plus pyrotinib, p <0.001), 19.0 vs. 17.0 months (first-line- vs. second-line inetetamab plus pertuzumab, p=0.096), and 14.0 vs. not reached months (first-line- vs. second-line inetetamab plus chemotherapy, p=0.229). The inetetamab plus pertuzumab group had the longest survival compared with inetetamab plus pyrotinib and inetetamab plus chemotherapy group in first-line (mPFS, 19.0 vs. 15.0 vs. 13.0 months) and second-line (mPFS, 17.0 vs. 10.0 vs. not reached months) settings. The ORR was 54.4% (95%CI, 49.0%-59.7%), with 14 (4.3%) complete response (CR) and 150 (45.6%) partial response (PR). Besides, 148 (45.0%) patients had stable disease (SD) for a disease control rate (DCR) of 99.4% (95%CI, 97.8%-99.8%). Grade 3 or higher adverse events (AEs) were observed in 29.5% of all 329 patients and mainly included diarrhea (38/329, 11.6%), white blood cell decreased (32/329, 9.7%), myelosuppression (18/329, 5.5%), neutrophil count decreased (7/329, 2.1%). No treatment-related deaths were reported. Compared with the other two subgroups, the incidence AEs of any grade (205/329, 62.3%) and grade ≥3 (80/329, 24.3%) in inetetamab plus pyrotinib group was the highest. Following the first- and second-line of

treatment, inetetamab- containing combinations demonstrated promising clinical activity and a manageable safety profile in patients with HER2-positive MBC, especially in the first-line treatment.

P5-03-15: EmpowHER 303: A phase 3 study to evaluate the efficacy and safety of zanidatamab vs trastuzumab with chemotherapy in patients (pts) with metastatic HER2-positive breast cancer who have progressed on, or are intolerant to, trastuzumab deruxtecan

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Background: HER2-targeted therapies have revolutionized the treatment of HER2-positive (HER2+) breast cancer (BC). However, there is a need for novel treatment options after progression on available therapies, including trastuzumab deruxtecan (T-DXd). Zanidatamab (zani) is a HER2-targeted IgG1-like bispecific antibody that binds two non-overlapping HER2 domains in a trans configuration, leading to crosslinking of adjacent HER2 molecules. In preclinical studies, zani was associated with multiple mechanisms of action, including HER2 internalization and downregulation, complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, and phagocytosis. As a monotherapy or in combination with other agents, zani has shown clinically meaningful efficacy against multiple HER2+ tumors, including metastatic BC (mBC). We describe an ongoing phase 3 study assessing zani in combination with chemotherapy in pts with HER2+ mBC whose disease has progressed on, or who are intolerant to, T-DXd.
Methods: EmpowHER 303 (NCT06435429) is a phase 3, randomized, open-label, multicenter study evaluating the efficacy and safety of zani vs trastuzumab (T) both in combination with physician's choice of chemotherapy in pts (≥18 years old) with histologically confirmed HER2+ mBC (per ASCO-CAP guidelines) and measurable disease (per RECIST version 1.1). Pts with tumors locally assessed as IHC 3+ can be randomized while HER2-positivity is centrally confirmed. Pts with tumors locally assessed as IHC2+/ISH+ cannot be randomized until their HER2 status is centrally confirmed. Eligible pts must have progressed on, or be intolerant to, prior T-DXd treatment and have received ≥2 but ≤4 lines of prior HER2-directed therapies for metastatic disease. Prior HER2-directed therapy after T-DXd (e.g. tucatinib-based regimen or trastuzumab emtansine) is allowed based on physician's choice, pts' eligibility, and institutional and local guidelines. Pts must be eligible to receive one of the following physician's choices of chemotherapy: eribulin, gemcitabine, vinorelbine, or capecitabine. Pts with central nervous system (CNS) metastases may be eligible if their CNS metastases have been treated or are clinically inactive. Pts must also have adequate hematologic parameters and hepatic function, LVEF ≥50% as determined by an echocardiogram or multiple gated acquisition scan, Eastern

Cooperative Oncology Group performance status of 0 or 1, and a life expectancy of at least 6 months. Exclusion criteria include known or suspected leptomeningeal disease, uncontrolled or significant cardiovascular disease, uncontrolled infection, history of hypersensitivity to monoclonal antibodies, or inability to receive zani or T. An estimated 550 pts will be randomized 1:1 to zani (1800 mg [pts <70 kg] or 2400 mg [pts ≥70 kg] intravenous [IV]) or T (8 mg/kg IV loading dose, then 6 mg/kg), + physician's choice of chemotherapy in 3-week cycles. Randomization will be stratified by number of prior lines of HER2-directed therapies, prior tucatinib use, geographic region, CNS metastases, and chemotherapy choice. Mandatory prophylaxes for diarrhea and infusion-related reactions are required starting cycle 1 for pts receiving zani + chemotherapy. The primary endpoint is progression-free survival (PFS) assessed by blinded independent central review (BICR). Secondary endpoints include overall survival, confirmed objective response rate, and duration of response (by BICR and investigator-assessed), investigator-assessed PFS, safety, patient-reported outcomes, pharmacokinetics, and immunogenicity for zani. Exploratory endpoints will assess biomarkers of treatment response/resistance.

P5-03-23: Unveiling Guillain-Barre Syndrome as a Paraneoplastic Sequelae of Breast Cancer

Rachelle Diane Maravilla, Agnes E. Gorospe

Paraneoplastic neurological syndromes (PNS) are immune-mediated conditions triggered by an underlying cancer. Classically, PNS have been correlated with onconeuronal antibodies that target intracellular proteins, showing limited direct pathogenic significance. Nevertheless, detection of such antibodies indicates a strong association with certain tumor types such as lung, ovarian, breast and lymphatic systems. PNS are extremely rare especially in subsets of breast cancer patients, and an incidence of 1-8 per 100,000 person-years. Symptoms include visual and gait disturbances, hypotonia, dysphagia, poor fine motor coordination, cognitive dysfunction, and epileptic seizures. We report a case of a 40-year old female with early-stage BRCA1 mutated, triple negative breast cancer. She underwent definitive surgery followed by adjuvant chemotherapy. However, after 4 cycles of dose-dense adriamycin and cyclophosphamide, evaluation scan showed tumor recurrence to the liver. She proceeded with Paclitaxel for 12 weeks with complete response of liver metastases. A month after, she presented with blank stares, generalized tonic-clonic seizures, dizziness and parieto-occipital headache. Cranial MRI showed multiple enhancing parenchymal foci in the left occipital, bilateral temporal lobes, pons and cerebellum. Among these, the largest lesions measured 1 x 2.4 x 1 cm and 1.7 x 2.5 x 2.1 cm in the right cerebellum. She was started on dexamethasone, leviteracetam and lacosamide, and was referred to Radiation Oncology. She then received 10 fractions of whole brain radiotherapy (WBRT), amounting to 3000 cGy. Despite this, however, her seizures persisted along with new onset bilateral lower extremity motor weakness (MMT 3-/5 on the right and 1/5 on the left). Leptomeningeal metastasis was considered but MRI of the spine was negative. Progression of neurologic deficits was noted, now with areflexia and bilateral upper

extremity weakness (MMT 4-/5, bilateral). Electromyography and nerve conduction velocity (EMG-NCV) showed probable early or beginning axonal polyneuropathy, absence of voluntarily activated motor units with apparently normal motor and sensory conduction velocities. Primary consideration at this time was Guillain-Barré Syndrome (GBS) specifically the acute motor axonal neuropathy (AMAN) variant. Cerebrospinal fluid was sampled which tested negative for infection. She was given 20 mg of intravenous immune globulin 5% (IVIG) for 5 days with minimal improvement of symptoms. At present, treatment plan is to give either targeted therapy for BRCA1 mutation or antibody-drug conjugate to address her malignancy and its possible underlying paraneoplastic process. For this case, the diagnostic confirmation of GBS secondary to the patient's concomitant breast carcinoma proved to be challenging especially since available data regarding the condition is limited. There is heterogeneity in timing, symptomatology, and presence of certain onconeural antibodies. These antibodies are usually seen in 60-70% of breast cancer patients exhibiting paraneoplastic symptoms. Hence, antibody testing may be helpful if detected but its absence cannot fully rule out PNS. To address this dilemma, four components were defined for the diagnosis of PNS: (1) presence of neurologic symptoms, (2) diagnosis of malignancy within 4 years from onset of neurologic manifestations, (3) exclusion of other neurologic diseases, and (4) at least one of the following: CSF analysis with inflammation and negative cytology, temporal lobe lesion on MRI, or epileptic activity in the temporal lobes by EEG. Since our patient fulfilled all four diagnostic components, the likelihood of her disease course being secondary to PNS is further strengthened. At present, however, there are no specific guidelines for such cases but treatment of the underlying malignancy is recommended.

P5-03-25: Trastuzumab deruxtecan (T-DXd) in leptomeningeal metastases from HER2-altered cancers

Qian Ma, David Rogawski, Toni Cao, Meaghan Roy-O'Reilly, Lilian Yao, Nova Xu, Seema Nagpal

Purpose: Emerging data suggest that trastuzumab deruxtecan (T-DXd) is an active treatment for brain metastases from HER2 + breast cancer. We aimed to characterize the activity of T-DXd in the treatment of leptomeningeal metastases (LM) from a range of HER2-altered cancers.
Methods: We reviewed neuro-oncology clinic records between July 2020 and December 2023 at Stanford Neuro-oncology clinic to identify patients who received T-DXd to treat LM.
Results: Of 18 patients identified, 6 had HER2 + breast cancer, 8 had HER2-low/negative breast cancer, 2 had HER2 + gastroesophageal cancer, and 2 had HER2-mutant non-small cell lung cancer (NSCLC). 10/18 (56%) patients had cytologically confirmed LM by CSF cytology or circulating tumor cell (CTC) capture. A partial response (PR) on MRI using the EORTC/RANO-LM Revised-Scorecard occurred in 4/6 (67%) patients with HER2 + breast LM, 2/8 (25%) patients with HER2-low/negative breast cancer, and 0/4 (0%) patients with HER2 + gastroesophageal cancer or HER2-mutant NSCLC. Median overall survival after

initiating T-DXd was 5.8 months. Survival after initiating T-DXd was numerically longer for HER2 + breast cancer patients compared with HER2-low/negative breast and HER2-altered non-breast cancer patients (13.9 months vs. 5.2 months and 4.6 months, respectively). Landmark analysis showed that patients with radiologic LM response to T-DXd by 2.5 months had longer survival than non-responders (14.2 months vs. 2.6 months, HR 0.18, 95% CI 0.05-0.63, $p < 0.05$), and landmark analyses at 3.5 and 4.5 months after starting T-DXd showed a similar but nonsignificant trend.
Conclusion: T-DXd induces LM responses in a subset of patients, and such responses may be associated with prolongation of survival. Prospective trials are needed to clarify the role of T-DXd in treating LM and which patients are most likely to benefit.
Keywords: Antibody-drug conjugate; HER2-altered cancers; Leptomeningeal metastases; Trastuzumab deruxtecan.

P5-03-26: Concurrent use of trastuzumab deruxtecan and radiation therapy in HER2-positive and HER2-low metastatic breast cancer: a single-center experience and review of the literature

Jihane Bouziane, Pierre Loap, Kim Cao, Sofiane Allali, Yacine Gounane, Loganadane Gokula, Laurence Escalup, Jean-Yves Pierga, Youlia Kirova

Purpose: Recent DESTINY-Breast trials have demonstrated trastuzumab deruxtecan's effectiveness in HER2-positive and HER2-low metastatic breast cancer. However, safety concerns remain regarding its combination with radiation therapy (RT). The purpose of this work is to assess the toxicity profile of combining trastuzumab deruxtecan and RT in patients with HER2-positive and HER2-low metastatic breast cancer to address these concerns.
Materials and Methods We conducted a retrospective study which included patients treated at Institut Curie Paris between November 2020 and January 2024. Patients with HER2-positive and HER2-low metastatic breast cancer who received concurrent trastuzumab deruxtecan and RT were identified. Data on patient demographics, treatment regimens, radiation doses, toxicity profiles, and treatment discontinuations were collected. Follow-up was conducted from the last day of radiotherapy until death or the last examination and toxicities were graded using the CTCAE V5.0.
Results The studied population includes all 33 patients with HER2-positive and HER2-low metastatic breast cancer who underwent concurrent treatment with trastuzumab deruxtecan and radiotherapy. The median follow-up was 11 months. The most common acute grade 1 toxicity was nausea. Grade 2 toxicities affected 21.2% of patients, including asthenia, mucositis, cardiac decompensation, and diarrhea. Trastuzumab deruxtecan discontinuation occurred in 5 patients due to systemic treatment-related toxicities, including nausea, thrombocytopenia, neutropenia, and cardiac decompensation. 21.2% of patients reported late toxicities, with nausea being the most prevalent.
Conclusion Our series of patients who received concurrent treatment of radiotherapy and

trastuzumab deruxtecan are showing acceptable toxicity. Larger prospective studies are needed to evaluate the toxicity and efficacy of this combination.

P5-03-27: Updated Real-World Evidence on the Efficacy and Safety of Inetetamab-Based Therapy in HER2-Positive Metastatic Breast Cancer Patients Previously Treated with Trastuzumab

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Background: Despite the clinical advancements made in the management of metastatic breast cancer (MBC), a notable void persists in the comprehensive evaluation of the therapeutic potential of inetetamab, a novel recombinant humanized anti-HER2 monoclonal antibody, among HER2-positive MBC patients who have previously undergone trastuzumab treatment. This study aims to bridge this gap by conducting a thorough, real-world analysis of inetetamab's efficacy and safety in this specific patient population.
Methods: A multicenter, retrospective, real-world study was conducted, enrolling 500 HER2-positive MBC patients between July 2020 and October 2023. Clinical and pathological data were systematically collected and analyzed to assess the progression-free survival (PFS), objective response rate (ORR), disease control rate (DCR), and the incidence of adverse events (AEs) among patients receiving inetetamab therapy.
Results: In the overall cohort, inetetamab therapy resulted in a median PFS of 8 months, with a median number of treatment lines being three. Notably, an ORR of 28.6% and a DCR of 89.2% were observed. Furthermore, patients receiving a combination regimen of inetetamab with tyrosine kinase inhibitors (TKIs) and chemotherapy achieved the longest PFS of 9.0 months, accompanied by an even higher ORR of 29.3%. The primary treatment-related AEs were neutropenia and leukopenia, both of which were generally well-tolerated by the patients.
Conclusions:
This study underscores the promising efficacy and safety profile of inetetamab in HER2-positive MBC patients with prior trastuzumab exposure, particularly when utilized in combination with TKIs and chemotherapy. These findings provide invaluable insights into the real-world application of inetetamab and serve as a foundation for further exploration of its optimal utilization strategies in clinical practice.

P5-03-28: Case report: interstitial lung disease in a paciente with metastatic breast cancer treated with radiotherapy and trastuzumab deruxtecan

Thamilyn Saruwatari, Marcelle Goldner Cesca, Monique Celeste Tavares

Introduction Pneumonitis or interstitial lung disease (ILD) is an adverse event associated with trastuzumab deruxtecan (T-DXd or DS-8201) described in up to 10 to 12% of cases, mostly classified as grade II or III. Fatal outcomes due to ILD and/or pneumonitis occurred in 1,0% and there are few cases reported in clinical practice.1 In this case report, we present a patient with metastatic breast cancer to the lung, pretreated, receiving T-DXd and pulmonary radiotherapy, who unfortunately progressed fatally with severe pneumonitis. Keywords: drug-induced pneumonitis; interstitial pneumonitis; trastuzumab-deruxtecan; radiotherapy Case report S.S, female, 64 years old, Asian ethnicity, ECOG 0, previously healthy. In May 2020, she began investigation for a nodular lesion in the right breast, core biopsy revealed invasive carcinoma of no special type, grade III, positive for ER 95%, PR 70%, HER2 3+, Ki67 80%, luminal B HER2 3+ phenotype. Initial staging examinations including total chest and abdomen CT showed multiple thoracic lymphadenopathies in the right axillary, right supraclavicular, right paratracheal, and subcarinal regions, multiple solid pulmonary nodules bilaterally suggesting secondary neoplastic involvement. Bone scintigraphy showed no evidence of bone metastases. Brain MRI revealed no lesions. In May 2020, she started first-line treatment for metastatic luminal B HER2 3+ breast cancer with docetaxel, trastuzumab, and pertuzumab (THP, administered IV every 3 weeks; pertuzumab 840mg on Day 1 of the first cycle followed by 420mg on Day 1 of subsequent cycles; trastuzumab 8mg/kg on Day 2 of the first cycle followed by 6mg/kg on Day 1 of subsequent cycles; docetaxel 75mg/m2 on Day 1 of each cycle). Response assessment after 3 months showed abnormal metabolic activity on PET CT (infraclavicular lymph nodes with SUV 4.4, mediastinal lymph nodes with SUV 5.6 and 8.1, subpleural pulmonary nodules with SUV 3.1 and 3.3, right breast lesion with satellite nodules and SUV between 3.9 and 17.3), and partial response by RECIST on total chest and abdomen CT. She continued THP until November 2020, when she experienced nodal disease progression and initiated second-line treatment with T-DM1, which she used for 6 months before switching due to further oncologic progression. Biopsy of the supraclavicular lymph node confirmed luminal B HER2 3+ phenotype. Third-line treatment was proposed with capecitabin and lapatinib (used for 4 cycles from May 2021 to August 2021), with disease progression noted in the breast, axillary lymph node, and pulmonary lesions. Fourth-line treatment with vinorelbin (August 2021 to November 2021) was initiated, with new nodal and pulmonary disease progression. Fifth-line treatment with trastuzumab and capecitabine was started and continued for 3 cycles (December 2021 to January 2022), but PET CT showed disease progression in the pulmonary and nodal regions. Sixth-line treatment with trastuzumab deruxtecan began in February 2022, with concurrent radiotherapy to pulmonary lesions (35 Gy in 10 fractions of 300 cGy) and mediastinal lymph nodes (30 Gy in 10 fractions of 300 cGy). After Cycle 27 of T-DXd, the patient presented to the emergency room with progressive dyspnea and syncope episode, oxygen saturation of 82% requiring 2L/min oxygen via nasal cannula. Vasovagal causes or central nervous system disease progression were ruled out, and transthoracic echocardiogram showed normal findings. Chest CT revealed consolidative and ground-glass opacities associated with septal thickening and sporadic bilateral fibroatelectatic changes, excluding viral and bacterial infectious causes. Evaluation by a pneumologist confirmed the diagnosis of T-DXd-associated pneumonitis.

Treatment was initiated with corticosteroids at 500 mg/day for 3 days, but clinical deterioration necessitated escalation to high-flow nasal cannula oxygen therapy, and a single dose of infliximab was administered. Corticosteroid therapy continued at 2 mg/kg/day. Unfortunately, the patient clinically deteriorated with progressive worsening of respiratory status and passed away after 36 days of hospitalization. Discussion Trastuzumab deruxtecan (also known as T-DXd and DS-8201) is an antibody–drug conjugate consisting of a humanized anti-HER2 monoclonal antibody linked to a topoisomerase I inhibitor payload through a tetrapeptide-based cleavable linker.2 Approximately 20% of women with breast cancer have tumors that overexpress human epidermal growth factor receptor 2 (HER2) and will receive anti-HER2 therapies.3 We present a case of pneumonitis and acute lung injury in a patient on T-DXd treatment. Trastuzumab-associated pneumonitis is not well described in the literature and the mechanism of trastuzumab-associated lung injury is not clear as well as risk factores, mainly after radiotherapy and polytreated patients. In the DESTINY-Breast04 Trial it has been documented that drug-related interstitial lung disease or pneumonitis occurred in 12.1% of the patients who received trastuzumab deruxtecan; In the trastuzumab deruxtecan group, the median time to onset in patients with interstitial lung disease or pneumonitis was 129 days.4 Similiar data was found in the DESTINY-Breast03 trial, where most cases of adjudicated drug-related interstitial lung disease or pneumonitis were mild or moderate. Cases of pneumonitis was identified in 27 patients (10.5%) who received trastuzumab deruxtecan (7 patients had grade 1 events, 18 had grade 2 events, and 2 had grade 3 events) and in 5 patients (1.9%) who received trastuzumab emtansine (4 had grade 1 events and 1 had a grade 2 event) (Table 2). No such events of grade 4 or 5 occurred in either treatment group, and most of the patients recovered by the time of the data cutoff. 5 When a case of pneumonitis is identified, discontinuation of T-DXd is recommended and early initiation of corticosteroid treatment is advised to minimize severity and morbidity. In some reported cases, prior thoracic radiotherapy increases the risk of acute lung injury in lung cancer patients.6 And identifying specific causative agents is challenging in oncology when drugs are given in combination regimens, or in association with thoracic radiotherapy, which is independently associated with lung fibrosis. Currently, there is no consensus regarding the use of T-DXd and radiotherapy, nor whether it could lead to cases of fulminant pneumonitis. Bibiliography: 1. ENHERTU. Prescribing information. Daiichi Sankyo, Inc.; 2024 2. Ogitani Y, Hagihara K, et al, Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. Cancer Sci. 2016;107(7):1039–1046. doi: 10.1111/cas.12966. 3. National Cancer Institute: Surveillance, Epidemiology, and End Results Program. Cancer stat facts: female breast cancer. 2021 (https://seer.cancer.gov/statfacts/html/breast.html)4. Shanu M, William J, et al, or the DESTINY-Breast04 Trial Investigators, Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med 2022;387:9-20 5. Javier C, Sung-Bae K, et al, for the DESTINY-Breast03 Trial Investigators, Trastuzumab Deruxtecan versus Trastuzumab Emtansine for Breast Cancer. N Engl J Med 2022;386:1143-1154 6. Gemma A, Kudoh S, et al. Final safety and efficacy of erlotinib in the phase 4 POLARSTAR surveillance

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P5-03-29: Impact of the PIK3CA mutation on clinical outcomes of patients with HER2+ metastatic breast cancer; a real-world study

Pegah Farrokhi

Introduction: Activating PIK3CA mutations occur in 30-40% of HER2-positive breast cancers, impacting the PI3K pathway essential for HER2 signaling. These genetic alterations are a known mechanism of resistance in HER2+ breast cancer and are linked to more aggressive tumors and poorer clinical outcomes. However, the clinical significance of PIK3CA mutations varies across treatment settings. In the neoadjuvant context, a correlation exists between PIK3CA mutations and reduced rates of pathological complete response. Conversely, in metastatic breast cancer (mBC), the prognostic and predictive value of PIK3CA mutations remains controversial, with conflicting results from various clinical trials. This heterogeneity in findings underscores the necessity for further investigation. The current study aims to elucidate the impact of activating PIK3CA mutations in HER2-positive mBC on clinical outcomes utilizing real-world data, thereby addressing the existing knowledge gap regarding the predictive and prognostic relevance in this patient (pt) population.
Methods: This was an observational cohort study utilizing the Flatiron Health-Foundation Medicine clinicogenomic database (FH-FMI CGDB), originated from ~280 US cancer clinics (~800 sites; primarily community oncology settings). Adult pts (≥ 18 years of age) diagnosed with HER2-positive mBC from January 1, 2011, to September 31, 2022, were included. Pts with clinical trial participation or less than 6 months of follow-up were excluded from the study. Descriptive statistics were used to summarize demographic and clinical characteristics. Overall survival (OS) and progression free survival (PFS) were assessed using the Kaplan-Meier method and Cox regression analyses.
Results: There were 1,325 HER2+ mBC pts included in the study. Activating PIK3CA mutations were identified in 44.7% of pts. Among pts, 148 (11.2%) pts were Black and 869 (65.6%) White pts. The median age was 63.0 years. The cohort contained 76.8% HR+/HER2+ and 23.2% HR-/HER2+ pts. The following alterations were also present in the tumors: BRCA1&2 (22.1%), ESR1 (17.3%), PTEN (9.9%), and AKT1 (4.4%). First-line treatment was administered to 86.7% of all pts. First-line HER2-targeted therapy was present in 49.9% of all patients; and use was higher in HR-/HER2+ (78.5%) pts compared to HR+/HER2+ (41.7%) pts. Pts with a PIK3CA mutation had shorter OS compared to wild-type pts but this difference was not significant in the overall population, (50.6 vs 57.1 months, long-rank= 0.073). However, among pts who received HER2 targeted therapy in the first-line treatment setting, those with a PIK3CA mutation demonstrated significantly shorter OS (45.8 vs 61.3 months, p=0.04) and PFS (9.0 vs 11.1 months, log-rank p=0.025) compared to wild-type pts. Cox regression analysis, after adjusting for demographic and clinical characteristics, further demonstrated that patients with activating PIK3CA

mutations who received first-line HER2 targeted therapy had significantly lower OS rates (HR=1.45, p=0.008) and PFS (HR=1.39, p=0.002) compared to PIK3CA wild-type pts. Conclusions: Based on our results, activating PIK3CA mutations are associated with lower overall and progression-free survival in HER2+ mBC patients who received first-line HER2 targeted therapy. Studies are warranted assessing the impact of targeting both the PI3K/AKT/mTOR and HER2 pathways to individualize treatment and mitigate resistance to HER2-directed therapy thereby improving clinical outcomes in this pt population.

P5-03-30: Real-world data on the use of trastuzumab deruxtecan in breast cancer patients at a tertiary hospital in Ourense, Spain.

Leticia Iglesias Rey, Paula Sampedro Domarco, Jesús García Gómez, Jesús García Mata

Acoording to the European Medicines Agency (EMA), Enhertu is for unresectable or metastaic Her2-positive breast cancer previously treated with antiHer2, and breast cancer with low Her2 expression that is unresectable or metastatic after chemotherapy in the metastatic or if there is recurrence during or within 6 moths after the end of adjuvant chemotherapy.
Our group evaluated patients with recurrent or metastatic breast cancer treated with trastuzumab deruxtecan at the Ourense University Hospital Complex, from May 1, 2021 to May 31, 2024. 19 female patients were included, 85% with ductal infiltrant carcinoma breast. At diagnosis, 52% were postmenopausal women. Initial staging showed that 10% were in stage I, 48% in stage II, 37% in stage III, and only 5% in satge IV.
Ehertu was administered most frequently in the thrid line of treatment or later in 63% of cases; 42% had previously received another ADC (TDM-1, sacituzumab govitecan, etc). 53% of the patients had a good general condition (ECOG 0) at the beginning of treatment. The most common metastases were lymph node and bone, although 32% had lung and liver metastases. The mean number cycles administered was 9 (range 1-31), and it was necessary to reduce the dose in 42% of patients to continue treatment. Thirteen patients continue with the same treatment, five changed therapeutic lines due to disease progression and one by their own decision. Of the 19 patients, 17 are still alive, indicating low mortality since the start of treatment with Enhertu.
It should be noted that 6 patients (31%) were initially diagnossed as her2 negative (1+,2+/ISH -) and throughout the course of the disease they transformed into Her 2+++, wich highlights the importance of rebiopsy of patients throughout their illness, in order to provide the most appropiate treatment. 7 patients were Her2+++ patients from the debut, and 6 patients were treated in the indication of Her2 low (Her2 1+,2++/ISH-)
100% of Her2 +++ patients initially received a pertuzumab+trastuzumab+taxane regimen (Cleopata regimen). In Her2 negative patientes (1+,2++/ISH-) at diagnosis, the most common first-line treatment was cyclin inhibitors with aromatase inhibitors, given that they were hormone receptor-positive patients.
Conclusions: These data undeline the effectiveness of trastuzumab deruxtecan in a heterogeneous and highly pretreated population. More follow-up is required to evaluated

the impact on overwall survival, but preliminary results indicate that it is an effective treatment with tolerable toxicity in a high percentage of patients with advanced breast cancer. This drug should be considered in all who present any percentage positivity (measured by immunohistochemistry) in Her 2.

P5-04-02: The Breaking Point: Understanding Healthcare Costs, Financial Strain, and Coping Mechanisms Used by Metastatic Breast Cancer Patients with Medicare Coverage

Fran Castellow, Megan Schoonveld-Diaz, Kathleen Gallagher, Rebekah Angove

Background: Cancer-related expenses, including provider services, medications, insurance premiums and non-medical expenses like transportation and food, continue to rise, forcing patients to shoulder an ever-growing out-of-pocket financial burden. Metastatic breast cancer (mBC) treatments are among the most expensive due to the need for lifelong therapy, regardless of income or insurance. The Inflation Reduction Act of 2022 (IRA) includes several consequential provisions aimed at reducing drug spending and thereby increasing access to more affordable pharmaceuticals for millions of Americans, including instituting an out-of-pocket (OOP) maximum in the Part D prescription drug benefit that is being phased in during the 2024 and 2025 plan year. Beginning in 2024, IRA encompasses measures that reduced the OOP patient responsibility to around $3300 and eliminated the 5% cost share in the catastrophic coverage phase. The purpose of this brief survey was to ask questions to better understand financial stress, experiences with healthcare costs and coping mechanisms experienced by patients in the past 12 months.
Methods: This cross-sectional analysis used data from a nationwide survey distributed in June 2024 by Patient Advocate Foundation (PAF). Inclusion criteria included a valid email address, age >19, self-reported mBC diagnosis and Medicare coverage. Descriptive statistics were calculated using medians and IQR for continuous variables and frequencies for categorical variables.
Results: Of 103 female mBC patients, 38% were <56 years, 32% identified as non-white, 57% were disabled, 83% had a household income <$50,000, 75% household of 1-2 people, and 27% lived in a rural location. Most patients (91%) were currently in treatment. Primary sources of household income were disability payments (55%) or Social Security income (36%). One-third (33%) reported a decrease in household income over the past 12 months. Average monthly healthcare spending included provider visits ($154; IQR $351), pharmacy prescriptions ($101; IQR $209.5), and insurance premiums ($174; IQR $250.50). Over half of reported increases in at least one of these costs in the past 12 months. Coping mechanisms for increased healthcare costs included; credit cards (42%), receiving charitable co-pay assistance (37%), lifestyle changes (36%), payment plan/charity assistance from provider (33%), or delaying care/treatment (26%). Sixty-one percent reported already being financially stressed and a $40 monthly increase in healthcare costs would be unaffordable. When asked about behaviors related to medication adherence, 43% reported if costs increased, they would change the way they are filling or taking

prescriptions.
Conclusion: For mBC patients, there is a low threshold of increased financial burden that, when reached, forces them to start making difficult choices about their care plan adherence and daily living expenses. The insights received from the survey respondents show that they are experiencing higher healthcare costs, including medication costs, despite the lowering of the OOP maximums. To mitigate the impact of rising healthcare costs on low-income cancer patients insured through Medicare, it is essential for policymakers, healthcare providers, and community organizations to collaborate to design solutions that address the total costs of care, and ensure access to support services, and charitable financial assistance programs. Additionally, raising awareness about the challenges faced by this vulnerable population and advocating for policies that address their needs can help alleviate some of the burdens imposed by inflation. While the IRA is a step in the right direction, its financial impact on mBC patients insured through Medicare appears to be limited.

P5-04-03: The impact of neighborhood-level social determinants on breast cancer mortality after a second primary breast cancer.

Ifeoma Nwigwe, Michael Desjardins, Kala Visvanathan

Background: Our team has shown that younger versus older women, and both Blacks and Hispanics breast cancer (BC) survivors versus White women have a higher mortality after a second cancer. We have also shown that county-level neighborhood poverty is associated with increased cancer mortality after a BC diagnosis. In this study, we assess to what extent neighborhood-level socioeconomic status (SES) contributes to high BC mortality after second cancers and compare the association to BC mortality after a first cancer. We hypothesize that social determinants are critical to address in women who develop a second cancer despite now being part of the health system.
Methods: Women diagnosed with a first BC and/or second cancer after first BC between 2007 and 2019 were identified from the Maryland (MD) Cancer Registry. Census tract-level neighborhood SES environments for poverty and median income were determined using local spatial autocorrelation analysis with Local Moran's I of MD's five-year census-tract estimates from the American Community Survey. MD census tract estimates above or below the state mean were identified as high (H)- or low (L)- tracts, respectively. We defined four neighborhood environments for each SES indicator as H-tracts among H-tracts (HH-tracts); L-tracts among L-tracts (LL-tracts); H-tracts among LL-tracts (HL-tracts); L-tracts among HH-tracts (LH-tracts). Women at least 20 years of age diagnosed with a local or regional primary BC, received surgery, and survived more than 365 days were assigned a tract-level neighborhood SES environment for each SES indicator associated with their first and second BC diagnosis. Time-to-event analyses were used to compare BC deaths across each tract-level SES indicator neighborhood environment separately. The data was analyzed using Multivariate Cox proportional hazard regression models adjusted for age at diagnosis,

tract median income, cancer stage, treatment, and Estrogen Receptor status. Models were also stratified by race/ethnicity (Non-Hispanic White, Non-Hispanic Black), age at diagnosis (<50, 50-69, =>70), and treatment (hormone therapy and chemotherapy).
Results: The analytical population included 15,149 MD women diagnosed with early-stage BC, of which 2,436 developed a second cancer, and BC deaths after first BC and second cancer diagnosis was 2039 and 315, respectively. We detected a 66% (HR=1.66, 95%CI=1.09, 2.53), 14% (HR=1.14, 95%CI=0.66,1.97), and 15% (HR=1.15, 95%CI=0.73,1.81) increase in BC death for women in LL, LH, and HL median income census tracts when compared to HH median income census tracts in the neighborhood income model after second cancer diagnosis. A 30% (HR=1.30, 95%CI=0.89, 1.90), 31% (HR=1.31, 95%CI=0.90, 1.93), and 20% (HR=1.20 95%CI=0.80, 1.79) increase in BC deaths for women in HH, LH, and HL poverty census tracts when compared to LL poverty census tracts was observed in the neighborhood poverty model after second cancer diagnosis. Notably, we observed increased BC death for women in LL, LH, and HL income neighborhood environments as well as in HH and HL poverty neighborhood environments at first BC diagnosis though not as robust as the second cancer neighborhood environment observations. Finally, neighborhood-level SES environments at first BC diagnosis were not statistically associated with BC death upon second diagnosis.
Conclusion: Overall, our findings highlight the importance of neighborhood-level SES, such as poverty and median income, at the time of second cancer diagnosis as a strong indicator of BC mortality after second cancer, possibly even more than after a first BC. These findings provide initial evidence of the need for continued surveillance of neighborhood-level SES factors after a first BC diagnosis, and early interventions to improve survival among this group of high-risk women.

P5-04-04: Comprehensive Analysis of Breast Cancer Treatment Delays in Brazil: A Six-Year Study

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Background: Breast cancer is the most common cancer among women in Brazil and timely treatment initiation is crucial for improving prognosis and survival rates. Despite the 60-day rule established in 2012, which mandates the start of treatment within 60 days of diagnosis, significant delays continue to be reported.
Objectives: This study aims to assess the interval between diagnosis and the commencement of breast cancer treatment in Brazil from 2017 to 2022. Secondary objectives include examining the association between the duration before treatment and both the type of treatment and cancer stage, as well as analyzing annual trends in treatment initiation times.
Methods: This ecological study employed a descriptive observational approach, analyzing data from the Oncology Panel of the Brazilian Unified Health System's Department of

Informatics (DATASUS). The data encompassed female breast cancer cases across Brazil's regions and states over a six-year span (2017-2022). Variables analyzed included time to treatment initiation, cancer staging, and type of therapeutic intervention. Trends in time-to-treatment were evaluated using chi-square tests and joinpoint regression, with the Annual Percent Change (APC) quantifying changes over time. Given the study's reliance on public datasets, ethical committee approval and informed consent were not required.
Results: The study identified 237,073 breast cancer cases in women from 2017 to 2022. During this period, 24.4% of patients began treatment within 30 days, 21.3% waited between 31 and 60 days, 16.8% between 61 and 90 days, and 37.2% took more than 91 days, with statistically significant differences ($p < 0.001$). Radiotherapy displayed the longest delays, with 85% of patients waiting over 61 days to commence treatment, followed by chemotherapy (57.2%) and surgery (30.8%), each with significant variances ($p < 0.001$). Patients in early stages (0, I, and II) experienced longer delays compared to those with advanced cancer (stages III and IV), with significant timing differences ($p < 0.001$). Trend analysis revealed a decrease in patients starting treatment after more than 121 days, but also a decrease in those starting within 30 days (APC -1.15, $p = 0.461$), and an increase in the 61 to 91 days bracket (APC 4.03, $p = 0.027$), surpassing the 31 to 60 days bracket (APC 3.27, $p = 0.080$).
Conclusion: The study highlights significant delays in the initiation of breast cancer treatment for most women in Brazil, often exceeding the recommended 60-day threshold and leading to treatment at more advanced disease stages. The longest delays were observed in radiotherapy, and patients with early-stage cancer faced the longest wait times. Trend analysis indicates an elongation in treatment initiation times post-implementation of the 60-day rule, underscoring the need for targeted efforts to expedite the onset of breast cancer treatment nationwide.

P5-04-05: Impact of racial and socioeconomic disparities on overall survival in Invasive Lobular Carcinoma

Toru Yoshino, Ryota Sato, Stnaley Lipkowitz, Takeo Fujii

Background: Invasive Lobular carcinoma (ILC) is the second most common histology and accounts for 10-15% of all breast cancers. ILC has molecular and clinical characteristics distinct from Invasive Ductal Carcinoma (IDC). The loss of E-cadherin expression is a hallmark of ILC and clinically ILC has a linear growth pattern, resulting in delaying radiographical detection with larger sizes and more lymph node involvement at the time of diagnosis. Although ILC has better short-term outcomes than IDC, its overall survival (OS) is reported to be worse due to late recurrences and resistance to chemotherapies. In breast cancer in general, it is well known that racial and socioeconomic disparities are crucial factors associated with poor prognosis. However, the impact of them on the survival outcomes of ILC in particular is not well understood, regardless of the unique features of ILC. In this study, we investigated how the racial, social economic factors as well as clinicopathological factors affect OS in ILC. Our primary objective was to test the association

between racial and socioeconomic factors and OS among patients with ILC.
Methods:The data was obtained from the Surveillance, Epidemiology, and End Results (SEER) Plus Data 17 registry. Female patients with ILC who were diagnosed from 2010 to 2015 were included. Patients with missing racial information were excluded. Age (50 or more), race, tumor grade, subtype, stage, types of surgery of the primary site, types of systemic therapies, types of radiation therapies, median household income, geographic location, and marital status were collected. Racial categories were classified into White (W), Black or African American (B), American Indian or Alaska Native (AIAN), Asian (A), and Native Hawaiian or Other Pacific Islander (NHPI) based on the U.S. Office of Management and Budget (OMB) standard. Univariate and multivariate Cox proportional hazards models were used to test the association between variables of interest and OS. P value less than 0.05 was considered as statistically significant.
Results: A total of 33,239 patients with ILC were included in the analysis. Among them, 28,401 (85.4%) were W, 2,780 (8.4%) were B, 1,708 (5.1%) were A, 176 (0.5%) were AIAN, and 174 (0.5%) were NHPI. B and NHPI were diagnosed with more advanced stage (Stage III and IV; 25.5% [B], 24.2% [NHPI] vs 20.8% [W]). B and AIAN had lower household incomes in ILC patients (<$65,000; 51.3% [B], 48.8% [AIAN] vs 34.8% [W]). Sixty percent of B were unmarried compared to 39.3% in W.In the multivariate analysis, lower household incomes and unmarried status remained significantly associated with short OS, which is consistent with prior reports. Interestingly, B, AIAN, or NHPI race or distant geographical location were not a significant factor in OS among this ILC patient population (B race; HR 1.00, 95% CI, 0.92-1.09, P=0.97; AIAN race; HR 1.1, 95% CI, 0.76-15.7, P=0.62; NHPI race; HR 1.07, 95% CI, 0.74-1.54, P=0.72; non-metro non-adjacent to metro location; HR 0.96, 95% CI, 0.93-1.16, P=0.56).
Conclusions: Our study demonstrated that lower household incomes and unmarried marital status but not race or geographical location were significant prognostic factors associated with short OS. This suggests that addressing the challenges in financial and social support could be effective to improve the OS of the patients with ILC. Biological differences of ILC in each race need to be investigated further.

P5-04-06: Demographic differences in an SHG-based prognostic indicator.

Tresa Elias, Edward B Brown IV, Bradley Turner

Improved prognostication of metastasis and prediction of therapeutic efficacy can enhance patient-tailored care. Recent studies suggest that several second harmonic generation (SHG)-based measurements of fibrillar collagen properties in the primary tumor may help improve current prognostication and prediction methods. One of these SHG-based measurements is the ratio of forward-scattered to backward-scattered SHG signal (F/B) from collagen pixels, which has been shown to be prognostic of metastasis when measured in the tumor stromal interface. Another SHG-based measurement is the variability in overall orientation of imaged collagen fibers, i.e. the average fiber angle variability (FAV), which has been shown to be prognostic of metastasis when measured in the tumor bulk.
For an improved metastasis prognostication tool to have clinical impact, a multicenter trial

must eventually take place. Unfortunately, minorities are typically underrepresented in research cohorts. Here we explore how patient race affects these prognostic signals in breast cancer. This will provide insight into the influence of patient demographics on the design of any future trial of these prognostic methods. Archived formalin fixed paraffin-embedded sections of 246 estrogen receptor positive invasive ductal carcinoma (IDC) were acquired, with 19 African-Americans (AAs) and 227 Caucasian-Americans (CAs). Sections were imaged with SHG in both the forward-scattered and backward-scattered direction. Specifically, three regions in the tumor-stromal interface (for F/B analysis) and three regions in the tumor bulk (for FAV analysis) were imaged. This sample set behaved similarly to previous sets, with F/B values in the tumor bulk being lower than in the tumor-stromal interface (10.55±4.88 vs 15.04±5.78, respectively, $p<0.0001$). In terms of patient demographics, we found that AA IDC tumors displayed a statistically significantly lower F/B than CA IDC tumors (11.98±5.27 vs 15.34±5.68, respectively, $p=0.014$). FAV was not different between the two groups (47.15±8.93 vs 46.01±9.20, respectively, $p=0.60$). The lower F/B found in AA patients is indicative of a more aggressive phenotype which is consistent with literature evidence of similar demographic differences in other tumor properties. Since we now see that SHG F/B varies with race, it is possible that the relationship between SHG F/B and metastatic outcome also varies with race. If this is true, then training a prognostic algorithm with a random cohort of patients (who would be overwhelmingly Caucasian-Americans) would disproportionately yield inaccurate predictions for demographics other than Caucasian Americans. Therefore, it should be a priority to recruit enough African-American patients for this future research cohort to develop an SHG-based prognostic algorithm that equally serves both demographics. If it is found that the relationship between SHG F/B and metastatic outcome does vary with race, then different prognostic algorithms can be developed for different demographic groups.

P5-04-07: Assessing COVID-19 vaccination status and symptoms in persons with a history of breast cancer

Iqra Siddiqui, Nikita Nikita, MD, Ana María López

Background: Persons with a history of cancer are at higher risk for adverse COVID-19 outcomes due to their immunosuppressed status. Vaccination to COVID-19 has proven effective in decreasing complications from COVID-19 infection. This study explored the vaccination status of persons with a breast cancer diagnosis receiving cancer care who had received the COVID vaccine 1st dose/2nd dose and/or COVID booster along with their consequent symptoms

Methods: Persons with a cancer diagnosis receiving care at a cancer center clinical site were randomly invited to complete a voluntary survey regarding their experience with COVID vaccinations via email, the electronic medical record, or during an in-person appointment. Paper surveys were completed in the clinic, placed in a sealed collection bin, and collected

by research staff weekly. Responses were anonymous. Participants were asked if they had received their 1st/2nd COVID-19 vaccine, their symptoms, and if they had received or intended to receive the subsequent booster. The study was conducted between September 2021 to September 2022. An EMR review of vaccination uptake was conducted to assess representativeness of our sample. We used a crude logistic regression model to assess the differences in post-vaccination symptoms reported by persons with breast cancer who were vaccinated. We stratified the results by race (Black versus white, non-Hispanic) and age (< 65 years and >=65 and older).

Results: Of the 532 persons with breast cancer who completed the COVID vaccination survey, 155 (31.6%) were Black and 335 (68.4%) were white, non-Hispanic. 255 (47.9%) were <65 years of age and 277 (52.1%) were greater 65 years of age. We found that patients older than 65 years of age were less likely to experience fever (by 58.8%), pain (by 58.3%), fatigue (by 48.9%), and headache (by 49.4%). Person over 65 years of age were 55.9% more likely to have received their second COVID vaccination and 67.3% more likely to have received their booster. Persons who identified as Black reported a different symptom profile--61.9% less likely to experience fever and 56.3% less likely to report fatigue following vaccination compared to their white, non-Hispanic counterparts.

Conclusion: In our data set, persons with a history of breast cancer older than 65 years were less likely to have experienced fever, pain, fatigue, and headache after COVID vaccination. Black persons were less likely to report fever and more likely to report pain. Anticipating differences in COVID-19 vaccination symptoms allows for the development of tailored education and resources for self-management and care.

P5-04-08: The role of sexual orientation in mammogram screening adherence among deaf, deaf blind, and hard of hearing women.

Erika Bergeron, Sowmya Rao, Poorna Kushalnagar

Background: Deaf, deafblind, and hard of hearing (DDBHH) women using American Sign Language (ASL) experience disparities in cancer screening and cancer-related health outcomes. There is a need for data on breast cancer screening among DDBHH women, specifically considering differences in sexual orientation, and whether they adhered to U.S. Preventive Task Force screening guidelines or not. The study objective is to assess the role of sexual orientation on mammogram adherence among DDBHH women adjusting for other characteristics. Methods: Using the NCI Health Information National Trends Survey in ASL (Kushalnagar et al., 2015), we surveyed adult participants to assess the U.S. Preventive Services Task Force (USPSTF) guidelines' age-appropriate adherence to mammogram. We used Fisher's Exact test to assess the independent relationship of sexual orientation with mammogram. Further, we obtained odds ratios (OR) and 95% confidence intervals (CI) from a multivariable logistic regression evaluating the same relationship adjusting for age,

race (Non-Hispanic White, Others), education (less than college, college graduate, post-graduate), marital status (married/living with partner, divorced/widowed/separated/never married) and whether they had a pap test (no, yes). A two-sided p<=0.05 was considered significant. Results: Survey response rate within three weeks of mail out in July 2023 was 17%. Participants were recruited in-person between September and November 2023 and virtually in March and April 2024. A total of 293 DDBHH adult participants (45-74 years) answered screening questions for breast cancer in ASL and English. Within this group, 91.5% self-reported screening adherence for mammogram. In a published study, similarly aged hearing women reported 75.6% adherence in the English version of the National Health Interview Survey (NHIS). After adjusting for correlates within the DDBHH sample, LGBTQA+ respondents were less likely (not statistically significant) to have a mammogram than straight persons [0.43 (0.16, 1.20); p=0.11]; adherence increased with increasing age [OR (95% CI): 1.17(1.08,1.26); p<0.001]. Discussion: With the 91.5% adherence rate, the DDBHH community has greater adherence to the recommended screening guideline compared to the general population of hearing adults. Yet, within this group, those identifying as LGBTQA+ exhibit lower adherence to breast cancer screening guidelines. Bridging these gaps is essential to achieve the Healthy People 2030 target of 80.3% screening adherence. Such research will aid the Center for Deaf Health Equity in actively working with the DDBHH community, particularly among LGBTQA+ individuals, to improve strategies for increasing breast cancer screening.

P5-04-09: A nationwide database study breast cancer in younger women: Chile 1997-2023

Claudio Salas, Iris Delgado, Yanara Bernal

Introduction: In Chile, official databases related to mortality and hospitalization record all events. The Chilean healthcare system is segmented, with 80% of the population belonging to the public system and 20% of higher-income individuals accessing the private system, which provides greater access to healthcare and personalized technology. Objective: to describe the evolution of the magnitude and risk of mortality and hospitalization of BC in women, according to epidemiological variables of time, place, person and socioeconomic level with an emphasis on women those < 40 years of age (younger). MATERIAL AND

Method: Descriptive analytical study with official secondary databases from Chile, to examine mortality from years 1997 to 2023, and hospitalization from 2001 to 2021, both sources having nearly 100% of total population coverage. Study variables: time (year of event occurrence), place of residence (urban/rural), person (age), and C50 code (ICD-10) to identify BC cases. Socioeconomic status was proxied by educational level for mortality and health insurance (public/private) for hospitalization. Due to the number of cases for mortality, the evolution was studied over three-year periods (triennia), and annually for hospitalization. Crude and age-adjusted rates were calculated, and the percentage change was analyzed using regression models with the Joinpoint Regression Program V5.2

software. All rates were calculated according to age range variables, place and health insurance. Data reading and analysis was carried out using Software R V6 (2023).

Results: Between 1997-2023, 35,540 women died from BC, of which 1,507 were <40 years old and 33,772 were 40 years old or older. In the 1997-1999 triennia, the crude mortality rate of women aged 40 and over was 20 times higher than that younger woman; at the end of the period (2021-2023) this ratio had decreased to 12 times. During the years studied, the risk of dying from BC increased by 19.2% in younger women, while in women 40 years of age and older, the risk decreases by 21%. From 1997 to 2014, the percentage change in mortality over triennia for in younger women was not significant. However, from 2015-2017 triennia onwards, the trend showed a statistically significant percentage increase of 10.3%. Regarding the analysis by place of residence, mortality in younger women increased in rural areas than in urban areas. Regarding education, younger women, on average, more than 3 years of schooling compared to those 40 years old and older. In relation to hospitalization between 2001-2021 there were a total of 179,999 events, with 9.5% (16,818) are younger women. The increase in magnitude was 300% in the group <40 and 320% in those aged 40 and over. The risk of hospitalization in younger women increased 1.5 times during the period, while for older women, it increased by 1.13 times. The analysis of insurance membership showed that women in the private system had greater access to hospitalization than those in the public system. This inequity was more accentuated in younger women, with a gap of 2.4 times greater access to hospitalization for those in the private system compared to the public system. In older women, this gap was 1.8 times.

Conclusions The analysis of public data includes the last 27 years, a period in which an increase in mortality from BC in younger women of 19% has been observed, increasing from 2015 onwards. The rise in mortality from BC in young women is multifactorial, it is diagnosed in more advanced stages, more aggressive tumor biology with a predominance of triple-negative disease, but it could also be influenced by an increase in incidence, as indicated by BC hospitalization rates. This analysis reveals significant gaps in the Chilean healthcare system, indicating higher mortality rates from BC cancer in young women from rural areas and within the public health sector. It provides empirical evidence to support targeted interventions for women with limited access to healthcare.

P5-04-10: Age at Breast Cancer Diagnosis and Associated Demographic Factors: An Epidemiological Study in Brazil

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Background: In recent years, shifts in the median age at breast cancer (BC) diagnosis have been observed in various countries. Multiple factors influence these changes, including demographic shifts, sex, lifestyle modifications, and genetic factors. As populations age and demographics change, there may be significant impacts on these trends. Lifestyle factors such as diet, exercise, and environmental factors also play a role. Genetic research has

emphasized the role of inherited factors in BC age onset. This study aims to analyze the trends in median age at BC diagnosis in Brazil, focusing on sociodemographic aspects like the distribution of the Human Development Index (HDI), sex, and variations among different racial and ethnic groups based on race/skin color.
Patients and methods: Data from 32 Brazilian Population-Based Cancer Registries spanning 1990 to 2020 were used to analyze BC median age trends based on age at diagnosis, sex, racial group, and geographical region. Racial and skin color information was collected through self-declaration aligning with categories like white, indigenous, yellow (Asian), black, and mixed race set by the Brazilian Institute of Geography and Statistics. Due to limited HDI data for Brazilian states and municipalities in 2000 and 2010, a mean HDI estimated for 2005 was utilized. Variations in the mean HDI for the year 2005 were observed across municipalities, with classifications ranging from low HDI (0.50 to 0.59) to moderate HDI (0.60 to 0.69), high HDI (0.70 to 0.79), and very high HDI (0.80 to 1.00). Analysis of median age trends employed Joinpoint models, with the Joinpoint Regression Program providing confidence intervals to assess the null hypothesis of APC = 0 and p-values to evaluate potential trend changes. Statistical significance was determined at $p < 0.05$.
Results: Between 1990 and 2020, there were 227,896 new documented cases of breast cancer (BC). After excluding 7,850 cases with missing sex or age data, 225,436 cases were analyzed. Among these cases, 99.2% were female and 0.8% were male, resulting in a ratio of 1 man for every 120 women diagnosed. The median age at diagnosis in Brazil was 56 years. Municipalities with lower and moderate HDI levels had a lower median age at diagnosis of around 53 years. Conversely, regions with higher and very high HDI levels showed a higher median age at diagnosis, ranging between 56 and 58 years, respectively. Black and Indigenous patients had a younger median age at diagnosis (54 years) compared to white patients (58 years). The median age at diagnosis of breast cancer has shown an increase over the last decade, attributed to improvements in HDI, with the most significant rise observed in the northern region of Brazil.
Conclusion: This study highlights significant differences in the median age at breast cancer diagnosis across different regions, with earlier onset associated with lower HDI areas. Furthermore, Black and Indigenous patients exhibited a younger median age of diagnosis, reflecting previous research that has shown disparities in BC within the Brazilian population concerning race.
Keywords: Breast cancer; median age; disparities; incidence.

P5-04-11: Quality of life and psychosocial disparities between ethnically and religiously diverse population with advanced breast cancer

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Background: In Israel all residents are covered by universal health care and yet disparities in health care access and utilization exist. Jerusalem is the largest and poorest city in Israel,

with significant with significant Jewish ultra-orthodox and Arab communities. These groups have shared reproductive factors, similar patriarchal community structures and often live in poverty. No research has been performed on these populations in the advanced breast cancer (ABC) setting with respect to quality of life (QOL) and psychosocial wellbeing

Methods: Women with ABC were prospectively recruited after consenting and self-identified their religion & level of religiosity. The following measurement tools were implemented: QOL - Functional Assessment of Cancer Therapy-Breast (FACT-B) Version 4, Spiritual wellbeing - The FACIT-Sp, Depression and Anxiety - Patient-Reported Outcomes Measurement Information System and demographic questionnaire. Comparisons between groups were conducted and the role of religion and depression in the explanation of differences of QOL between the groups was tested by moderated mediation model.

Results: 160 ABC patients were recruited. 39 (24.4%) identified themselves as Ultra-Orthodox Jews, 35 (21.9%) as Arabs and 81 (50.6%) as non-ultra-orthodox Jewish. Groups differed in their health related QOL($F(2,150) = 10.49$, $p<.001$); Spiritual wellbeing ($F(2,150) = 26.09$, $p<.001p<.001$) and depression ($F(2,134) = 9.61$, $p<.001$).
Ultra-Orthodox patients reported higher levels of QOL and spiritual wellbeing compared to the other groups and lower levels of depression compared to the Arab patients. Arab patients reported lower levels of QOL and higher levels of depression compared to the other groups. In addition, they reported lower levels of spiritual wellbeing compared to Ultra-Orthodox patents.
There were significant differences between groups in physical ($F(2,152) = 13.96$, $p<.001$); functional ($F(2,151) = 7.12$, $p<.001$); emotional ($F(2,151) = 8.81$, $p<.001$) and breast cancer wellbeing ($F(2,152) = 10.21$, $p<.001$) as measured by FACT-B.
Throughout the different domains of QOL Arab patients did worse (except the social/family subscale) than the Ultra – Orthodox patients. In addition, they reported on lower levels of QOL than the general population in total QOL, physical and functional wellbeing. Ultra – orthodox patients reported on higher levels of QOL in comparison to the general population in Total QOL, emotional well- being and breast cancer wellbeing.
The study's moderated mediation model was found to be significant ($F (2,126) = 121.16$, R-sq = 65.8%, $p < 0.000$). The differences between Arab and Jewish patients in Health related QOL were found to be mediated by depression, with group-depression relationship moderated by religiosity levels.

Conclusions: Significant health care disparities exist amongst women with ABC in terms of QOL, psychosocial and spiritual wellbeing. Although the current study examined two minority groups who have shared low socio-economic status, similar reproductive habits (multiple pregnancies from an early age), paternalistic and patriarchal community structure and challenges in health care access for cultural-ethnic-social reasons, different patterns of well-being were found. Ultra-Orthodox patients reported superior health related QOL, spiritual wellbeing, and lower levels of depression compared to other groups. Arab patients

reported poorer health related QOL, higher levels of depression and lower levels of spiritual wellbeing and religiosity compared to Ultra-Orthodox patients. Further research is needed to create effective, culturally appropriate interventions.

P5-04-12: Comparative Analysis of Female Breast Cancer: Incidence, Mortality, and 5-Year Survival Rates Across Races and Diagnosis Stages in Different Age Groups

Navneet Kaur

Background: Significant advancement has been made in breast cancer care, resulting in improved outcomes, largely due to enhanced screening and targeted management. However, these benefits are not equally distributed across all population subgroups. Factors such as genetics, access to healthcare, racial identity, social determinants of health, education, and clinical trial enrollment opportunities all contribute to persistent disparities in breast health. This study aims to compare female breast cancer incidence, mortality, and 5-year survival rates based on age, race, and stage at diagnosis.
Method: Data were extracted from the SEER 22 registries database, focusing on age, stage at diagnosis, and race. Rates are per 100,000 and age-adjusted to the 2000 US Standard Population (19 age groups - Census P25-1130). Mortality data was sourced from U.S. Mortality Data (1969-2022), National Center for Health Statistics, CDC. We compared incidence, mortality, and 5-year survival rates across different racial groups and age categories.
Results: The incidence rate in Hispanic women was 101.2 (100.5-101.9), in Black women was 129 (128.3-130.3), and in White women was 139.0 (138.5-139.4). The rate for localized breast cancer was 60.7 (60.2-61.2), 74.1 (73.3-74.8), and 93.7 (93.3-94.1) in Hispanic, Black, and White women, respectively. The rate for distant breast cancer was 6.4 (6.2-6.5), 11.5 (11.2-11.8), and 7.5 (7.4-7.6) in Hispanic, Black, and White women, respectively. The incidence rate in women aged 15-39 years was 19.8 (19.4-20.3), 27.2 (26.4-28.0), and 24.3 (23.9-24.7), and for ages 40-64 years was 175 (174.2-177.3), 218.4 (216.1-220.7), and 234.5 (233.3-235.7) for Hispanic, Black, and White women respectively. For women aged more than 75 years, the incidence was 291.6 (286.0-297.4), 408.8 (401.0-416.7), and 454.8 (451.8-457.8) for Hispanic, Black, and White women, respectively.
The mortality rate was 13.7 (13.5-13.9), 26.8 (26.5-27.1), and 19.4 (19.3-19.5) in Hispanic, Black, and White women respectively. For ages 15-39 years, the mortality rate was 1.8 (1.7-1.9), 3.8 (3.6-4.0), and 2.0 (1.9-2.1). For ages 40-64 years, the mortality rate was 17.8 (17.4-18.2), 37.1 (36.4-37.7), and 22.7 (22.5-22.9). For ages 65-74 years, the mortality rate was 43.4 (42.0-44.9), 85.0 (83.2-87.0), and 63.3 (62.6-63.9) in Hispanic, Black, and White women, respectively.
The 5-year survival rates (%) for Hispanic, Black, and White women were 88.4 (88.1-88.7), 83.8 (83.4-84.3), and 93.0 (92.8-93.1) respectively. Age- and race-adjusted 5-year survival rates were as follows: for ages 15-39, 84.6% (83.5-85.6) for Hispanic, 79.7% (78.3-81.0) for Black, and 89.1% (88.5-89.7) for White women. For ages 40-64, 89.4% (89.1-89.8) for

Hispanic, 84.6% (84.1-85.1) for Black, and 93.4% (93.2-93.6) for White women. For ages 65-74, 91.2% (90.4-91.9) for Hispanic, 86.3% (85.3-87.2) for Black, and 94.4% (94.1-94.7) for White women. For ages 75 and over, 79.7% (78.0-81.3) for Hispanic, 78.7% (76.7-80.5) for Black, and 90.6% (90.0-91.2) for White women.
Conclusion: White women had the highest incidence rate of breast cancer, followed by black and Hispanic women. Localized breast cancer incidence was highest in white women, while distant breast cancer incidence was highest in black women. Young black women (ages 15-39) had the highest incidence rates for their group. Mortality rates were highest among black women across all age groups. Overall and age-adjusted 5-year survival rates were lowest for black women, followed by Hispanic and white women.

P5-04-14: Variants of PEAK1, SLC2A4RG, PALB2, and XIRP2 and their relationship with Genetic Ancestry across Breast Cancer Patients from different Racial/Ethnic groups.

Grace Vélez Crespo, Julie Dutil, Vivian Colón López

Breast cancer is the second leading cause of cancer deaths worldwide, affecting 1 in 8 women. Disparities in breast cancer incidence and mortality have been observed across different racial and ethnic groups, with Hispanic and African American women experiencing higher incidence and mortality rates. Differences in the genomic landscape of breast cancer patients have been linked to early age at diagnosis, worse disease progression, drug resistance, and relapse.
Using the All of Us research hub, one of the largest biomedical databases, we are investigating genetic variants and the genetic ancestry related to PEAK1, PALB2, SLC2A4RG, and XIRP2 across racial and ethnic groups to identify potential variations that can contribute to differences observed among these groups. These genes are involved in DNA repair, epithelial-to-mesenchymal transition (EMT), and cell cycle regulation. PEAK1, for instance, regulates cell proliferation, promotes invasion and metastasis in various cancers, including breast cancer, and is associated with drug resistance. PALB2 functions as a tumor suppressor involved in homologous recombination repair, with pathogenic variants linked to hereditary breast cancer in Hispanics. SLC2A4RG acts as a tumor suppressor by regulating the cell cycle and has been studied in glioblastoma and breast cancer. XIRP2, known for its roles in cardiovascular diseases, is implicated in breast cancer metastasis and colon cancer promotion.
Despite identifying many genes involved in breast cancer development and metastasis, there remains a need to understand genetic variability across different populations to develop better diagnostic tools and improve treatment for breast cancer patients. Our cohort includes 6,056 cases of breast cancer, from which our preliminary results showed that Hispanics and African Americans are diagnosed with breast cancer at an earlier age (median = 64 and 67 years, respectively) compared to Non-Hispanics (median = 71 years). Using a genetic ancestry approach and in accordance with other studies, we identified that the majority of our Hispanic population are related to the Admixed American ancestry, with

smaller portions of European and African ancestry. By accessing the All of Us research hub's control tier for genetic data, we identified the frequency of genetic variants of PEAK1, PALB2, SLC2A4RG, and XIRP2. Using a Minor Allele Frequency (MAF) of 0.01 as a parameter, we identified 27 variants for PEAK1, 12 for PALB2, 2 for SLC2A4RG, and 99 for XIRP2.
Our current and upcoming analyses include associating the identified genetic variants with ancestry and race/ethnicity among cases already identified in our cohort and dataset, and comparing them with our control groups to identify possible genetic variants involved in breast cancer development across the different populations under study.

P5-04-15: Examining the association between biological age, stress, and resilience among diverse breast cancer survivors using the NIH All of Us Dataset

Bathsheba Aklilu, Erica Tate, Dr. Cathy Samayoa

Breast cancer disparities exist by race and ethnicity. This includes disparities in age at diagnosis, quality of life, and health outcomes. These disparities may be due to chronic stress, which can result in premature aging. Telomere shortening, a hallmark of biological aging, is a promising biomarker that has been associated with adversity, chronic stress, and health outcomes. However, most studies lack racial and ethnic minorities and thus biological age among diverse breast cancer survivors remains unknown. This study aims to investigate the factors associated with telomere length attrition among diverse breast cancer survivors using the NIH All of Us research dataset. Using Python and Jupyter Notebook within the NIH All of Us dataset, we will examine whole genomic data to quantify telomere length via a short-read whole genomic sequencing average telomere length analysis. We will also examine the relationships between telomere length and stress and resilience factors. We hypothesize that telomere length will be shorter among racial and ethnic minorities, and also in women who report higher levels of stress. We expect telomere length to be positively associated with resilience. This study will provide a greater understanding of the biological mechanisms driving breast cancer health inequities.

P5-04-16: Stromal tumor-infiltrating lymphocytes-based model predicts efficacy for neoadjuvant therapy in HER2-low early breast cancer

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Human epidermal growth factor receptor 2 (HER2)-low early breast cancer (EBC) is less sensitive to neoadjuvant therapy (NAT) and lacks effective biomarkers to predict treatment response. Existing prediction models and biomarkers for pCR in HER2-negative EBC patients are not tailored specifically for HER2-low EBC and often involve complex variables that are challenging to obtain. To date, only one messenger RNA-based multi-omics prediction model has been reported, showing excellent performance in predicting pCR to

NAT in HER2-low EBC. However, this model is complex and difficult to implement. Thus, there is an urgent need to develop predictive models based on easily available measurements to identify HER2-low EBC patients who can mostly benefit from NAT. In this study, we evaluated baseline sTIL numbers and composition in HER2-low EBC and found that sTIL levels were higher and the proportion of immune-promoting cells was higher in patients achieving pCR following NAT, particularly notable in those treated with neoadjuvant chemotherapy and immunotherapy. Furthermore, we investigated the correlation between baseline stromal tumor-infiltrating lymphocytes (sTILs) levels and pathological complete response (pCR) status in 293 eligible HER2-low EBC patients across two prospective studies. Our analysis revealed that patients achieving pCR following neoadjuvant chemotherapy exhibited higher levels of sTILs and a greater proportion of immune-promoting cells compared to non-pCR patients. Subsequently, we randomly divided patients into a training set (n=205) and a validation set (n=88) to develop and validate a sTILs-based predictive model for pCR, which incorporated sTILs, lymph nodal status, hormone receptor status, and Ki67. With favorable discrimination, calibration, and clinical value, the areas under the curve of the model (0.884 in the training set; 0.891 in the validation set) were significantly higher than that of the model without sTILs (0.811; 0.785; both $P<0.05$) and the sTILs-alone model (0.774; 0.792; both $P<0.05$) for predicting pCR. Importantly, patients with a high nomogram score (≥ 106) had significantly better disease-free survival and overall survival compared to those with a low nomogram score (< 106). Additionally, the model also exhibited an encouraging predictive ability for pCR in HER-low/HR-negative EBC patients who received neoadjuvant chemotherapy plus immunotherapy. In summary, HER2-low EBC patients achieving pCR demonstrated elevated sTILs levels and an anti-tumor immune-inflammatory microenvironment at baseline, potentially contributing to the improved efficacy of NAT. The sTILs-based model exhibited excellent predictive ability, offering potential for personalized treatment strategies and improved prognosis in HER2-low EBC patients.

P5-04-17: Association Between Ki-67, Body Mass Index, and Stromal Tumor-Infiltrating Lymphocytes in Hormone Receptor-Positive, HER2-Negative Breast Cancer Patients

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Background: Stromal tumor-infiltrating lymphocytes (sTILs) and the tumor microenvironment are a growing research focus in breast cancer. Previous studies established the prognostic role of sTILs in triple-negative and human epidermal growth factor receptor 2 (HER2)-positive tumors, but the role of sTILs remains unclear in hormone receptor-positive (HR+) tumors. A preclinical study demonstrated that obesity exacerbates immune aging, with diet-induced obese mice showing higher frequency of CD8+ TILs, higher PD-1 expression, and decreased Ki-67; donor cells from healthy obese human patients also showed higher PD-1 expression and lower proliferation rate [1]. Hypothesizing that similar patterns might be seen in patients with HR+ HER2-negative

breast cancer, we assessed for associations between Ki-67, body mass index (BMI), and sTILs in a cohort of these patients who did not receive neoadjuvant systemic therapy.

Methods: We performed a retrospective review of patients with HR+ HER2-negative breast cancer who underwent upfront surgery at Mayo Clinic in Jacksonville, Florida, between January 1, 2022, and June 1, 2023. Height and weight were obtained from the initial breast oncology or breast surgery visit note in the medical record. BMI was calculated using the standardized National Institutes of Health calculator [2]. BMI was categorized as normal (BMI <25), overweight (25 ≤ BMI <30), or obese (BMI 30). sTILs and Ki-67 were evaluated by a breast pathologist. Continuous variables were summarized with the median and interquartile range. Pearson correlation coefficient was estimated to assess for an association between Ki-67, sTILs, and BMI. Wilcoxon rank sum test was performed to identify significant differences of Ki-67 or sTILs between BMI categories. All statistical tests were two-sided, and p-values <0.05 were considered statistically significant. Statistical analysis was performed using R Statistical Software (version 4.2.2; R Foundation for Statistical Computing, Vienna, Austria).

Results: A total of 176 patients were included in the analysis. A moderate correlation was seen between Ki-67 and sTILs percentage (%) with Pearson correlation coefficient of 0.37 (95% CI 0.21-0.52, p <0.001), but there was no significant correlation seen between BMI and sTILs % (r = -0.05, 95% CI -0.2-0.01, p=0.51) or BMI and Ki-67 (r = 0.12, 95% CI -0.06-0.29, p=0.18). When we compared BMI categories, there were no significant differences in Ki-67 (p=0.098) or sTILs % (p=0.735). However, there was a trend towards higher Ki-67 and lower sTILs % in the patients with obese BMI as compared to those with normal or overweight BMI. Median Ki-67 % was 11.9, 11.9, and 16.2 for patients with normal BMI, overweight BMI, and obese BMI, respectively. Median sTILs % was 5, 5, and 3.2 for patients with normal BMI, overweight BMI, and obese BMI, respectively. When grouped by normal/overweight BMI versus obese BMI, Ki-67 was significantly higher in the obese BMI group (11.9 vs. 16.2, p=0.033).

Conclusion: Tumors of HR+ HER2-negative breast cancer patients with BMI in the obese range had significantly higher Ki-67 compared to tumors of patients with lower BMI. Ki-67 correlated with sTILs %; however, we did not identify a significant association between BMI and sTILs %. These findings suggest that BMI 30 might be associated with a more aggressive luminal B subtype of HR+ HER2-negative breast cancer, but it might not be a useful predictive biomarker of benefit of immune checkpoint inhibitor therapy. Larger patient cohorts are needed to validate and clarify these findings.

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P5-04-18: Clinical Validation of an Ultra-Sensitive ctDNA NGS Assay in HR-Positive, HER2-Negative Breast Cancer Patients

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Introduction: Breast cancer remains the most common cancer diagnosis among women worldwide, with the majority of cases being hormone receptor (HR)-positive and HER2-negative. Although endocrine therapies have significantly improved patient outcomes, the risk of disease progression persists, sometimes even during the initial treatment phases. Current diagnostic techniques lack the sensitivity needed for patients with low tumor fractions and circulating tumor DNA (ctDNA) shedding in the early stages of disease or during early disease progression. This study presents the clinical validation of an ultra-sensitive ctDNA Next-Generation Sequencing (NGS) assay, for comprehensive genomic profiling in HR-positive, HER2-negative patients receiving endocrine therapy and experiencing minor progression.

Methods: PredicineCARE Ultra is an ultra-sensitive liquid biopsy assay featuring a proprietary NGS panel targeting key oncogenic genes. This assay utilizes ultra-deep sequencing, surpassing 100,000x coverage, allowing for the detection of genomic alterations with a sensitivity far exceeding that of standard ctDNA NGS assays, which typically achieve around 20,000x coverage. In this study, we enrolled 58 patients with advanced HR-positive, HER2-negative breast cancer. Plasma samples were collected after first-line aromatase inhibitor (AI) therapy and before initiating second-line treatment with Fulvestrant. These samples were analyzed using the ultra-sensitive ctDNA assay to detect potential residual or progressive disease and to profile gene alterations.

Results: Among the 58 patients, the ultra-sensitive assay detected positive tumor fractions in 43 individuals. The median tumor fraction among these 43 patients was 1.98%, ranging from 0.50% to 58.5%. In total, the assay identified 193 somatic mutations, 95 gene copy number variations (CNVs), and one FGFR3 gene fusion (FGFR3-BAIAP2L1). The five most frequently mutated genes were TP53 (25.9%), PIK3CA (20.7%), ATM (19.0%), ESR1 (17.2%), and BRCA2 (15.5%).
These results were compared against a down-sampling algorithm simulating standard ctDNA assays with 20,000x coverage. The PredicineCARE Ultra cfDNA NGS assay detected 31.3% more mutations (193 mutations vs. 147 mutations) and 30.1% more CNVs (95 CNVs vs. 73 CNVs). It was particularly effective in identifying mutations with allele frequencies (MAF) below 0.1%, uncovering 10 additional mutations not detected by standard sensitivity

assays, with the lowest MAF being 0.03% compared to the 0.10% threshold of standard assays. This increased sensitivity was notable in genes linked to targeted therapies and drug resistance, revealing 30.7% more variations in PIK3CA and 11.1% more in ESR1, with the lowest MAFs of 0.08% and 0.06%, respectively. Moreover, the FGFR3-BAIAP2L1 gene fusion was uniquely detected by the ultra-sensitive assay at a MAF of 0.09%.

Conclusion: The PredicineCARE Ultra ctDNA NGS assay showcased a superior ability to detect low tumor fractions and low-frequency mutations in HR-positive, HER2-negative breast cancer patients, significantly outperforming standard liquid biopsy assays. This enhanced sensitivity offers substantial benefits for early identification of treatment resistance and disease progression, potentially allowing for more timely and tailored therapeutic interventions.

P5-04-19: Gene variants in circulating tumor cells as markers of resistance in patients with metastatic breast cancer receiving CDK4/6 inhibitors

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Background: Predictive biomarkers for the stratification of patients with metastatic (M), hormone receptor-positive/HER2-negative (HR+/HER2-) breast cancer (BC) to receive CDK4/6 inhibitor (i) treatment before therapy start are still missing to protect patients with primary resistant disease from starting ineffective therapies and minimize unnecessary toxicities. Here, we aim to identify markers of primary and acquired resistance to CDK4/6i therapy by sequencing genomic (g) DNA of circulating tumor cells (CTCs) at baseline and at the time of progression.
Patients and Methods: CTCs were isolated from 10 ml EDTA blood of 33 HR+/HER2- MBC patients at baseline of CDK4/6i plus endocrine therapy (TX) and at the time of progression using positive immunomagnetic selection (AdnaTest EMT2/StemCell Select). mRNA-depleted CTC lysates were used to isolate gDNA by a newly established workflow (AllPrep DNA/mRNA Nano) and libraries were constructed with a customized QIAseq® Targeted DNA Pro Panel, targeting hotspots or all exonic regions of 20 genes (AKT1, AR, BCL11B, CXCR4, EPCAM, ERBB4, FAT1, FGFR1, FGFR2, HLA-A, KRAS, MAPK1, MET, MLH1, NF1, NFKBIA, RB1, STAT1, STAT3, YAP1). All consumables: QIAGEN, Germany. Pooled libraries were analyzed by 150bp paired-end sequencing on an Illumina NovaSeq instrument. Buffy coat DNA samples as germline controls were available from 24/33 patients. Statistical analysis was conducted by log-rank testing, multivariate Cox regression and two-sided exact Fisher test. Non-Responders were defined as patients showing a progression-free survival (PFS) of six months or less (primary resistance).
Results: Variants in all of the 20 genes were found by sequencing CTCs and matched buffy coat samples (mean UMI coverage: 665) – in total, 225 different variants were called. Comparing the prevalence of variants in CTCs at baseline and at the progressive disease time point revealed increased variant counts after CDK4/6i TX in MET and RB1,

respectively. Patients with at least one variant in BCL11B in their CTCs at baseline showed a significantly decreased PFS (p=.042). In contrast, patients with FAT1 p.S3554A or FAT1 p.I2718V detected in their CTCs at baseline had a significantly prolonged PFS (p=.046/.027). In CTCs at baseline, HLA-A variants [p.T345S (log-rank p=.034, Fisher p=.010), p.K335N (log-rank p=.011, Fisher p=.015), p.I306V (log-rank p=0.011, Fisher p=.015), p.R138H (log-rank p=.002, Fisher p=.010), p.Y123F (log-rank p=.011, Fisher p=.015), p.F33S (log-rank p=.015, Fisher p=.010)] significantly correlated with a reduced PFS and their prevalence was significantly different in Responders (n=19) compared to Non-Responders (n=13). HLA-A p.L180* in CTCs at baseline significantly related to a reduced PFS (p=0.011), but not to response. The prevalence of the latter variant increased after CDK4/6i TX in comparison to the baseline time point. Interestingly, HLA-A variants in the matched buffy coat samples did not significantly correlate with PFS. Patients with at least one variant in NFKBIA in their buffy coat showed a significantly prolonged overall survival (p=.019) – even significant in the multivariate Cox regression analysis (p=.024) accounting for age, number of metastases, therapy line, de novo/recurrent metastatic status and visceral or only non-visceral metastases.
Conclusion: BCL11B variants as well as different HLA-A variants in CTCs before therapy initiation might be primary resistance markers for CDK4/6i TX and thus, predictive markers for TX stratification. Acquired resistance could be related to variants in MET and RB1 as well as specific HLA-A variants. We further suggest two FAT1 variants in CTCs at baseline as markers to indicate sensitivity to CDK4/6i TX.

P5-04-20: Predictive and Prognostic Value of Magee Equation 3 and Tumor-Infiltrating Lymphocytes in HR+ HER2-Negative Breast Cancer: A Retrospective Cohort Study in a tertiary hospital in Sao Paulo, Brazil.

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Background: The Magee Equation 3 (ME3) is a mathematical model that correlates histopathological variables of breast cancer (BC) with the Oncotype DX recurrence score. ME3 also predicts pathological complete response (pCR) in patients with hormone receptor-positive (HR+) and HER2-negative (HER2-) BC undergoing neoadjuvant chemotherapy (NAC). Besides molecular tests, prognostic factors and tools, such as the Residual Cancer Burden (RCB) index and tumor-infiltrating lymphocytes (TILs), are valuable for predicting outcomes, especially the latter in triple-negative BC. This study aims to analyze overall survival (OS) and disease-free survival (DFS) in relation to ME3 scores and TIL levels in HR+ HER2- BC patients. Secondary objectives include evaluating the associations between ME3 and RCB, and between TILs and pCR.
Methods: A retrospective cohort study was conducted at the Instituto do Câncer do Estado de São Paulo (ICESP) from January 2011 to January 2024. The study included women with HR+ and HER2- BC, stages I-III, who completed NAC followed by surgical treatment. ME3 scores were categorized as low (<18), intermediate (18-25), and high risk (>25). Their

association with RCB categories [RCB-0 (pCR), RCB-1, RCB-2, and RCB-3] was evaluated using the Chi-square test. TIL percentages were classified as greater than 5% or less than 5%, and their association with pCR was also examined using the Chi-square test. OS and DFS were assessed using the Kaplan-Meier method (KM) and the Log-Rank test. All statistical analyses were performed using SAS, with a significance level set at $p < 0.05$.
Results: A total of 151 women were included, with a mean age of 50.3 years (range: 25-85). 61.6% of patients identified as white and the average BMI was 28.02 kg/m^2. Additionally, 11.9% were nulliparous and 49.0% were post-menopausal. Before NAC, 57.6% had tumors > 5 cm, 64.2% were node-positive (N+), and 84.7% of BC cases were invasive carcinoma of no special type, with an average Ki67 index of 21.7%. 21.8% (33/151) of cases had ME3 scores greater than 25, while 78.2% had ME3 scores less than 18 (59/151) or between 18-25 (59/151). All patients underwent the same NAC regimen (AC-T). Regarding surgical treatment, 60.3% underwent mastectomy, and 76.2% had level I and II axillary lymphadenectomy.
The median follow-up time was 82.7 months (range: 4-145 months). Median OS and DFS were not reached according to ME3 categories. There was a statistically significant association between DFS and ME3 categories (log-rank p=0.0473). At 60 months, the DFS rate for ME < 18 was 83.9%, 69.0% for ME 18-25, and 76.7% for ME > 25. In a Cox multivariate analysis, ME3 remained associated with DFS with an HR of 2.48 (95% CI 1.18-5.21) for intermediate risk (p=0.017) and an HR of 1.82 (95% CI 0.74-4.48) for high risk (p=0.193). This association was not observed for OS and ME3 (log-rank p=0.07). At 60 months, the OS rate was 94.6% for ME < 18, 82.5% for ME 18-25, and 83.3% for ME > 25. However, in a Cox multivariate analysis, ME3 was associated with lower OS in the intermediate risk group with an HR of 2.81 (95% CI 1.10-7.19, p=0.031). The KM curves and the log-rank test suggested no association between TILs and OS or DFS (p=0.733 and p=0.183 respectively).
There was a significant association between RCB and ME3 (p<0.001): 71.4% of patients with RCB-0 had ME3 > 25, 14.3% had ME3 scores of 18-25, and 14.3% had ME3 < 18. A significant association (p<0.001) was identified between pCR and TILs after NAC, indicating that higher TIL expression was associated with lower pCR rates.
Conclusion: ME3 is a free online tool that demonstrated significant predictive and prognostic value for DFS in our study, serving as a low-cost alternative to Oncotype DX. While our results indicate a trend towards a statistically significant association between ME3 and OS (p=0.07), this finding should be further evaluated in larger studies. TILs were not associated with OS or DFS in our cohort; however, our results showed an inverse association with pCR.

P5-04-21: Decoding breast cancer: unveiling the role of hallmark genes in tumor progression and prognosis

Balazs Gyorffy, Otilia Menyhart, William Kothalawala

The hallmarks of cancer framework offer a valuable paradigm for understanding the fundamental organizing principles shared across various cancers. However, the absence of a standardized gene set for these cancer hallmarks has led to inconsistent biological interpretations across different studies, complicating data comparison and integration efforts. In this study, we aimed to establish a consensus cancer hallmark gene set by integrating data from available mapping resources. We utilized this consolidated gene set to identify overrepresented cancer hallmarks associated with breast cancer progression.
By synthesizing data from seven independent projects, we identified 6,763 genes linked to the ten cancer hallmarks. Then, we performed a cancer hallmarks enrichment analysis for prognostic genes associated with overall survival in the KM-plotter database, including 494 genes with a hazard rate (HR) over one and 650 genes with a HR below one (at a False Discovery Rate below 10% and with a cutoff value over 100). "Evading immune destruction" was the most significant hallmark (n=55, p=3e-05), followed by "Tumor promoting inflammation" (n=51, p=7.3e-04), and "Resisting cell death" (n=101, p=0.01) when analyzing genes whose higher expression correlated to lower hazard rate. When examining the genes linked with higher HR values, no cancer hallmark reached statistical significance. The results can be validated using our established online tool (www.cancerhallmarks.com) that also allows the identification of cancer-associated hallmarks from new gene sets.
In summary, we established a consensus list of cancer hallmark genes. We identified evading immune destruction and tumor promoting inflammation as key cancer hallmarks linked to breast cancer prognosis. Our project enhances the utility of the hallmark concept as an effective organizational tool, facilitating the assignment of genes to specific biological functions.

P5-04-22: Prognostic value of circulating tumor DNA (ctDNA) in early triple-negative breast cancer (TNBC): A systematic review and meta-analysis

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Introduction: TNBC accounts for ~15% of all breast cancers and carries the worst prognosis and the highest risk of recurrence relative to other breast cancer subtypes. There is an unmet need to identify novel biomarkers to stratify the risk of recurrence and to optimize the utilization of neoadjuvant and adjuvant therapies. We performed a systematic review and meta-analysis to evaluate the prognostic value of ctDNA in patients with non-metastatic TNBC who have received treatment with curative intent.
Methods: A literature search was conducted using Ovid Medline, Elsevier EMBASE, Cochrane Central Register of Controlled Trials, and Web of Science Databases for publications up to 11/16/2023, which identified 3,526 studies. Results were uploaded to Covidence and assessed by two independent reviewers. Studies assessing the role of ctDNA

in predicting recurrence- free survival and/or overall survival (OS) were included. All recurrence outcomes were combined for this analysis. Statistical analysis was performed using Revman Web. Log-hazard ratios (HR) were pooled for studies reporting recurrence and death as a time-to-event outcomes. Odds ratios (OR) were calculated and pooled for studies reporting patient-level data on recurrence, death, and pathological complete response (pCR). The Cochrane Q chi-square test and I2 statistic was used to examine heterogeneity across studies. P values <0.05 and I2 >40% were considered significant. Quality assessment was performed using the Quality in Prognostic Studies tool. Prospero ID: CRD42023492529.
Results: A total of 16 publications (n=1,106 TNBC patients) were included: 8 prospective observational studies (n=361), 7 clinical trials (n=703), and 1 prospective case-control study (n=42). Tumor-informed assays were used by 10 studies (n=561), while 5 used tumor-agnostic assays (n=509), and 1 did not specify (n=36). ctDNA analysis was done using polymerase chain reaction (PCR) in 5 studies (n=327), and next-generation sequencing (NGS) in 9 (n=734), with 2 using other methods (n=45). For studies that reported recurrence as a time-to-event outcome, pooled log-HR showed that post-neoadjuvant ctDNA+ is associated with a higher likelihood of disease recurrence (HR 3.79; 95%CI 2.62-5.48). Similarly, for studies that reported patient-level data, pooled OR showed that post-neoadjuvant ctDNA+ is associated with higher odds of disease recurrence (OR 6.59; 95%CI 3.40-12.75). Subgroup analysis separating ctDNA+ before surgery versus after did not significantly impact the results. Pooled log-HR also revealed that ctDNA+ in the post-neoadjuvant setting is associated with worse OS (HR 3.47; 95% CI 2.17-5.56). Furthermore, ctDNA+ after neoadjuvant therapy (before surgery) is associated with lower odds of achieving pCR (OR 0.12; 95%CI 0.04-0.36). ctDNA+ during or after adjuvant chemotherapy is similarly associated with a higher likelihood of disease recurrence (HR 7.51; 95%CI 4.80-11.74) and worse OS (HR 7.96; 95%CI 1.59-39.78).
Conclusions: We found that ctDNA+ in the post-neoadjuvant setting (both before and after surgery) is correlated with a higher likelihood of disease recurrence and worse OS. These findings suggest that ctDNA has potential prognostic value in early TNBC. However, it remains unclear if therapeutic intervention for patients who are ctDNA+ can improve outcomes. While there is a strong correlation between ctDNA+ and worse outcomes, many of the results included in this meta-analysis are observational or secondary endpoints of clinical trials. The studies are heterogeneous in terms of treatments and methodologies for ctDNA assessment. While more studies are needed before incorporating ctDNA into clinical practice, the findings of this meta-analysis are reassuring and show the promise of ctDNA as a biomarker.

P5-04-23: Genomic Investigation of Angioma in Mammary Cancer

Rachel Geiser, Lorinda Baker, Peaches Ulrich, Albert Wendt

We aim to identify a genomic driver leading to overexpression of benign hamartomatous growths seen in the excessive thoracic angiomas of breast cancer patients. In our practice, we have observed that 4% of Caucasian postmenopausal and 1% of premenopausal breast

cancer patients demonstrate large numbers of cutaneous angiomas, largely on the anterior trunk. Genetic analysis of "cherry angiomas" have found that they frequently carry specific somatic missense mutations in the GNAQ and GNA11 (Q209H) genes. Tumorigenesis has been correlated with abnormal expression and receptors, and associated G proteins (GNAS, GNAQ, or GNA11). Oncogenic mutations in G protein coupled receptors and guanine protein encoding genes have been identified in a significant number of tumors such as uveal melanoma and other malignancies. If a common genomic driver is identified in these breast cancer patients with excessive thoracic angiomas, there may be targeted treatment available for this genetic tumor subset in the future. To test the hypothesis that a driver for increased number of angiomas may also be a driver for developing breast cancer, ten breast cancer patients with 100 or more thoracic angiomas were consented to have somatic whole exome sequencing of their archival breast cancer tissue through CARIS Life Sciences. At the time of abstract submission, the batch of 10 patients' tumor samples have been sent to CARIS for sequencing. Once the results are received, we will include further specific information on distribution of angiomas in Caucasian women in pre-and post-menopausal breast cancer patients.

P5-04-24: ATV-1601 is a Potent and Selective Allosteric Inhibitor of AKT1E17K and Shows Profound and Durable Regressions in AKT1E17K-Driven Patient Derived Xenograft Models

Shomit Sengupta, Anna C. Schinzel, Aaron Coffin, Truc Pham, Benjamin Brigham, Gianna Iantosca, Rajiv Govindaraj, Josepha LaPointe, Joann Prescott-Roy, David Church, Gordon Murray, Thomas Roddy, Anil K. Padyana, Marion Dorsch, Maria-Jesus Blanco

Background: The AKT pathway modulates cell survival and proliferation in response to insulin and growth factor signaling. A gain-of-function mutation in the AKT1 Pleckstrin-Homology domain from glutamic acid to lysine (E17K) leads to constitutive activation resulting in tumorigenesis. AKT1E17K driver mutations are predominantly found in ER+/HER2- breast cancer and occur as a mechanism of acquired resistance to PIK3CAi treatment in these tumors. Additionally, AKT1E17K mutations are found in triple negative breast cancer, endometrial cancer, prostate cancer and meningiomas among other solid tumors. The therapeutic benefit of inhibiting the AKT/PI3K pathway has been well established in the clinic. However, AKT/PI3K pathway inhibition has been shown to lead to a broad range of adverse events including hyperglycemia, which in the case of pan-AKT inhibitors is primarily driven by inhibition of AKT2. To maximize tolerability and efficacy in AKT1E17K mutant patients, we have developed ATV-1601, a novel orally bioavailable, non-covalent, allosteric AKT1E17K inhibitor.
Methods: ATV-1601 was developed using structure-based drug design and driven by incorporating molecular interactions observed between AKT1E17K and mutant-selective metabolite binders discovered using Atavistik's proprietary Atavistik Metabolite-Protein Screening (AMPS) technology. A combination of biophysical, biochemical, and cell-based methods were used for lead optimization. Potency and selectivity were assessed in BaF/3

cells engineered to be dependent on AKT1E17K or AKT2 in the absence of IL-3. A PK/PD relationship was established in vivo using a CDX model. AKT1E17K target engagement was measured by phospho-AKT1E17K and off-target activity was measured by phospho-AKT2, insulin and glucose levels. In vivo efficacy was assessed in cancer patient derived xenograft (PDX) models.
Results: ATV-1601 is potent on AKT1E17K and highly selective over AKT2 in a BaF/3 cellular proliferation assay. Biophysical characterization of ATV-1601 binding kinetics demonstrated significant differences between AKT1E17K and AKT2. In vivo, ATV-1601 shows good oral bioavailability and exposure across species. In CDX models ATV-1601 demonstrated a strong dose-proportional exposure and achieved >90% AKT1E17K inhibition for 24 hours. AKT2-driven hyperglycemia was not detected across multiple in vivo studies. ATV-1601 demonstrated profound tumor regressions with long durations in ER+/HER2- and triple negative breast cancer PDX models and is well-tolerated in multiple preclinical species at the projected efficacious exposure.
Conclusion: ATV-1601 demonstrates exceptional efficacy with a favorable in vivo tolerability profile and data supports advancing toward clinical development.

P5-04-25: Detection of Human Epidermal Growth Factor Receptor 2 (HER2) in Cultured Circulating Tumor Cells (CTCs) Isolated from Peripheral Blood of Breast Cancer Patients

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Background: Overexpression of HER2 is indicative of a subset of breast cancers that are characterized by rapid growth and poor prognosis. The advent of HER2-targeted therapies has transformed the treatment landscape, making accurate detection and quantification of HER2 expression critical for guiding treatment decisions. Typically, HER2 status is determined through invasive tissue biopsies, which pose risks to patients and logistical and financial burdens. Analysis of CTCs from liquid biopsies offer a minimally invasive alternative, but the rarity of CTCs in peripheral blood and the technical challenges associated with their isolation and characterization have hindered the widespread adoption of this technique for protein biomarker analysis. In response to these challenges, we propose a novel approach to enhance the detection and quantification of HER2 using liquid biopsies by cultivating CTCs ex vivo, thereby amplifying their numbers to a level that allows for more reliable detection.
Methods: Adult breast cancer patients at least 18 years of age with tumor size of at least 1 cm were prospectively enrolled for this study. Breast tumor tissue was acquired via core needle biopsy and tested for HER2 protein expression following the hospital's standard procedures for immunohistochemistry (IHC) and, if needed, for HER2 gene amplification with fluorescence in situ hybridization (FISH) as recommended per the ASCO-CAP Guideline. The peripheral blood mononuclear cell (PBMC) layer was isolated from whole blood for culturing of CTCs on the 3D organoid culture platform, Rapid, Reproducible, Rare

Cell 3D Expansion (R3CE) (AcroCyte Therapeutics, Inc., New Taipei City, Taiwan). After 7 days of culture, a multiplex immunofluorescence (IF) staining protocol targeting cell nuclei (DAPI), CD45, EpCAM, and HER2 was employed to identify and enumerate HER2-positive CTCs. Mean CTC counts were compared using Student's t-test.
Results: Ten female breast cancer patients with a mean (standard deviation) age of 55 (8) years were included in the study. One (10%), 5 (50%), and 4 (40%) patients had stage I, II, and III cancer, respectively. Two (20%) and eight (80%) patients had grade 2 and 3 cancer, respectively. Based on tumor tissue pathology, three (30%) were HER2-positive (IHC 3+). FISH was conducted for two patients with IHC 2+, and both were negative. For the remaining 5 patients, 4 were IHC 1+ and one was IHC 0. After 7 days of culturing, CTCs were identified for 8 (80%) of the patients. Mean CTC counts were higher for grade 3 cancer [261 (435)] than grade 2 cancer [80 (113)], but the difference was not statistically significant (p=0.33). Positive HER2 IF staining was observed for all cases in which CTCs were identified. The percentage of CTCs with positive HER2 staining ranged from 38% to 100%, with a median of 77%. No significant difference in percentage of CTCs with positive HER2 staining was observed between patients who were HER2-positive and HER-2 negative by tissue pathology.
Conclusion: Our study demonstrated the feasibility of culturing and IF staining CTCs from breast cancer patients for HER2 analysis. Additional investigations of the association between HER2 expression in CTCs and tumor tissue are needed to realize the potential of CTCs for guiding treatment with HER2-targeted therapies.

P5-04-26: Prevalence of BRCA1, BRCA2, and PALB2 genomic alterations among 924 Taiwanese breast cancer assays with tumor-only targeted sequencing: extended data analysis from the VGH-TAYLOR study

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Background: The discovery of BRCA1 and BRCA2 genes in 1994 and 1995 led to increased focus on the homologous recombination (HR) repair pathway in cancer research and therapy. Preclinical studies in 2005 demonstrated that poly ADP-ribose polymerase (PARP) inhibitors selectively target BRCA-deficient cells. Numerous phase III clinical trials have shown that PARP inhibitors can improve treatment outcomes in both early and advanced germline BRCA1/2 (gBRCA1/2)-mutant breast cancer, as well as achieve a better quality of life compared to cytotoxic chemotherapy.
However, the prevalence of gBRCA1/2 mutations is estimated to be only 2-5% in the unselected general population. Therefore, it's important to identify patients beyond gBRCA1/2 carriers whose cancers may be sensitive to PARP inhibition. Studies in prostate and ovarian cancer have suggested that some patients with somatic BRCA1/2 (sBRCA1/2) mutations or mutations in HR-related genes other than BRCA1/2 may benefit from PARP inhibitors.

Among HR-related genes, PALB2 is responsible for loading RAD51 onto ssDNA, stimulating RAD51-mediated strand exchange and D-loop formation via the BRCA complex (BRCA1-PALB2-BRCA2-RAD51). Germline PALB2 mutation is estimated to be present in about 1% of breast cancer patient populations and is associated with an increased risk of breast and pancreatic cancer.
The epidemiology of breast cancer differs between Taiwanese (ethnically Han Chinese origin) and Caucasian populations. Taiwanese breast cancer patients have a younger median age of disease onset and carry a greater risk for disease progression and shorter interval to secondary contralateral breast cancer than Western women. As the early onset and bilaterality of breast cancer are more likely to be related to genetic predisposing factors, it is important to identify potential genetic alterations underpinning Taiwanese patients.

Methods: This study aimed to assess the prevalence of BRCA1, BRCA2, and PALB2 mutations in Taiwanese breast cancer patients using targeted sequencing on tumor-only samples. The Institutional Review Board of Taipei Veterans General Hospital approved the study (protocol number: 2018-09-007A). Written informed consent was obtained from all participants prior to enrollment.
A total of 879 consecutive breast cancer patients, representing 924 assays, were enrolled in the study. Patients were assigned to different groups based on their treatment plans: Group 1 [planned to receive surgery as the first-line treatment and followed by adjuvant therapy], Group 2 [planned to receive neoadjuvant therapy as the first-line treatment and followed by surgery], and Group 3 [diagnosed with de novo and treatment naive stage IV breast cancer, or stage IV breast cancer with recurrence beyond three years after surgery].
Formalin-fixed paraffin-embedded specimens were assayed using the Ion Torrent Oncomine™ Comprehensive Assay v3 (Thermo Fisher Scientific, Waltham, MA), enabling the detection of 161 cancer-related genes and identification of single nucleotide variants (SNVs), copy number variations (CNVs), gene fusions, and indels.
Variants were filtered with the Oncomine™ Knowledgebase Reporter and the online VariED tool to filter out Taiwan Biobank polymorphisms. The mutational consequences of filtered variants were determined using the ClinVAR database, OncoKB™, and SNPnexus.
Reflex germline testing was conducted for patients with pathogenic or likely pathogenic variants using whole exome sequencing (WES) or whole genome sequencing (WGS) through blood sample collection.
To distinguish germline from somatic mutations with tumor-only sequencing, algorithms including the LOH-germline inference calculator (LOHGIC) and the somatic-germline-zygosity (SGZ) method were employed as alternatives for patients not ready for germline testing.

Results: Of the 924 assays, 281 were positive for mutant BRCA1, BRCA2 and PALB2 in 130 patients. These mutations impacted 27 patients (3.1%) for BRCA1, 76 patients (8.6%) for BRCA2, and 46 patients (5.2%) for PALB2. In total, genetic alterations were noted in 14.8%

(130 patients) of the cohort.
The BRCA1 mutation cohort was associated with a higher proportion of advanced stages compared to those without. The BRCA2-mutant patients showed a higher incidence of family history of ovarian cancer, resulting in a significant difference in the number of mutant patients with a family history of ovarian cancer.
In terms of IHC phenotypes, 13 (2.3%) of the BRCA1 mutant breast cancers were HR+/HER2−, 3 (3.3%) were HR+/HER2+, 2 (2.6%) were HR−/HER2+, and 9 (6.9%) were HR−/HER2−. For BRCA2 mutated cases, 51 (9.0%) were HR+/HER2−, 9 (9.9%) were HR+/HER2+, 6 (7.9%) were HR−/HER2+, and 9 (6.9%) were HR-/HER2-. Among the PALB2 mutated patients, 30 (5.3%) were HR+/HER2−, 4 (4.4%) were HR+/HER2+, 3 (3.9%) were HR−/HER2+, and 9 (6.9%) were HR−/HER2−.
Co-occurrence was found between BRCA1/2 in 13 samples (log2 odds ratio: > 3, p-value < 0.001, and q-value < 0.001), BRCA1 and PALB2 in 8 samples (log2 odds ratio: > 3, p-value < 0.001, and q-value < 0.001), and BRCA2 and PALB2 in 8 samples (log2 odds ratio: 2.401, p-value < 0.001, and q-value < 0.001). Five patients had both BRCA1/2 mutations, 1 had both BRCA2 and PALB2 mutations, none had both BRCA1 and PALB2 mutations, and 7 had all three mutations.
The study analyzed various genetic variants and identified 176 amino acid (AA) changes. There were four variants that did not notice any AA change, and three novel splice site variants (BRCA1 c.5256+1G > A, BRCA1 c.5215+1G > A, and BRCA2 c.-38-3CAG > C) were identified. Notably, 60.2% (106) of the discovered AA changes were not documented in either ClinVAR or the Oncomine™ Knowledge database. Using the OncoKB™ for annotation, 171 (97.2%) AA changes were found to have clinical implications.
The most common mutations were p.K654fs (3 cases) for BRCA1, p.N372H (26 cases), p.S2186fs (5 cases), p.V2466A (5 cases), and p.X159_splice (5 cases) for BRCA2, and p.I887fs (30 cases) for PALB2. It should be noted that although p.N372H was observed 26 times in BRCA2-mutated assays, it has been confirmed to be a benign variant.
Reflex germline testing was conducted for patients until November 20, 2023. Specifically, 48 cases (36.9%) were identified with pathogenic or likely pathogenic variants via tumor-targeted sequencing, as classified by the ClinVar database, and were subsequently recalled for further investigation through WGS or WES. Among 130 cases examined, 7 cases (5.4%) completed WGS uncovering crucial genetic variations. None harbored germline mutations in BRCA1/2 and PALB2. Another 20 cases (15.4%) were reached and 9 had completed WES (6.9%). The study group encountered enormous challenges including loss of follow-up and 14 were deceased.
The reflex germline testing results showed that three variants (BRCA1 c.1969_1970del, BRCA1 c.3629_3630del, BRCA2 c.8755-1G > C) were classified as Pathogenic/Likely pathogenic (P/LP) by ClinVar and as likely loss-of-function or likely oncogenic by OncoKB. One variant (PALB2 c.448C > T) was not listed in ClinVar, but OncoKB annotated it as likely loss-of-function or likely oncogenic. Additionally, there were 2 cases (1.5%) of germline mutations with uncertain significance, and 5 cases (3.8%) with benign germline alterations, emphasizing the genetic intricacy involved in breast cancer development.
Using alternative methods as the results of LOHGIC and SGZ analyses for determining

germline and somatic mutations, among the 281 samples, 40 were identified as germline mutations, and 169 samples (60.1%) were of somatic origin. Borderline cases comprised 26 samples (9.3%), and 46 samples (16.4%) were unclassifiable due to missing data on tumor purity.

Discussion: This study presents one of the largest cohorts of breast cancer patients with BRCA1, BRCA2, and PALB2 mutations detected through tumor-only sequencing in Taiwan. The high detection rate of breast cancer susceptibility genes could lead to more patients undergoing germline testing and receiving appropriate treatments.

The study revealed a significant tendency of co-occurrence between BRCA1/2, BRCA1-PALB2, and BRCA2-PALB2 mutations. This finding may have implications for understanding breast carcinogenesis and creating genetic panels for predicting and prognosing hereditary breast cancer.

Tumor-only targeted sequencing has gained increasing attention due to its ability to reveal pathogenic/likely pathogenic variants in genes associated with cancer predisposition and potential therapeutics with a higher level of coverage. The Dana-Farber/Harvard Cancer Center study found that over half (52.9%) of the tumor-identified P/LP patients did not meet any personal or family history criteria for clinical genetic testing (CGT). These results show the potential of tumor-only sequencing in detecting P/LP mutations in cancer predisposition genes across malignancies.

Both germline and somatic alterations can affect treatment decisions and outcomes. The results of the TBCRC-048 and TBB trials suggested further exploration of PARP inhibitors in metastatic or advanced breast cancers with HR-associated mutations beyond BRCA1 and BRCA2. Identifying additional biomarkers to expand this treatment in somatic BRCA1/2-mutant or HR-related-gene-mutant advanced breast or ovarian cancers could significantly benefit patients who would otherwise receive chemotherapies as the only regimen.

The significance of reflex germline testing cannot be overemphasized. However, the extent of reflex testing conducted to date is still limited. This has led to the exploration of alternative methods to determine whether the reported mutation is of germline or somatic origin. The refined LOHGIC and SGZ methodologies are designed to assess three crucial factors: tumor purity, allele frequency, and confirmation of a diploid genome. Although these algorithms are useful, they have their limitations; in an analysis of 130 cases, 10 could not be conclusively classified as either germline or somatic due to varying origins of the mutations.

Accurate interpretation of genetic variants is critical in both clinical and research settings. The classification criteria can vary between submitters, and the evidence for a particular variant may be conflicting, leading to difficulties in unbiased interpretation. Numerous studies have explored potential indicators for reinterpreting pathogenic variants within specific databases, as well as across distinct platforms. The reinterpretation of cancer predisposition genes requires a multidisciplinary effort involving clinicians, genetic counselors, bioinformaticians, and researchers.

This study has several strengths, including being the first large-scale analysis of its kind in

Taiwan and Asia, the availability of data on family history, molecular subtypes, and early or advanced breast cancer status, and the utilization of updated annotation databases and guidelines during interpretation and annotation. However, it also has limitations, including limited additional germline sequencing with compromised recalled rates, varying sample sizes among different clinical groups, focus on only three genes, and the need for more in-depth evaluation of clinical outcomes and subgroup analyses.

Conclusion: This study reported a cohort of Taiwanese breast cancers harboring mutations in BRCA1, BRCA2, and PALB2 through tumor-only sequencing, which underscores the impact of these genes on breast cancer risk and potential therapeutic opportunities. Tumor-only sequencing has enabled a greater number of patients to uncover their genomic alterations, offering additional insights for management strategies. These include recommendations for germline testing and the prospective utilization of PARP inhibitors to augment treatment efficacy. Nonetheless, supplementary germline testing remains critical, and investigating alternative methods for distinguishing whether variants are germline or somatic origin are invaluable. The use of updated annotation and rigid guideline follow-up and the integration of multiple types of genomic data can help improve the accuracy of cancer risk assessment and inform personalized prevention and treatment strategies.

P5-04-27: A Novel Patient-Derived Xenograft Model of Inflammatory Breast Cancer

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Background: Inflammatory breast cancer (IBC) is one of the most aggressive forms of breast cancer, but few models exist for studying experimental therapeutics. Inflammatory breast cancer research urgently needs additional models to allow pre-clinical testing of experimental therapeutics. The objective of our study was to establish and characterize a novel patient-derived xenograft (PDX) from a HER2 positive IBC patient refractory to neoadjuvant chemotherapy to better understand the biology of IBC resistance.
Methods: We derived a novel PDX from a patient with hormone receptor negative, HER2 positive IBC refractory to neoadjuvant chemotherapy with Docetaxel, Carboplatin, Trastuzumab, and Pertuzumab (TCHP). Tumor was implanted into NOD SCID gamma mice and used for serial propagation of PDX. We performed short-tandem repeat (STR) profiling, plotted tumor growth curves for mice treated with alpelisib/everolimus vs. untreated, and immunohistochemistry (IHC). Paired Student's t-tests were used to compare tumor growth curves. We used 10X genomics for single cell transcriptome analysis of 1000 cells derived from the PDX, ctDNA, whole exome and RNA sequencing to further characterize the genetic landscape of the PDX model.
Results: Tumor next generation sequencing (NGS) showed a mutation in PIK3CA (H1047R), a variant associated with resistance to HER2 targeted therapy and chemotherapy, but sensitivity to everolimus. Using STR profiling, we were able to prove the PDX and primary

tumor were derived from the same patient. By the 3rd generation transplant, 15/15 orthotopically transplanted mice produced PDX. The mean tumor size was 619 mm3. Five mice were then treated with a combination of alpelisib and everolimus for 20 days to block signaling of the PI3K/AKT/mTOR pathway. Tumor growth was compared over 21 days. After treatment with alpelisib/everolimus, the tumor size decreased significantly (869.9mm3 vs. 45.8mm3, p = 0.006). No mice (0/15) had metastasis on necropsy at the time of meeting humane endpoints.

We performed IHC to assess the intensity of protein expression in the treated vs untreated tumors. All tumors strongly expressed JAK2. The tumor appeared to have changed receptor expression after neoadjuvant TCHP (from HER2 positive to triple negative). The group treated with alpelisib/everolimus had higher expression of PIK3CA, CD24, and HER2. Only the treatment group expressed HER2 and ALDH1. E-cadherin, PIK3CA, and CD24 were stronger in the treatment group when compared to control.

10X Genomics UMAP (Uniform Manifold Approximation and Projection) plots showed a snapshot of the transcriptome with 6 unique clusters in the PDX. RNA Seq showed genes with differential expression between primary tumor and PDX. Whole exome sequencing identified copy number variants and single nucleotide variants.

Conclusion: We established a PDX of a HER2 positive IBC tumor with a PIK3CA hotspot mutation (H1047R) refractory to trastuzumab. Few PDX models have been created to study IBC. Alpelisib/everolimus decreased tumor growth in our model. JAK2 was strongly expressed in both treated and untreated tumors. TCHP resistant tumor cells downregulated HER2 expression, which was re-expressed after treatment with alpelisib and everolimus. Targeting the PI3K/mTOR pathway may be useful to overcome resistance in HER2 positive IBC with a H1047R mutation in PIK3CA.

P5-04-28: Subtype by PAM50 changes after Neoadjuvant Endocrine therapy, from A Phase III Randomized, Double-Blind, Neoadjuvant Study of Hormonal Therapy plus Palbociclib versus Hormonal Therapy plus Placebo in ER+HER2- Operable Breast Cancer

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Background: Luminal B breast cancer has heterogeneity which leads to unmet medical needs for cure. HER2-enriched type identified by PAM50 is occasionally found in luminal B subtype breast cancer by immunohistochemistry, leading to resistance to endocrine therapy (ET). To understand biological change following ET and to predict efficacy of ET, and consider additional therapies, we analyzed sequential samples from a neoadjuvant ET trial for exploratory analysis.

Patients and Methods: This study was conducted as translational research for a phase III

randomized, double-blind study of neoadjuvant hormonal therapy with or without palbociclib in untreated pre/peri- and post-menopausal women with operable, hormone receptor-positive (estrogen receptor and/or progesterone receptor), HER2-negative breast cancer (NCT03969121). Other major inclusion criteria included tumor size ≥ 15mm, T1c-3N0-1, Ki67 LI ≥14% by central assessment, and no previous history of radiotherapy or systemic therapy for breast cancer. Patients were randomly assigned 1:1 to receive 16 weeks of hormonal therapy plus palbociclib (125mg/day, 3W1R) or hormonal therapy plus placebo. Hormonal therapy consisted of letrozole for post-menopausal patients and tamoxifen plus LH-RH agonist for pre/peri-menopausal patients. The co-primary endpoints included PEPI score and EPclin Risk Score, a score combining EndoPredict® molecular score with clinical factors. The primary outcome was reported previously that the addition of palbociclib to neoadjuvant hormonal therapy did not improve efficacy measured by PEPI score. From 141 cases in the trial, 60 samples from 20 cases, who had sufficient tissue from biopsies at screening, cycle 1 day 15 (C1D15) and surgery, were analyzed by PAM50 for intrinsic subtype and risk of recurrence (ROR).
Result: The distribution of cases (subtype and Ki-67) is shown in Table 1 and 2. Among 20 cases, 13 cases were in Palbociclib arm, and 7 in Placebo arm. There were 3 Her2-enriched cases in Palbociclib group (1 case at both screening and surgery, 1 case at screening only, 1 case at surgery only). As for ROR, there were no low-risk cases, 4 intermediate-risk cases, and 16 high-risk cases at screening. There was a shift to lower scores at C1D15: 10 low-risk cases, 9 intermediate-risk cases, and 1 high-risk cases. By surgery, however there seemed to be some rebound, but still lower than before treatment: 8 low-risk cases, 6 intermediate-risk cases, and 6 high-risk cases. Average of ROR was 69 at screening, 33 at C1D15, and 38 at surgery. In analysis of 17 luminal A/B cases (without 3 HER2-enrich cases), baseline data are not significantly different between Palbociclib arm and Placebo arm. Ki-67 at C1D15 was lower in palbociclib arm than in placebo arm (Average 1.5% (95% CI 0.4-3.4), 6.0% (1.5-10.6) respectively, p=0.027). At surgery, there were 8 PR cases and 2 SD cases in palbociclib arm, 3 PR cases and 4 SD cases in placebo arm. There were no significant differences between the two arms in Ki67(%), PEPI score, EP clin risk score and ROR at surgery.
Conclusion: Neoadjuvant endocrine therapy for 16 weeks changes subtype classification by PAM50 from luminal B to luminal A and the ROR drastically. Greater effects were noted for the combination, not statistically significant, but this warrants further investigation in a larger cohort. There were 3 HER2-enriched cases with poor response, but this was not significant because of the small sample size. These 3 cases probably reflect tumor heterogeneity on the baseline biopsy. Neoadjuvant 16 weeks endocrine therapy may work to screen for ET resistance, and help select a switch in therapy, or those benefitting most from Palbociclib. Further research will be necessary including PAM50, Oncotype Dx and those with high Ki67.

Clinical trial identification: NCT03969121
Funding: Pfizer Inc.

P5-04-29: Investigating the expansion of 95-gene signature (Curebest® 95GC Breast) indication for predicting the risk of recurrence in patients with ER-positive and lymph node-positive breast cancer.

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Background: Gene profiling technology, which assesses multiple genes, has garnered attention as a research tool for personalized cancer treatment and its use is recommended in several international guidelines.
Currently, postoperative adjuvant therapy for ER-positive and HER2-negative breast cancer includes a wide range of options, including anthracyclines and taxanes, CDK4/6 inhibitors, and TS-1, in addition to hormone therapy.
21-gene signature assay categorizes patients into two groups for assessing adjuvant chemotherapy efficacy: RS 0-25 and RS 26 or higher for N1 patients. However, the broad clinical range of RS 0-25 complicates treatment decisions when relying solely on this cutoff. And over-treatment of the good prognosis group cannot be ruled out. Moreover, in premenopausal patients with N1, optimal prognostic tools have not been established.
Curebest™ 95GC Breast (95GC) provides a 95-gene signature that stratifies patients into two groups: high-risk (95GC-H) and low-risk (95GC-L), predicting the recurrence risk and is clinically indicated only for ER-positive, HER2-negative, node-negative invasive (ER+/HER2-/N0) invasive breast cancer.
This study aims to investigate the applicability of 95GC in N1 cases and also to discuss the correlation between RS of 21-gene signature assay and 95GC determination.

Methods: We reviewed our institutional databases to identify patients with ER-positive, HER2-negative, node-positive breast cancer who had 21-gene signature assay data available and who had undergone definitive surgery between April 2005 and May 2018 and adjuvant endocrine therapy without any cytotoxic agents. We included only patients for whom archival FFPE tissue from definitive surgery was available. We excluded patients with pathological node-negative, distant metastatic disease, and male patients. The Fisher exact test was used to compare variables between 95GC groups. A Kaplan-Meier estimate with a log-rank test was used for survival analysis.

Results: A total of 58 patients from our institution were initially included in the analysis; one patient was excluded due to indeterminate HER2-ISH results, resulting in a final cohort of 57 patients including premenopausal patients.
When the index cutoff value to distinguish between the 95GC-H and 95GC-L groups was set at 50, the 5-year recurrence-free survival (RFS) rate and the lower limit of the 95% confidence interval were significantly higher in the L group. The L group showed a markedly better prognosis compared to the H group (92.8% and 64.8%; p=0.0063). This

cutoff value was consistent with the reference value used for N0 patients.
We classified 48 patients (84.2%) as 95GC-L; 9 patients (15.8%), as 95GC-H.
The median follow-up duration was 87.3 months. There were no statistical differences in age, menopausal status, T stage, nuclear grade, histological grade, or PR status between the 95GC-H and 95GC-L groups.
There were 6 patients (10.5%) of recurrence within 5 years after surgery and 2 of which were distant metastases, both in the 95GC-H group. 5-year distant recurrence-free survival (DRFS) was significantly better in the 95GC-L group than in the 95GC-H group (100% and 77.8%; p=0.0164).
The sensitivity and specificity of 95GC for predicting recurrence were 44.4% and 89.6%, respectively. Comparable calculations were conducted for the RS0-25 and RS≥26 groups of 21-gene signature assay, revealing sensitivity and specificity values of 11.1% and 100%, respectively. These results indicate that 95GC demonstrates a higher sensitivity equivalent index compared to 21-gene signature assay.

Conclusions: 95GC can predict recurrence risk in patients with ER+, HER2-, N1 breast cancer. It also suggested that it may be able to predict the risk of recurrence better than RS of 21-gene signature assay.

P5-04-30: Holographic and molecular characterization of early disseminated cells from breast cancer patients

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Growing evidence suggests that cancer cells disseminate into blood vessels at an early stage, seeding metastatic sites in breast cancer. These early-stage tumor cells that lodge or extravasate at metastatic sites can enter dormancy, marking a potential source of late recurrence and therapy resistance. Thus, the presence of early disseminated cells poses risks to patients but also holds potential benefits for early detection and opportunities for possibly curative interventions. We evaluated this in a cohort of women with newly diagnosed early-stage breast cancer (Stage 0, 1, 2). Blood samples were collected prior to initiation of therapy and analyzed by Astrin Biosciences' AI-empowered proprietary holographic imaging platform combined with in-flow protein marker expression for the presence of disseminated tumor cells. The platform was previously trained on holographic signatures (encoding both optical and morphological signatures) of 100 million+ individual cells and could differentiate healthy from cancer cells with greater than 99% accuracy. Preliminary data from this study revealed that blood samples from the majority of early breast cancer patients exhibited disseminated cancer cells. Gene expression patterns from the enriched cancer cells were further profiled via quantitative PCR using a selective gene panel consisting of breast-specific and cancer-specific genes. We were able to identify selective sets of breast and cancer-specific genes in these patients confirming breast origin with cancer-like features. In summary, we utilized holographic imaging coupled with

proprietary deep learning approaches to identify early disseminated cells in women who are undergoing screening for breast cancer. Molecular analyses of these cells confirmed breast cancer origin. Combined, this work enables Astrin Biosciences to develop a two-pronged assay consisting of holographic plus molecular characterization of disseminated cells for early detection of breast cancer.

P5-05-01: Real World Treatment Patterns and Outcomes in Her-2 Positive Metastatic Breast Cancer Patients with Brain Metastases

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Background: Approximately 50% of HER2+ metastatic breast cancer (MBC) patients will develop brain metastases (BMs). While traditional treatment for BMs consisted of radiation +/- surgery, optimal treatment now involves a multi-disciplinary approach of local and CNS-penetrating Her-2 targeted systemic therapies since their FDA approval in 2020. First line systemic therapy for HER2+ MBC is trastuzumab (H), pertuzumab (P), and Taxol (T). Subsequent therapy options include trastuzumab emtansine (T-DM1), tucatinib/H/capecitabine triplet therapy (TUC), and trastuzumab deruxtecan (T-DXd). This study aimed to examine real-world treatment patterns and clinical outcomes of HER2+ breast cancer BMs.
Methods: This single institution retrospective study included HER2+ MBC patients with BMs treated between Jan 2017 - Feb 2024, with at least 6 months of follow-up after BM diagnosis. Primary objective was to evaluate treatment sequence and duration after BM diagnosis. Overall survival (OS) was defined from date of BM diagnosis to date of death. Systemic progression-free survival (PFS) and CNS-PFS were defined from systemic therapy initiation date to systemic or CNS disease progression, respectively. Kaplan-Meier method was used for survival analysis. Time to systemic therapy discontinuation (due to toxicity or progression) was also evaluated. Radiation necrosis (RN) was diagnosed by MRI perfusion imaging +/- biopsy and multi-disciplinary review.
Results: 78 patients with HER2+ MBC with BM met the eligibility criteria, including 36 (46.2%) patients with BMs at initial MBC diagnosis and 42 (53.8%) subsequently diagnosed with BMs. Median number of systemic therapies received prior to BMs diagnosis was 3 (range 0-13). Patients had a median of 2 BMs (range 1-40) at diagnosis, and median total BM volume was 7.29 (range 0.09-105.22) cm3. 72 (92.3%) patients received stereotactic radiosurgery (SRS); 25 (32.1%), WBRT; and 40 (51.3%), neurosurgical resection. Of 129 total SRS courses, 40 (31.0%) were treated with 18-22 Gy in 1 fraction (fxn), 67 (51.9%) with 21-24 Gy in 3 fxns, and 17 (13.2%) with 25-30 Gy in 5 fxns. For patients with a diagnosis of BM before 2020 (n=45), the 1st systemic therapy prescribed after BM diagnosis consisted of HPT (n=6; 13.3%), T-DM1 (n=2; 4.4%) or other (n=37; 82.3%). After 2020 (n=33), the 1st systemic therapy prescribed after BM diagnosis was HPT (n=4; 12.1%), T-DM1 (n=2; 6.1%), TUC (n=8; 24.2%), T-DXd (n=8; 24.2%), or other (n=11; 33.4%). The

median duration for each systemic therapy was 6.0 mo, 12.9 mo, 7.3 mo, and 3.2 mo for T-DM1, TUC, T-DXd, and other treatments, respectively (p=0.27). For the entire cohort, median OS was 36.8 mo (95% CI 21.9-79.2), median systemic PFS was 13.4 mo (95% CI 8.7-16.0), and median CNS-PFS was 14.7 mo (95% CI 11.3-25.5). Twenty-three (29.5%) patients developed RN (any CTCAE grade) with a median time to RN after initial SRS treatment of 26.5 months (range 2.0-220.5 months). Of the patients with RN, 7 (30.4%) received antibody-drug conjugates (ADC) concurrently with SRS.
Conclusions: Since 2020, TUC and T-DXd were increasingly prescribed as initial systemic treatment after BM diagnosis. The combination of SRS and HER2 targeted systemic therapies in this cohort resulted in excellent survival outcomes but some risk of RN. Interestingly, TUC trended toward a longer treatment duration than T-DM1 or T-DXd. Further investigation into reasons for discontinuation of each systemic therapy (toxicity vs. progression) is warranted. Limitations of this study include the retrospective design, small cohort size, and limited follow-up. Future studies and longer follow-up will allow for a better understanding of how treatment patterns affect survival outcomes and patient quality of life.

P5-05-02: Pharmacokinetic profile and preliminary efficacy of GQ1001, a next generation HER2-targeting ADC, combined with pyrotinib in pretreated patients with HER2-positive metastatic breast cancer: A phase 1b clinical trial

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Background: GQ1001 is a novel HER2-targeted antibody-drug conjugate (ADC) that was generated by conjugating trastuzumab to DM1 via a unique open-ring containing linker and the enzymatic site-specific conjugation technology, which significantly improves homogeneity and stability of the study drug[1]. Preclinical studies showed robust anti-tumor activities in multiple HER2+ PDX and CDX models, and excellent pharmacokinetics and safety profiles in rats and cynomolgus monkeys. Phase 1a study (NCT04450732) showed superior tolerability and promising antitumor efficacy in heavily pretreated HER2-positive advanced solid tumors[2]. In vivo study also demonstrated a synergistic anti-tumor efficacy of GQ1001 combined with pyrotinib, an irreversible pan-HER2 receptor tyrosine kinase inhibitor (TKI)[1]. Thus, a phase 1b study (NCT05575804) was designed to investigate the safety and preliminary efficacy of the combination of GQ1001 and pyrotinib in pretreated patients with HER2-positive metastatic breast cancer.

Methods: In the “3+3” dose-escalation phase, the dose of GQ1001 was 6.0 mg/kg, 7.2 mg/kg and 8.4 mg/kg intravenously in a 21-day cycle, and pyrotinib was taken orally in a fixed dose of 320 mg once daily in 28-day cycles. The dose escalation or de-escalation was based on the incidence of specified dose-limiting toxicities (DLTs) in the initial dose group. DLTs were observed for the first 21 days. Blood samples were collected at pre-dose, within 10min

of EOI of cycle1 and cycle3 for GQ1001 and TAb; collected at pre-dose, 4h and 24h after dose in D1 and D8 for pyrotinib; and collected integrally at pre-dose, within 10min of EOI, 0.5h, 2h, 8h, 24h, 48h, 72h, 168h, and 336h after dose from different patients. Concentrations of GQ1001, TAb and pyrolizinib in serum and fDM1 in plasma were quantitated using enzyme-linked immunosorbent assay (ELISA) and liquid chromatography and tandem mass spectrometry separately.

Results: A total of 15 HER2-positive metastatic breast cancer patients received GQ1001 and pyrotinib treatment. All patients (15/15) had failed on previous trastuzumab (or its biosimilars) containing therapy. And 33.3% of the patients (5/15) had been treated with pertuzumab. Dose escalation has been completed with no DLT reported. In HER2-positive advanced breast cancer, the combination of GQ1001 and pyrotinib (320mg, qd) did not significantly change the plasma concentrations of GQ1001 at BI and the end of infusion and each time point of DM1 in Cycle1and Cycle3 compared with GQ1001 alone. Compared with pyrotinib alone (320mg, qd), there was no significant change in the plasma concentration of pyrotinib at D1-4 hours after a single dose. The results showed that there were no significant changes in the plasma concentrations of GQ1001, DM1 and pyrotinib after the combination of GQ1001 (6.0mg/kg, 7.2mg/kg, 8.4mg/kg, ivgtt, 3W) and pyrotinib (320mg, qd). At the data cut off date (June 30th, 2024), the median follow-up time was 8.61(95CI% 6.83-15.08) months. Ten patients were still on going, with the longest treatment duration of 523 days.The 12-month-PFS rate was 73.5% and the objective response rate (ORR) was 80% (12/15) in all patients enrolled. At 6.0 mg/kg, 7.2 mg/kg and 8.4 mg/kg dose level, the ORR was 75% (3/4), 100% (5/5), 66.7% (4/6), respectively. No new safety signal was observed.

Conclusion: No significant changes were observed in the plasma concentrations of GQ1001, DM1 and pyrotinib after the combination of GQ1001 and pyrotinib. This combinaiton demonstrated promising preliminary antitumor activity among pretreated patients with HER2-positive metastatic breast cancer.
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P5-05-03: Neratinib-based combination treatments for patients with HER2-positive breast cancer brain metastases

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Background: Breast cancer brain metastases (BCBM) contribute to substantial morbidity and mortality in patients (pts) with HER2+ metastatic BC (mBC). The central nervous system (CNS) is a common site of recurrence in pts with HER2+ BC, and there is an ongoing need for safe and effective treatments. Neratinib (N), an oral, irreversible pan-HER tyrosine kinase inhibitor, is FDA approved for the extended adjuvant treatment of early-stage HER2+ BC and in combination with capecitabine (C) for HER2+ advanced or mBC. We summarize prospective data of N-based combinations in HER2+ BCBM.
Methods: We conducted a literature review of N combinations for HER2+ BCBM, focusing on prospective trials reporting CNS outcomes. CNS-specific data were extracted from studies restricted to pts with untreated/active BM (TBCRC 022; NCT01494662) or asymptomatic/stable BM (NALA [NCT01808573] and NEfERT-T [NCT00915018]). TBCRC 022, a dedicated HER2+ BCBM trial, assessed N combinations in pts with no prior lapatinib (L; Cohort 3A; N+C) or with prior L (Cohort 3B; N+C), in pts with previously untreated BM (Cohort 4A; N+T-DM1), and in pts progressing after CNS-directed therapies with no prior T-DM1 (Cohort 4B; N+T-DM1) or in pts with prior T-DM1 (Cohort 4C; N+T-DM1). Also available were descriptive CNS outcomes for 1) pts with baseline CNS metastases previously treated with HER2-directed therapy in NALA (N+C vs L+C) and 2) metastatic treatment-naive pts in NEfERT-T (N+paclitaxel [P] vs trastuzumab [T]+P). Endpoints included CNS objective response rate (CNS-ORR) per composite/volumetric (TBCRC 022) or RECIST (NALA and NEfERT-T) (assessed locally or centrally), or ORR per Response Assessment in Neuro-Oncology-Brain Metastases (RANO-BM; assessed centrally). Cumulative 1-year incidence of intervention for CNS disease or incidence of progressive CNS disease were reported for the NALA CNS subgroup.
Results: The analysis includes 212 pts: 93 with untreated/active BCBM from TBCRC 022; 101 (16.3% of ITT) from NALA; and 18 (3.8% of ITT) from NEfERT-T. No pts had received prior tucatinib and only 2 pts in TBCRC 022 cohort 4 had received prior trastuzumab deruxtecan. Across all three studies, 131 pts had measurable/target CNS lesions at baseline and were evaluable for CNS-ORR (TBCRC 022: n=49 in Cohort 3 and n=44 in Cohort 4; NALA: n=32; NEfERT-T: n=6). In TBCRC 022, CNS-ORRs were 49% (18 of 37 pts in Cohort 3A; N+C), 33% (4 of 12 pts in Cohort 3B; N+C), 50% (3 of 6 pts in Cohort 4A; N+T-DM1), 29% (5 of 17 pts in Cohort 4B; N+T-DM1), and 24% (5 of 21 in Cohort 4C; N+T-DM1). In NALA, CNS-ORRs were 26% (5 of 19 pts; N+C) vs 15% (2 of 13 pts; L+C). In NEfERT-T, CNS-ORRs were 100% (3 of 3 pts; N+P) vs 33% (1 of 3 pts; T+P). In TBCRC 022, RANO-BM ORRs were 24% (9 of 37 pts; Cohort 3A), 17% (2 of 12 pts; Cohort 3B), 33% (2 of 6 pts; Cohort 4A), 35% (6 of 17 pts; Cohort 4B), and 29% (6 of 21 pts; Cohort 4C). In the NALA CNS subgroup (n=101), 1-year cumulative incidence of intervention for CNS disease was 26% for N+C vs 36% for L+C, and 1-year cumulative incidence of progressive CNS disease was 26% (N+C) vs 42% (L+C). Diarrhea was the most common grade ≥ 3 toxicity.
Conclusions: Data from prospective trials of N-based combinations show consistent intracranial activity across various treatment settings in untreated/active and asymptomatic/stable HER2+ BCBM. Clinical guidelines and real-world analyses further support the use of N combinations for HER2+ BCBM. Notably, N+T-DM1 had intracranial efficacy in pts with previously untreated BM and pts who had undergone multiple local

CNS-directed and/or systemic therapies, including prior T-DM1. In addition to the established CNS activity of tucatinib and trastuzumab deruxtecan, the combination of N with other FDA-approved drugs represents a promising approach to the treatment of HER2+ BCBM in clinical practice, although the optimal sequence of treatments is not known.

P5-05-04: Final results of a phase II trial evaluating paxalisib with trastuzumab for patients (pts) with HER2 positive metastatic breast cancer with active brain metastases.

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Background: HER2+ breast cancer brain metastases (BCBM) have frequent alterations in the PI3K/Akt/mTOR pathway. Mutations in PIK3CA or PTEN loss are associated with resistance to anti-HER2 therapies and worse prognosis. Preclinical data from patient-derived xenografts (PDX) models of HER2+ BCBM showed that inhibition of PI3K and mTOR led to durable responses regardless of PIK3CA mutation status. Paxalisib is a potent, brain-penetrant inhibitor of class I PI3K and mTOR. We report the final results of a phase II trial evaluating paxalisib with trastuzumab for pts with HER2+ BCBM (NCT03765983).
Methods: Eligible pts had HER2+ active BCBM with at least 1 measurable (≥10 mm) CNS lesion, ECOG PS 0-2, and any prior lines of therapy. The trial had two cohorts: Cohort A: a single-arm, two-stage, phase II cohort with safety run-in; and Cohort B: a pre-surgical window cohort. Pts initially received paxalisib 45 mg orally daily and trastuzumab 8 mg/kg loading dose, then 6 mg/kg every 3 weeks. Primary endpoint for Cohort A was objective response rate (ORR) in the CNS per Response Assessment in Neuro-Oncology Brain Metastases (RANO-BM) criteria. Secondary endpoints included progression-free and overall survival (PFS, OS), safety and pt-reported outcomes. Research blood and cerebrospinal fluid with optional tumor biopsy were requested at baseline, on-treatment and at progression. Cohort A planned to enroll 37 pts in a Simon two-stage design. If ≥4 responses were seen at the second stage, the regimen would be considered successful. This design had 90% power with alpha ≤10%. Cohort B planned to enroll 10 patients.
Results: Between February 2019 and March 2024 we enrolled 17 pts to Cohort A and 0 to Cohort B. Median age was 44.3 years, 70.6% White, 17.6% Black, and 66.7% of tumors were hormone receptor-positive. Among the 17 pts with BCBM, 14 pts (82.4%) also had extracranial disease. Pts had a median of 8 prior lines of therapy (range 3-14). Five of 17 pts (29.4%) had prior craniotomy, 12/17 (70.6%) had prior stereotactic radiation, and 6/17 (35.3%) had prior whole-brain radiation. Two of 5 pts enrolled in safety run-in had dose-limiting toxicities including fatigue, diarrhea, anorexia and dehydration; and subsequent pts (n=12) were enrolled to paxalisib dose level -1 (30mg daily, RP2D). Within the first stage, 0/12 pts (0%) had response in CNS and the trial closed for futility. One pt (8.3%) had clinical benefit through 18 weeks per RANO-BM. Six pts (50%) had stable disease as best RANO-BM response. The ORR by RECIST 1.1 was 0%, with 1 unconfirmed partial response

and 8 pts (66.7%) with stable disease. Site of first progression was CNS in 5 pts, CNS and extracranial in 1 pt, and 5 pts had clinical deterioration. Median bi-compartmental PFS was 7.4 weeks, and median OS was 16.5 months. Fourteen of 17 pts (82.4%) had treatment-related adverse events (TRAEs) of grade (G) ≥2. The most common TRAEs G ≥2 were fatigue (47.1%; 0% G≥3), diarrhea (29.4%; 11.8% G≥3), hyperglycemia (29.4%; 11.8% G≥3), and mucositis (23.4%; 5.9% G≥3). Molecular analyses of blood, cerebrospinal fluid and tumor tissue will be presented at the meeting.
Conclusions: In this heavily pre-treated population of pts with HER2+ active BCBM, the combination of paxalisib 30 mg daily with trastuzumab was feasible, with a toxicity profile consistent with a class effect of PI3K/mTOR inhibitors. However, it was associated with minimal clinical activity.

P5-05-05: Incidence and Risk Factors of Brain Metastasis in HER-2 Positive Primary Breast Cancer: A Retrospective Analysis

Anu Gaba, Hallie Thompson. Abe E Sahmoun

Introduction: Breast cancer (BC) is the most prevalent malignancy and the second leading cause of cancer-related death among women worldwide. Despite the progress in early diagnosis through screening and effective treatment, BC recurrence and metastasis remain a significant risk for lower survival. Brain metastasis (BM) occurs in nearly one-third of HER-2+ patients.
BM is associated with both poor quality of life and prognosis. The blood–brain barrier and the limited activity of anti-HER-2+ therapy in the brain microenvironment have contributed to the susceptibility of patients with HER-2+ primary BC to BM development.
The aim of this study was to identify modifiable risk factors associated with increased risk for developing BM in HER-2+ primary BC.
Methodology: This was a retrospective study that examined demographics, clinical, and treatments variables in HER-2+ primary BC women. Bivariate analyses were conducted comparing women with HER-2+ BC who developed BM to women with metastasis to other organs using t-test for continuous variables and Chi-square or Fisher's exact tests for categorical variables. All significance tests were two-sided and p-value < 0.05 was considered significant.
Results: 42 women diagnosed with HER-2+ primary BC were analyzed. 27 (64%) of women developed BM. The mean age (±SD) was 51.5 (12.9) years for women with BM and 51.3 (14.1) years for no BM (p=0.96). There was no association between race and BM occurrence (White: 64% vs. other: 67%; p=1.00). The median tumor size (IQR) was 18.5 (12-31) mm for women with BM and 27.5 (13-50) mm for no BM (p=0.26). There were no significant differences in cancer histology, hormonal receptors positives (ER+/PR+), or BC management. Liver metastasis was associated with increased risk of brain metastasis (82% vs. 18%; p=0.04). The estimated median time to BM was 1.4 (95% CI: 0.9-2.7) years. Almost half 18 (42.9%) of women died. The estimated median time to death was 4.3 (95% CI: 2.2-5.2) years.

Conclusions and Significance: This descriptive small study found that diagnosis of liver metastasis was associated with increased risk of BM. Brain MRI use in women diagnosed with liver metastasis could be beneficial to cancer. Future studies should use the National Cancer Database to have the adequate power to detect other risk factors for developing BM. Lastly, examining differences in pathological molecular biology by comparing the differences in the breast primary lesions of patients with BM and those without BM could be helpful in predicting the occurrence of BM.

P5-05-06: Factors Influencing Long-Term Remission in HER2-Positive Metastatic Breast Cancer Patients Treated with Trastuzumab-based Therapy: A Single-Center Retrospective Analysis

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Introduction: Approximately 20% of individuals diagnosed with breast cancer progress to metastatic disease. Overexpression of the HER-2 oncogene occurs in 15-20% of breast tumors and typically signifies a more aggressive tumor biology. Trastuzumab therapy has been linked to prolonged survival, especially among patients who achieve more than 2 years without disease progression while on treatment. This study examines baseline characteristics and identifies factors associated with extended response or remission to initial trastuzumab-based therapy, focusing on patients who have benefited from treatment for more than 5 years.

Methods: We retrospectively identified 105 patients diagnosed with HER2-positive recurrent and de novo metastatic breast cancer between 2015 and 2019 from the Tawam Hospital pharmacy database. The primary endpoint of the study was the time to tumor progression. We categorized patients based on their response to trastuzumab-based therapy, specifically comparing those with treatment durations of 5 years or more to those with less than 5 years. Univariate and multivariable logistic regression models, incorporating variables with a p-value ≤ 0.25 from unadjusted analysis, were employed to explore the association between achieving a time to tumor progression greater than 5 years and various risk factors. Data analysis was conducted using R software version 4.3.1

Results: The median age at diagnosis of metastatic disease was 47 years (interquartile range, 37–53 years). Among the patients, 78 (74%) remained in remission for less than 5 years, while 27 (26%) achieved remission for 5 years or more, with 22 of these patients continuing therapy at the time of abstract preparation. In univariate analysis, factors significantly associated with achieving a time to tumor progression greater than 5 years included age at diagnosis (OR = 1.04, 95% CI [1.00-1.09], p = 0.030), T3-4 stage (OR = 3.11, 95% CI [1.17-8.68], p = 0.023), N2-3 stage (OR = 0.31, 95% CI [0.08-0.96], p = 0.042), ECOG performance status of 2-3 (OR = 0.22, 95% CI [0.03-0.82], p = 0.022), recurrent disease (OR

= 0.16, 95% CI [0.04-0.45], p < 0.001), and presence of brain metastases (OR = 0.07, 95% CI [0.00-0.37], p < 0.001). In multivariate analysis, age at diagnosis (adjusted OR = 1.06, 95% CI [1.01-1.12], p = 0.030), grade 3 diagnosis (adjusted OR = 0.26, 95% CI [0.06-0.99], p = 0.048), recurrent disease (adjusted OR = 0.22, 95% CI [0.05-0.80], p = 0.021), presence of central nervous system metastases (adjusted OR = 0.06, 95% CI [0.00-0.45], p = 0.004), and treatment interruption (adjusted OR = 8.50, 95% CI [2.30-38.1], p = 0.001) remained significantly associated with achieving a time to tumor progression greater than 5 years. Cardiac toxicity associated with trastuzumab-based therapy was observed in 2 patients within the study cohort, necessitating treatment interruption in one case and discontinuation in another.

Conclusion: The study identifies factors associated with prolonged remission (>5 years) in HER2-positive metastatic breast cancer patients treated with trastuzumab based therapy. Older age at diagnosis, lower disease stage and grade, better performance status, Denovo metastastases, and no brain metastases predict favorable outcomes. These findings underscore the importance of early diagnosis, treatment adherence, and tailored management approaches for improving long-term prognosis in this group.

P5-05-07: A prospective, multicenter, single-arm clinical study of inetetamab combined with pyrotinib and capecitabine/vinorelbine in the treatment of HER2-positive metastatic breast cancer resistant to previous trastuzumab treatment

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Background: Inetetamab is an innovative Fc-modified monoclonal antibody targeting human epidermal growth factor receptor 2 (HER2). Pyrotinib is a tyrosine kinase inhibitor (TKI). Theoretically, the combination of a large-molecule monoclonal antibody and a small-molecule TKI can exert a better antitumor effect. In the study, inetetamab in combination with pyrotinib and capecitabine/vinorelbine was used to treat HER2-positive metastatic breast cancer (MBC) that was previously resistant to trastuzumab. This study evaluated the efficacy and safety of the treatment regimen and conducted an exploratory analysis of the clinicopathological characteristics of the benefiting population.

Methods: The prospective, multicenter study enrolled 40 patients with HER2-positive MBC who received inetetamab in combination with pyrotinib and capecitabine/vinorelbine between December 2020 and December 2023. All enrolled patients were previously resistant to trastuzumab treatment. The primary endpoint was progression-free survival (PFS) estimated using the Kaplan-Meier method. The secondary endpoints were objective response rate (ORR), disease control rate (DCR), and safety.

Results: Among all patients receiving treatment, the median number of treatment lines was

3, and the median PFS was 8.2 months (95% CI 2.860-13.540). The correlation between different clinicopathological characteristics and mPFS was assessed using the Kaplan-Meier method. The results showed that significant associations with mPFS were observed for estrogen receptor (HR) positivity (P = 0.019), disease-free survival (DFS) > 24 months (P = 0.005), absence of lung metastasis (P = 0.017), Eastern Cooperative Oncology Group (ECOG) performance status score of 0-2 (P < 0.001), ≤3 lines of inetetamab treatment (P < 0.001), secondary resistance to trastuzumab (P = 0.011), no prior TKI treatment (P < 0.001), no prior antibody-drug conjugate (ADC) treatment (P < 0.001), and no prior cranial radiotherapy (P = 0.026). Multivariate analysis using the Cox model revealed that the independent prognostic factors affecting mPFS were no prior TKI treatment (HR = 0.260, 95% CI 0.079-0.859, P = 0.027) and ≤3 lines of inetetamab treatment (HR = 0.128, 95% CI 0.025-0.654, P = 0.014). In this study, the ORR was 62.5%, and the DCR was 77.5%. The Fisher's exact test was used to examine the correlation between different clinicopathological characteristics and ORR. The results suggested significant associations with ORR for DFS > 24 months (P < 0.001), ECOG performance status score of 0-2 (P = 0.009), ≤3 lines of inetetamab treatment (P < 0.001), secondary resistance to trastuzumab (P = 0.021), no prior TKI treatment (P = 0.003), no prior ADC treatment (P = 0.008), and no prior cranial radiotherapy (P = 0.018). The most common adverse reactions of all grades in this study were diarrhea (95.0%), leukopenia (45.0%), neutropenia (42.5%), anemia (37.5%), fatigue (40.0%), increased aspartate aminotransferase (AST) (37.5%), increased alanine aminotransferase (ALT) (35.0%), and nausea (30.0%). The incidence of severe adverse reactions of grade 3 or above was relatively low (all < 20%).

Conclusion: The combination of inetetamab with pyrotinib and capecitabine/vinorelbine can serve as a good treatment option for HER2-positive MBC that is resistant to trastuzumab. In particular, patients who have not received TKI treatment before and have ≤3 lines of inetetamab treatment can experience more significant benefits in terms of PFS. All adverse events (AEs) are controllable and tolerable.

Clinical trial information: ChiCTR2300074366.
Research Sponsor: None.

P5-05-08: Inetetamab-base regimens for patients with HER2-positive metastatic breast cancer and brain metastases: a real-world retrospective study

Liping Chen

Background: Although huge progresses have been achieved in treating HER2-positive BC, HER2-positive metastatic breast cancer (MBC) remains incurable, nearly 70% to 75% MBC patients will progression after first-line treatment with trastuzumab, posing a disproportionate health burden on patients and presenting a substantial unmet medical need.

Breast cancer metastasizes to the brain is a late event, which will happen to approximately 50% of patients with HER2+ breast cancer, with a median survival of 7 to 18 months after diagnosis. In recent years, a growing number of small-molecule TKIs produce an antitumor effect on the brain. However, whether monoclonal antibodies plus small-molecule TKIs can exactly enhance the treatment efficiency of breast cancer with brain metastasis patients requires further verification.

Inetetamab is a Chinese-origin recombinant anti-HER2 monoclonal antibody with aminoamides modified Fc segment which optimizes the antibody-dependent cellular cytotoxicity (ADCC) effect. Herein, we assessed the efficacy and safety of inetetamab-containing regimens in patients with HER2-positive metastatic breast cancer (MBC), particularly focused on the benefits by patients with brain metastases.

Methods: We retrospectively reviewed the medical records of patients with HER2-positive MBC who received inetetamab-containing regimens as a salvage treatment at any line setting from December 2020 and April 2024.The primary end point was progression-free survival (PFS) in the total population (TP). Secondary end points included PFS in the subgroup with brain metastases, objective response rate (ORR), disease control rate (DCR), and safety. The study is ongoing, but recruitment is complete.

Results: At the data cutoff date of June 1,2024, a total of 90 patients were enrolled in this analysis. Median follow-up duration was 7.2 months (IQR 3.6–13.1). The median PFS reached 12 months (95% confidence interval [CI] 7.3 to 17 months) in the TP. The ORR was 46.7 % (42/90), and the DCR was 92.2 % (83/90). The median PFS of first-line, second-line, third-line or above were not reach,15.9 months (95% CI 11.9 to NA months),5.9 months (95% CI 4.4 to 12.2 months), respectively. Cox univariate and multivariate analyses demonstrated that first- and second-line was the significant favorable prognostic factor for PFS (treated as first- and second-line vs third-line or above: 15.9 vs 5.9 months, p=0.0021). 30 out of 90 patients had brain metastases, the median PFS in the subgroup with brain metastases reached 12 months (95% CI 4.7 to 19.3 months). Notably, in this subgroup, the median PFS had no significant differences with overall patients (p=0.568), ORR was 53.3% (16/30), DCR was 96.7% (29/30). Furthermore, subgroup analysis revealed a median PFS of 12 months (95% CI 5.5 to 18.5 months) in patients with brain metastases radiotherapy (N=22). The most frequently combine target regimen was pyrotinib or apatinib (29/30, 96.7%).

The most common treatment-related adverse events (frequency ≥ 10%) were diarrhoea (52.2%), vomiting/ nausea (20%), fatigue (20%), and leukopenia (18.9%). Grade≥ 3 treatment-related adverse events mainly were diarrhea (12.2%). No grade 4 diarrhea or cardiac-related events were reported.

Conclusion: Inetetamab offers a promising option and a manageable safety profile for HER2-positive MBC who pretreated with multiple-line therapies. Meanwhile, Inetetamab plus small-molecule TKIs regimens show the activity in brain metastases population, which deserve further validation in a larger group trial.

P5-05-09: Trastuzumab deruxtecan combined with pyrotinib in first-line HER2-positive unresectable or metastatic breast cancer: an exploratory, multi-center, single-arm, phase Ib/II study (TROPHY)

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Background: Trastuzumab deruxtecan (T-DXd), a human epidermal growth factor receptor 2 (HER2)-directed antibody-drug conjugate (ADC), has demonstrated remarkable efficacy in the second-line or later treatment of HER2-positive (HER2+) metastatic breast cancer (mBC) and its exploration as the first-line (1L) treatment (DESTINY-Breast09) is ongoing. Combining T-DXd with pertuzumab may further enhance the efficacy seen with T-DXd monotherapy. Pyrotinib, an irreversible, pan-HER receptor tyrosine kinase inhibitor (TKI), has also been approved in combination with trastuzumab and docetaxel for the 1L treatment of HER2+ mBC and is widely used in China. As preclinical and clinical studies have shown the synergistic effects of HER2 ADCs combined with TKIs (Olson D, et al. Cancer Res Commun 2023; Borges VF, et al. JAMA Oncol 2018), this study evaluates the efficacy and safety of the promising combination regimen of T-DXd plus pyrotinib for the 1L treatment of HER2+ mBC in China (TROPHY, NCT06245824). Here we will report the results from phase Ib of this study.
Methods: This multi-center, single-arm, phase Ib/II study was conducted in 8 sites in China and is comprised of a dose finding stage (phase Ib) followed by a dose expansion stage (phase II). Patients with no prior chemotherapy or HER2-targeted therapy in the metastatic setting who have experienced relapse with a disease-free interval (DFI) > 6 months from the completion of neoadjuvant or adjuvant treatment to advanced or metastatic diagnosis are eligible for enrollment. Asymptomatic or treated brain metastases is allowed. The primary objective of phase Ib was to assess safety and tolerability and determine the recommended phase 2 dose (RP2D). Patients were followed up beyond the 21-day dose-limiting toxicity (DLT) period for safety events. The primary objective of phase II was to evaluate the efficacy of T-DXd plus pyrotinib as the 1L treatment of HER2+ mBC.
Results: As of 28 Jun 2024, 5 HER2+ mBC patients were treated with T-DXd 5.4 mg/kg every 21 days (Q3W) plus pyrotinib 400 mg (n=2) or 320 mg (n=3) once daily as starting dose, with a median duration of follow up of 3.5 months (range, 0.7-5.4). The median age of enrolled patients was 47 years (range, 33-68), and all of them had an ECOG performance status (PS) of 1, 60% (3 of 5) with visceral metastases. The only DLT was grade 3 anorexia, which occurred during the first cycle in 1 patient who received 400 mg of pyrotinib. This was followed by a de-escalation to the pyrotinib 320 mg cohort, where no DLT occurred. The most common treatment-emergent adverse events (TEAEs) included diarrhea (100%, 5 of 5), nausea (100%, 5 of 5), vomiting (100%, 5 of 5), anorexia (60%, 3 of 5), alanine aminotransferase (ALT) increased (60%, 3 of 5), aspartate aminotransferase (AST) increased (60%, 3 of 5), and weight loss (60%, 3 of 5), etc. Grade 3 TEAEs included anorexia and ALT increased (in 1 patient in the pyrotinib 400 mg cohort), and diarrhea (in 1 patient in the pyrotinib 320 mg cohort). There was no grade 4 or 5 TEAEs. All 4 patients whose tumors were evaluable had achieved partial responses.

Conclusions: Pyrotinib 320 mg was identified as RP2D. The combination regimen of T-DXd plus pyrotinib showed a manageable safety profile with preliminary antitumor activity as the 1L treatment in patients with HER2+ mBC and will be explored in phase II stage.

P5-05-10: Real-world treatment patterns and clinical outcomes of first-line therapy and first-line maintenance therapy in patients with human epidermal growth factor 2-positive metastatic breast cancer

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Background: In patients with HER2+ MBC, treatment guidelines recommend first-line (1L) combination therapy with a taxane, trastuzumab, and pertuzumab (THP), followed by 1L maintenance therapy with trastuzumab and pertuzumab. Real-world data on treatment patterns and clinical outcomes with 1L therapy and, in particular, 1L maintenance therapy in patients with HER2+ MBC are limited. Furthermore, while brain metastases (BM) are less common at 1L initiation, up to 50% of patients with HER2+ MBC will eventually develop BM, often during 1L maintenance.
Objective: To describe patient characteristics and treatment patterns overall, and clinical outcomes with THP, in patients with HER2+ MBC, including patients with BM prior to or at 1L initiation, receiving 1L therapy and 1L maintenance therapy in the real-world setting.
Methods: This retrospective cohort study included female patients (≥18 years old) diagnosed with HER2+ MBC between January 2012 and February 2024 in the Flatiron Health Metastatic Breast Cancer Enhanced Datamart who received 1L therapy in real world clinical practice settings. Patients with BM were those with BM prior to or at 1L initiation. Key outcomes for patients who received 1L THP, including time to discontinuation (TTD), time to next treatment (TTNT), and overall survival (OS), were assessed from 1L initiation date (index date) until data cut off or death using the Kaplan-Meier method. Induction duration for patients who received 1L THP was defined as time from index date until 20 days after last administration of taxane. Maintenance duration was defined as time from 21 days after last administration of taxane until the end of 1L maintenance with trastuzumab and/or pertuzumab.
Results: A total of 4739 patients with HER2+ MBC received 1L treatment and met inclusion criteria, including 606 patients with BM at or prior to initiating 1L therapy. At 1L initiation, 2382 (50.3%) patients received dual HER2-targeted therapy, 1727 (36.4%) single HER2-targeted therapy, 310 (6.5%) chemotherapy alone, and 320 (6.8%) other regimens. In patients with BM, 234 (38.6%) received dual HER2-targeted therapy, 319 (52.6%) single HER2-targeted therapy, 28 (4.6%) chemotherapy alone, and 25 (4.1%) other regimens. Overall, 1878 (39.6%) patients received 1L THP, and their median (95% CI) TTD was 14.0 (13.0–15.0) months, median (95% CI) TTNT was 15.0 (14.0–16.0) months, and median (95% CI) OS was 57.0 (53.0–63.0) months. In the subset of patients who had BM at or before 1L initiation who received 1L THP (n = 147, 24.3%), median (95% CI) TTD was 12.0 (9.6–14.0) months, TTNT 12.0 (9.7–14.0) months, and OS 29.0 (26.0–40.0) months. Of those

who received 1L THP, 1289 of 1878 patients (68.6%), including 87 of 147 (59.2%) with BM, received 1L maintenance therapy. Median (IQR) duration of induction therapy was 4.2 (3.6–4.9) months overall and 4.2 (3.5–5.6) months in patients with BM. Median (IQR) duration of maintenance therapy was 9.4 (4.1–22.6) months overall and 6.9 (3.3–18.2) months in patients with BM.
Conclusion: Patients with HER2+ MBC were treated with a range of 1L regimens during the study period. Less than half of the patients received guideline-recommended 1L THP; of these, real-world outcomes including OS were poorer in patients with BM compared with the overall group. While median duration of induction therapy was similar between the overall cohort and patients with BM, patients with BM had a shorter duration of maintenance therapy than patients overall. These data highlight the unmet need for effective 1L treatment options in patients with HER2+ MBC, particularly patients with BM. Ongoing clinical trials evaluating HER2-targeting regimens in the 1L maintenance setting may offer additional options for improved outcomes in this treatment landscape.

P5-05-11: Systematic review of ADCs vs chemotherapy in 2L+ Her2+ mBC

Bruno Larvol, Mark Gramling

Antibody-drug conjugates (ADCs), which combine monoclonal antibodies with cytotoxic payloads, have revolutionized cancer treatment by providing more effective and selective therapies with less toxicity than traditional chemotherapy.
A systematic search in LARVOL CLIN- a database of 100k+ trials, 95k+ Kaplan-Meier (KM) curves, and 15k+ Hazard Ratios (HRs)- was conducted to identify trials comparing ADCs and chemotherapy in 2L+ for Her2+ metastatic breast cancer (mBC). A meta-analysis was performed on phase 3 trials using digitized KM data and HRs extracted from forest plots. Biomarker subgroup populations were evaluated in the context of ADC response and were verified using VERI, a precision oncology database.
Only 5 trials had mature survival data on ADCs vs chemotherapy. All trials used trastuzumab as the antibody, and ADCs varied in payload and linker. TH3RESA and EMILIA assess T-DM1 (trastuzumab emtansine); DESTINY-Breast02 and DESTINY-Breast04 evaluate T-DXd (trastuzumab deruxtecan); and TULIP examines T-Duo (trastuzumab duocarmazine). The primary outcome in all these trials was progression-free survival (PFS) and for TH3RESA and EMILIA Overall survival (OS) as well. The evaluated ADCs prove their efficacy in treating Her2+ mBC compared to the respective controls (HR PFS: 0.52* [TH3RESA]; 0.69* [EMILIA]; 0.3* [DESTINY-Breast02]; 0.36* [DESTINY-Breast04]; 0.64* [TULIP]; and HR OS: 0.68*. [TH3RESA]; 0.75* [EMILIA]; 0.69* [DESTINY-Breast02]). However, ADC benefit may be higher for T-DXd compared to T-Duo (ORR: 74.1% vs 27.2% [DESTINY-Breast02]; 52% vs 16% [DESTINY-Breast04] and 27.8% vs 29.5% [TULIP]). In contrast to standard chemotherapy, patients benefit from T-DM1 and T-XDd regardless of PIK3CA mutation status (HR PFS for PIK3CA mutated vs PIK3CA wild-type: 0.44* vs 0.47* [TH3RESA]; 0.45* vs 0.74 [EMILIA]; 0.6* vs 0.5* [DESTINY-Breast04]) and PTEN expression levels (HR PFS for increased PTEN expression vs decreased PTEN expression: 0.4* vs 0.49* [TH3RESA]; 0.78 vs 0.55* [EMILIA]). Regarding Her2 expression, while DESTINY-Breast04

evaluated Her2+ low mBC, patients with higher mRNA expression levels benefit most from ADC treatment (HR PFS for Her2 expression high vs Her2 expression low: 0.68* vs 0.4* [TH3RESA]; 0.64* vs 0.65* [EMILIA]; 0.55* vs 0.48* [DESTINY-Breast04]).
ADC-targeted therapies open significant new opportunities with more than 200 trials in phase 3. T-DM1 with DM1 as payload and T-DXd with deruxtecan are approved for Her2+ mBC patients after anti-Her2 regimens. However, T-Duo with seco-DUBA as payload, despite fulfilling its PFS primary outcome, did not reach OS statistical significance, causing FDA approval to be paused. Regardless of PIK3CA mutations and PTEN loss, which could be expected to drive Her2 pathway activation, T-DM1 and T-DXd are effective. While T-DM1 and T-DXd demonstrate enhanced benefit with relatively elevated Her2 mRNA levels, bot∫h remain effective even in cases of low Her2+ mBC.

P5-05-12: Real-world outcome of patients with HER2-positive metastatic breast cancer who were treated with inetetamab-containing regimens: A multicenter retrospective analysis conducted in China

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Background: Inetetamab is a novel anti-HER2 humanized monoclonal antibody with the same Fab segment as trastuzumab, but its Fc segment is modified by amino acids to provide stronger antibody-dependent cell-mediated cytotoxicity(ADCC). This study aimed to observe real-world outcome of inetetamab-containing regimens in HER2-positive metastatic breast cancer (MBC), and explore more effective and safe treatment strategies for these patients(pts).
Methods: This was a multicenter, retrospective, real-world study. A cohort of HER2-positive MBC pts who received inetetamab-containing regimens between November 2020 and March 2023 was evaluated. The primary endpoint was progression-free survival (PFS), according to the Response Evaluation Criteria in Solid Tumors, version 1.1. Secondary endpoints included objective response rate (ORR) and disease control rate (DCR). Adverse events (AEs) were graded according to the National Cancer Institute Common Toxicity Criteria version 5.0.This trial has been registered with the Chinese Clinical Trial Registry under the number ChiCTR2200061413.
Results: A total of 136 pts were included in the final analysis. The median age of the enrolled pts was 53 years (range 27–90 years). 15 pts had de novo stage IV cancer (11%), and 121 pts had recurrent disease (89%). The median number of treatment lines administered was 3 (range 1–11). In total, 136 pts were included: 47 pts received ≤2nd-line treatment, and 89 pts received ≥3rd-line treatment. Most pts had received other anti-HER2 therapies prior to receiving inetetamab. 94.9% of pts had received trastuzumab(129/136), and 28.7% had received both trastuzumab and pertuzumab(39/136). Additionally, 67.6% of pts had prior treatment with trastuzumab and HER2-TKIs in anti-HER2 regimens (92/136).The median PFS(mPFS) of the entire cohort was 5.7 months (95% CI 4. 97-6.44 months). The ORR was

33.1% (45/136) and the DCR was 75% (102/136). The mPFS of the ≤2nd-line treatment group was 9.8 months (95% CI 3.35-16.25 months), which was significantly better than ≥3rd-line treatment group at 5 months (95% CI 3.87-6.13 months, P＜0.001). The mPFS was 12.07 months in the subgroup that previously received trastuzumab without TKIs(95% CI 8.46-15.68 months). This was significantly longer than the mPFS of 5.4 months in the subgroup that had received trastuzumab with HER2-TKIs (95% CI 4.62-6.18 months, P=0.001).The most common adverse events (AEs) were leukopenia (31.6% [43/136]), neutropenia (27.2%[37/136]), diarrhea (19.1%[26/136]), nausea (16.2%[22/136]), and fatigue (13.2% [18/136]). Among these, grade 3 or 4 AEs were leukopenia (15.4% [21/136]), neutropenia (11.7% [16/136]), and diarrhea (8.1% [11/136]). No treatment-related serious adverse events or deaths occurred.
Conclusion: Inetetamab, a novel anti-HER2 therapeutic option, has demonstrated encouraging efficacy with manageable toxicity in patients with HER2-positive metastatic breast cancer. It could be considered as an alternative treatment option for these patients.

P5-05-13: Assessment of efficacy and pulmonary toxicity of Trastuzumab Deruxtecan in HER2-positive and HER2-low Metastatic Breast Cancer in United Arab Emirates

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Background: Trastuzumab deruxtecan (T-DXd) is a HER2-directed antibody-drug conjugate indicated for the treatment of unresectable or metastatic HER2-positive (IHC 3+ or ISH positive) breast cancer who have received a prior anti-HER2-based regimen. T-Dxd is also indicated for unresectable or metastatic HER2-low (IHC 1+ or IHC 2+/ISH-) breast cancer, following prior chemotherapy in the metastatic setting or recurrent disease within 6 months of adjuvant chemotherapy. This study aims to evaluate the efficacy and safety of T-DXd in treating HER2-positive, and HER2- low metastatic breast cancer (MBC) patients in a real-world clinical setting. In the T-DXd pivotal clinical trials, response assessments were conducted every 6 weeks with CT or MRI and outside of a clinical trial setting, this frequent imaging is not practically feasible and is not reimbursed. We sought to review the incidence of pneumonitis in a real-world setting and to assess if the 3-monthly response assessment scans would be sufficient to rule out asymptomatic pneumonitis.
Methods: A retrospective analysis was conducted on 100 patients diagnosed with HER2-positive (IHC 3+ or ISH positive) or HER2-low (IHC 1+ or IHC 2+ and ISH negative) MBC treated with T-DXd at 5.4mg/kg every 21 days, with standardised dose adjustments as required. Treatment was continued indefinitely until disease progression or development of intolerable side effects. Responses were assessed using RECIST v1.1 criteria, and toxicity was determined using the CTCAE version 5.0. Data analysis was performed using the SPSS IBM software version 26.

Results: The median age was 47 years (range: 29-86); 92% were <65 years; 99% were

female. All patients had an ECOG performance status of 0-1at baseline. Eighty two percent of patients were HER2-positive (IHC 3+ or ISH positive), while 18 % were HER2-low (IHC 1+ or IHC 2+ and ISH negative). Seventy nine percent had visceral disease, 31% had brain metastases at baseline, 47% were hormone receptor positive (HR+), 55% had grade 3 disease, and 57% had high Ki-67 of > 20%. Sixty nine percent of patients had received T-DXd in the 2nd and 3rd line setting (34% and 35%, respectively). Partial response, complete response and stable disease was observed in 65%, 10% and 9% of patients, respectively. Overall survival at 12 months and 24 months was 81.5% and 59.5%, respectively. Progression-free survival (PFS) at 12 months and 24 months was 73% and 43.5%, respectively. Median PFS was 24 months (95% CI 16.90-31.09). Only one patient developed grade 3 pneumonitis suggesting that perhaps the 12-weekly assessment imaging is sufficient for the vast majority of patients.

Conclusions: Our real-world experience confirms that the efficacy of T-DXd in UAE is consistent with published data from published phase 3 clinical trials. The incidence of ILD is much lower than anticipated and until further data is available it may be reasonable to continue using the 12-weekly assessment scans to monitor patients for ILD. Further studies are needed to determine the optimal imaging frequency for monitoring ILD.

P5-05-14: Real-World Treatment Patterns and Clinical Outcomes of Inetetamab combined with pyrotinib plus chemotherapy in Her-2 negative metastatic breast cancer patients : a multi-center retrospective study

Huanhuan Zhou, Chao Deng, Lu Gan, Fan Li, Xuefeng Xu, Yanman Fang, Xiaowei Qi, Weigang Bian, Zeshun Yu, Zhanhong Chen, Xiaojia Wang

Background: Inetetamab is an innovative anti-HER2 monoclonal antibody. Studies indicated that the combination of the use of dual anti-HER2 components with complementary mechanisms of action will improve anti-tumor effect. The study aims to investigate the efficacy and safety of inetetamab combined with pyrotinib plus chemotherapy for HER2 positive metastatic breast cancer (HER2+MBC).

Methods: From July 2020 to December 2022, 99 HER2+MBC patients received at least two cycles of inetetamab combined with pyrotinib plus chemotherapy treatment regimens in in multicenters in China. Data cut-off April 2023. The primary endpoint was progression-free survival (PFS), secondary endpoints were objective response rate (ORR) and safety.

Results: In the overall population, the median PFS was 12.3 months (95% CI: 10.9-13.6), the ORR was 53.5%. Of the 99 patients in this cohort, 47(47.5%) patients received this treatment as first line (First-line subgroup) and 52（52.5%） patients as second line

(Second -line subgroup) , respectively. The median PFS in the first-line subgroup was significantly longer than that in the second-line subgroup (15.0 months [95% CI: 13.6-15.8] vs. 10.3 months [95% CI: 9.6-10.9], p<0.001). In First-line subgroup, the combination regimen of albumin-bound paclitaxel provided longer median PFS than other chemotherapy (15.0 months [95% CI: 13.6-NA] vs. 11.1 months [95% CI: 8.6-NA], p=0.012). In addition, the study included 19 patients with brain metastases. The mPFS of patients with versus patients without brain metastases was 10.5 (95% CI: 9.5-12.9) months versus 13.0 (95% CI: 11.2-15.0) months (p= 0.052). 4 patients with brain metastases in the first-line subgroup had longer mPFS of 15.6 (95% CI: 10.0-NA) months. The most common grade ≥3 treatment-related adverse events observed were leukopenia (19.2%), diarrhea (18.2%), myelosuppression (7.1%) and neutromenia (4.0%).

Conclusions: This study shows that Inetetamab combined with pyrotinib plus albumin-bound paclitaxel might be a safety and promising therapy for HER2-positive MBC, Including patients with brain metastases.

P5-05-15: Intrinsic subtype expression between durable and poor responder during anti-HER2 treatment in triple-positive breast cancer

Jieun Lee, Kabsoo Shin, Sung Hak Lee, Jae Ho Byun

Background: HER2 positive breast cancer is defined as HER2 overexpression by immunohistochemical stain or amplification of HER2 based on in situ hybridization, irrespective of ER or PR status. In Hormone receptor positive and HER2 positive breast cancer, there are crosstalk between estrogen receptor signaling pathway and HER2 pathway, and there may be difference of tumor response and biology compared to HR negative and HER2 positive breast cancer. In this study, we analyzed the intrinsic subtype of HR positive, HER2 positive breast cancer based on prediction analysis of microarray 50 (PAM50) and Breast Cancer 360 panel (nanostring) between durable and poor responder in first-line docetaxel + trastuzumab + pertuzumab (THP) treated patients.

Methods: Baseline tumor tissue was collected in 12 HR(+), HER2(+) metastatic breast cancer patients. Durable responders were defined as patients showing response over 35 months, and poor responders were defined as patients showing response less than 12 months to first-line THP. Molecular subtype was analyzed based on PAM50 and Breast Cancer 360 Panel.

Results: Total 6 durable responders and 6 poor responders with adequate tumor tissue were enrolled for the study. Before the analysis, authors hypothesized that durable responders would show HER2 enriched subtype and poor responders will show luminal subtypes. In final analysis, durable responders, 4 patients were classified as HER2 enriched, and 2 patients were luminal A subtype. In poor responders, 3 patients were Her2 enriched, 2 patients were luminal A, and 1 patient was classified as luminal B. Based on PAM50, patients were clustered based on cell differentiation, tumor inflammation signature (TIS)

and MHC2 expression. Durable responders showed relatively high expression for ER signaling and cytokine & chemokine expression.

Conclusions: Other than HER2 pathway, ER pathway and inflammatory signal may influence the tumor response of HR(+), HER2 (+) breast cancer. Further clinical trials should be designed based not only on HER2 status, ER status and other molecular subtypes should be considered to maximize the clinical benefit.

P5-05-16: Real-world analysis of inetetamab-based therapy for the treatment of HER2-positive advanced breast cancer.

Cailing Lin, Xiangqin Huang, Wei Chen, Zhiwu Lin

Background: Inetetamab is an Fc segment-modified innovative anti-HER2 monoclonal antibody. It has been proven to be effective and safe in HER2-positive advanced breast cancer. Here, we investigated the efficacy and safety of inetetamb-based therapy for HER2-positive metastatic breast cancer.

Methods: In this observational real world study, we investigated the medical records of eligible women who aged 18 years or older with pathologically confirmed HER2-positive recurrent or metastatic invasive breast cancer, and received at least two cycles of inetetamab combined with different treatment regimens in Fujian provincial hospital from August 2020 to present. The primary endpoint was progression-free survival (PFS) estimated by the Kaplan-Meier method. The sec- ondary endpoints were the objective response rate (ORR), disease control rate (DCR), and safety.

Results: A total of 91 patients were enrolled and 68 were included in the efficacy analysis. The patients' median age at enrollment was 54 years, 42 patients (46.2%) had hormone receptor-positive disease and 44 patients (48.4%) had visceral metastasis. The median number of treatment lines administered was two. Among them, the proportion of patients treated with trastuzumab was 80%. The median PFS was 14.0 months (95% confidence interval [CI] 11.5 to 19.0 months). Patients treated with first-line regimens benefited the most, with a median PFS of 18.0 versus (vs.) 14.0 vs. 7.5 months (first-line- vs. second-line- vs. third-line and above), ORR was 83.82% (57/68) and DCR reached 98.5% (67/68). Among 91 patients, 80 (87.9%) experienced adverse reactions (AEs). The most common AE was diarrhea, occurring in 37 patients (40.7%). No treatment-related serious adverse events or treatment-related deaths occurred.

Conclusion: Inetetamab-based therapy demonstrated promising efficacy and safety in HER2-positive metastatic breast cancer patients. Further research and comparative analysis should be necessary for therapeutic benefits.

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Disclosure: All authors have declared no conflicts of interest.

P5-05-17: Trastuzumab deruxtecan for HER2-positive and HER2-low breast cancer brain metastasis: Results from a single-institution retrospective study.

Chunfang Hao, Jie Zhang, Xiaochen Jia, Chao Xu, Yuehong Zhu

Background: Trastuzumab deruxtecan (T-DXd) has demonstrated promising outcomes in patients with breast cancer brain metastases (BMs), as evidenced by the results from the DEBBRAH, ROSET-BM, and TUXEDO-1 trials, as well as pooled analyses from the DESTINY-Breast01/02/03 studies. However, limited data exist regarding the treatment of BMs patients with T-DXd in real-world clinical settings in China. Therefore, this study aims to evaluate the impact of T-DXd therapy on HER2-positive and HER2-low metastatic breast cancer patients with BMs in the real-world context.

Methods: We conducted a single-institution retrospective real-world analysis and obtained a total of 30 patients with HER2-positive and HER2-low metastatic breast cancer with BMs who were treated with T-DXd between July 2023 and July 2024.

Results: Thirty patients were included in this study, 26 with HER2 positive and 4 with HER2-low status. At data cutoff (July 7, 2024), patients were followed up for a median of 3.6 months (95%CI [2.0; 5.2]). Patients had received a mean of three prior lines of therapy (range 1-10) and the median age was 52.5 (range 32-72). All the patients were active BMs, 17% of whom had leptomeningeal disease, and 33% received prior radiotherapy for BMs. In the HER2-positive cohort, 65% received prior TKI and 38% prior T-DM1. The ORR was 76.9%, and the intracranial (IC)-ORR was 80.8%. The DCR was 92.3%, and the IC-DCR was 84.6%. The median PFS was 8.8 months (95%CI [1.8; 15.7]). The IC-ORR for patients with the largest lesion >2cm, those treated with TKI, those treated with T-DM1, and those with prior radiotherapy for BMs was 83.3% (5/6), 81.0% (17/21), 90.9% (10/11), and 85.7% (6/7), respectively. In the HER2- low cohort, the ORR, IC-ORR, DCR and IC-DCR were all 75%. Median PFS was not evaluable due to small patient numbers. Toxicities were in line with what previously reported in larger registered clinical trials.

Conclusions: Our real-world analysis indicated a clinically meaningful intracranial activity of T-DXd in HER2-positive and HER2-low breast cancer patients with active BMs, with ORR and IC-ORR higher than 70% in the real-world clinical practice.

P5-05-18: Characterizing Patient Experience and Decision Making Associated with Considering Stopping or Pausing HER2-targeted Treatment for MBC Population Using a Questionnaire

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Background: HER2+ targeted treatment for metastatic breast cancer (MBC) is one of the first successes of targeted therapy for advanced cancer, with some patients having years of disease control without any evidence of active disease progression. This has led to unanswered questions of how long HER2 targeted therapies should be continued. We aimed to characterize patient experience with anti-HER2 drugs, particularly in those with sustained disease stability.
Methods: We developed a questionnaire comprised of closed- and open-ended questions to elicit experiences of patients diagnosed with HER2+ MBC. Particular attention was directed at whether patients considered stopping or pausing HER2-targeting treatment after achieving no evidence of disease (NED) and the associated decision making process. We administered the questionnaire online via outreach to MBC patients through social media and nonprofit digital newsletters.
Results: A total of 124 people in the United States responded, 82 (66%) were diagnosed with de novo MBC and 42 (34%) were originally diagnosed with early-stage. Following initial use of a taxane +/- carboplatin + trastuzumab +/- pertuzumab, continued trastuzumab +/- pertuzumab was the most common treatment regimen among the surveyed. At least 95 (76%) had experienced =/>1 year of progression-free disease after starting treatment and 65 (52%) were NED at time of response. Eighty-five (68%) had reached NED after a median time on treatment of 6 months; among these patients, 45% stated they had either discussed or thought about discussing with their oncologist the possibility of stopping HER2-targeting treatment. Level of education was shown to be significantly associated with considering HER2 treatment termination, with more years post high school education corresponding with greater likelihood of having that discussion. In 32% (12/38) of cases, the discussion was initiated by the oncologist. Of these, 7 patients (18%) decided to terminate treatment. Among respondents who had considered stopping treatment, the most common reason given was side effects, followed by emotional toll of continuous treatment. Patients who achieved NED status reported that the lack of evidence for safety was the chief reason to continue treatment despite questioning its need. Many patients reported quality of life (QoL) challenges, including debilitating side effects (e.g. severe itch) and impact to their financial wellbeing. Patients' descriptions of their decision making processes ranged from "Since it's working we won't switch" to "…it was a matter of not killing myself over enduring the pain & itch" with most reporting that they made the decision together with their oncologist, although some reported that just the oncologist (11%) or just the patient (13%) made the decision.
Conclusion: Nearly half of surveyed patients who achieved NED considered stopping HER2-targeting treatment. However, the range of responses regarding the decision making process highlights a problem space in need of research attention to better characterize

barriers and facilitators associated with decision and patient-oncologist communication. We found that more educated patients were more likely to question continued HER2 treatment and that their clinicians were more likely to engage in these complex conversations. These findings indicate the potential for a treatment gap between subsets of patients that is not based on treatment efficacy nor QoL. There is a need for clinicians to be prepared for complex conversations with a high level of uncertainty and to conduct them in a way that is comprehendible to all patients to support shared decision making. Conversations about potential risks to staying on treatment or going off treatment are deserved by all patients, even if we have to wait for prospective studies, such as the ongoing Stop-HER2 (NCT05721248) and Free-HER (NCT05959291), to provide more definitive levels of these risks for the MBC population.

P5-05-19: Complete response with Alpelisib and Trastuzumab in a patient with ER/PR-negative, HER2-positive refractory metastatic breast cancer

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Introduction: Metastatic HER2-positive breast cancer is treated with regimens such as taxane with trastuzumab and pertuzumab followed by trastuzumab deruxtecan (T-DXd). The third line options are ado-trastuzumab emtansine (T-DM1), tucatinib with capecitabine, and trastuzumab. Mutations in the PIK3CA gene encoding PI3Kα have been reported in 12%-39% of HER2-positive breast cancers and are associated with worse prognosis. Alpelisib is an oral, α-specific PI3K inhibitor that is approved in combination with fulvestrant in hormone receptor positive, HER2-negative, PIK3CA-mutated advanced breast cancer following progression on or after endocrine therapy. Activating mutations in the PIK3CA gene are associated with resistance to anti-HER2 therapies. Combined inhibition of HER2 and PI3K overcomes this mechanism preclinically. We present a patient with estrogen and progesterone receptor (ER/PR) negative, HER2-positive, PIK3CA-mutated multidrug resistant refractory breast cancer who had a complete response to alpelisib and trastuzumab.
Case Presentation: A 63-year-old female patient underwent left mastectomy for a stage I (T1aN0M0) ER/PR-negative, HER2-positive breast cancer in 2014. Four years later, she developed a HER2-positive right axillary breast cancer which was treated with neoadjuvant chemotherapy followed by axillary nodal dissection and regional radiation therapy. Since 2018, right axillary nodal and chest wall metastases progressed on several lines of systemic therapy including lapatinib and capecitabine, trastuzumab and paclitaxel, trastuzumab and vinorelbine, fam-trastuzumab-deruxtecan-nhki (Enhertu), T-DM1, margetuximab with eribulin, and finally trastuzumab with capecitabine. A right chest wall biopsy demonstrated a high grade invasive ductal carcinoma that was ER/PR-negative and HER2-positive. Next generation sequencing revealed a PIK3CA mutation (p.H1047R). Treatment was changed to alpelisib and trastuzumab. After four months of treatment, she developed a complete response of cutaneous metastases with adverse effect of grade 2 mucositis. She continues to be under treatment and is doing well.

Conclusions: Alpelisib and trastuzumab can be a viable treatment option for patients with HER2-positive and PIK3CA-mutated metastatic breast cancer. Our case demonstrates the importance of obtaining next generation sequencing in patients with refractory breast cancer. Integrating precision medicine can elucidate mechanisms of resistance and allow treatment with combination and targeted therapies that can improve patient outcomes, similar to our patient presented in this vignette.

P5-05-20: Efficacy of Fam-Trastuzumab Deruxtecan-nxki (T-DXd) in Hormone Receptor-Negative, HER2-3+ Metastatic Breast Cancer: A Case Study

Aliya Khan, Evan Landau, Alan B Grosset, Samuel A. Kareff

Abstract: This case report evaluates the efficacy of fam-trastuzumab deruxtecan-nxki (T-DXd) in a 59-year-old woman with hormone receptor-negative, HER2-3+ breast cancer. Following initial treatment with the TCHP chemotherapy regimen (docetaxel, carboplatin, trastuzumab, and pertuzumab), the patient experienced disease progression. T-DXd was subsequently administered, resulting in significant tumor regression. This response enabled successful surgical resection and completion of adjuvant radiation therapy, marking a notable clinical outcome.

Introduction: Breast cancer in hormone receptor-negative, HER2-positive patients presents substantial treatment challenges. T-DXd, an antibody-drug conjugate (ADC), has shown promise for these patients, particularly those who have undergone prior anti-HER2 therapies. T-DXd is indicated for metastatic or recurrent HER2-positive breast cancer within 6 months of initial treatment. The frontline regimen of TCHP achieves overall response rates (ORR) of 70-80%. However, T-DXd has demonstrated superior efficacy to trastuzumab emtansine (T-DM1) in the DESTINY-Breast03 trial, making it a viable option for patients with disease progression.
According to updated assessment data, out of 246 patients treated with T-DXd, 240 (97.6%) achieved disease control. This includes:
Complete Response (CR): 21.1% (52 patients)Partial Response (PR): 61.0% (150 patients)Stable Disease (SD): 15.4% (38 patients)

Case Presentation: A 59-year-old woman with hormone receptor-negative, HER2-positive stage IIIB left breast cancer presented with large-volume disease. She commenced the first cycle of TCHP (docetaxel, carboplatin, trastuzumab, and pertuzumab), completing five cycles. However, subsequent ultrasound imaging of the left breast revealed extensive breast edema, skin thickening, and a pathologic right axillary lymph node, which, although slightly smaller than before, indicated ongoing disease activity.
A biopsy of a left breast periareolar lesion and a left flank lesion confirmed invasive ductal carcinoma involving the dermis with dermal lymphatic invasion. Immunohistochemistry

(IHC) results showed ER-negative, HER2 IHC 2+, and HER2 FISH-positive status for both specimens, consistent with invasive ductal carcinoma.
The left breast showed massive involvement with progressive cancer, indicating significant worsening of the disease. Consequently, the patient began second-line chemotherapy with fam-trastuzumab deruxtecan-nxki (T-DXd), completing twelve cycles.
An ultrasound indicated a 0.5 cm possible malignancy in the 3 o'clock position of the left breast and macrocalcifications or areas of fat necrosis in the 10 o'clock position. PET-CT scans showed that previously observed hypermetabolic lesions in the left breast and axilla were no longer seen, with improved soft tissue swelling and skin thickening, suggesting a significant reduction in disease burden, thereby facilitating the possibility of curative resection.
Following this regimen, the patient underwent bilateral mastectomy. Pathology revealed the right breast to have sclerosing adenosis and cysts with no malignancy, while the left breast contained two distinct foci of invasive ductal carcinoma (9 mm and 10 mm), with all margins negative and no skin involvement. Reactive lymph nodes indicated fibrosis and calcifications consistent with neoadjuvant therapy, with no metastatic carcinoma present (ypT1bpN0).
The patient experienced common side effects such as nausea and fatigue, which were manageable. However, the risk of interstitial lung disease (ILD) or pneumonitis necessitated regular CT scans and readiness to intervene with corticosteroids like prednisone.
The patient subsequently completed adjuvant radiation treatment. Despite an overall satisfactory outcome, she remains at high risk for potential future relapse, necessitating close follow-up.

Discussion: This case illustrates T-DXd's potential as an effective salvage therapy for hormone receptor-negative, HER2-positive breast cancer post-failure of first-line treatments. The patient's significant tumor regression facilitated surgical resection, previously unfeasible due to extensive disease. ADCs like T-DXd offer targeted delivery of cytotoxic agents, reducing systemic exposure and side effects, as seen in this patient's response. The EMILIA trial further reinforces T-DXd’s role as a critical option following T-DM1 progression.
This case adds to the growing evidence supporting T-DXd’s role in treating specific breast cancer subpopulations. Broader studies are necessary to validate these findings and refine patient selection. Exploring combination therapies with T-DXd could enhance treatment efficacy and outcomes.

Conclusion: Fam-trastuzumab deruxtecan-nxki (T-DXd) shows significant potential in treating hormone receptor-negative, HER2-positive breast cancer, achieving substantial tumor regression and clinical improvement. Advances in ADCs like T-DXd have transformed the therapeutic landscape, offering targeted, effective treatments with manageable side effects. While the results are promising, ongoing research and close follow-up are crucial to optimize treatment strategies and monitor for relapse in high-risk patients.

P5-05-21: Tumor/stromal infiltration of Natural Killer (NK) cells and overall survival (OS) in patients (pts) with metastatic (met) HER2+ breast cancer (BC).

Denis M Collins, Janet McCormack, Laura Ivers, Jose Javier Berenguer-Pina, Jo Ballot, Cecily Quinn, Darko Skrobo, Alex J Eustace, Naomi Walsh, Giuseppe Gullo, Janice Walshe, Aurelie Fabre, John Crown

Background: NK cells are innate immune cells capable of direct anti-tumour cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC can be mediated by autologous anti-tumour antibodies (aaAbs) produced by the adaptive immune response, or monoclonal antibody (mAb) therapies like trastuzumab that are used to treat HER2+ BC. While the majority of met HER2+ BC pts progress on treatment, higher stromal tumor immune infiltrate has been associated with longer OS. Utilising the "Thousand Patient HER-2 database" project at Saint Vincent's University Hospital (SVUH) Dublin, we have identified cohorts of trastuzumab-treated met HER2+ BC pts with OS survival times ranging from 0.3 months (mos) to 223.3 mos. This study examines the tumor and stromal NK cell levels in primary and met samples in the context of OS in this patient population.
Methods: Clinico-pathological data was available for formalin-fixed, paraffin-embedded biopsy specimens (n=52 primary, n=28 met biopsies). Immunohistochemical staining was conducted for NK cells using anti-CD56 (Agilent IR62861-2). A DAKO Link 48 Autostainer was utilised for staining. Slide analysis used an Aperio AT2 Digital Slide Scanner (Leica Biosystems), Aperio ImageScope 12.4 software (Leica Biosystems) and QuPath analysis software (University of Edinburgh). Following tumor area annotation, an algorithm identified tumor or stromal NK cells. Data was expressed as number of positively stained cells/mm2 tissue. NK cells were designated as "Present" (≥1 cells/mm2) or "Absent" (0 cells/mm2) in the tumor and stromal compartments. Survival studies utilized the Kaplan Meier method. HR= Hazard ratio, CI=Confidence interval, p,0.05 is significant. Non-parametric statistical tests were utilised for independent (Mann-Whitney test) and paired (Wilcoxon signed rank test) comparisons.
Results: NK cell levels were significantly lower in the tumor compartment vs the stromal compartment for both the primary ($p<0.001$) and met sites ($p=0.04$). Paired primary and met sample (n=23) NK cell levels were not significantly different within the tumor or stromal compartments. The presence of NK cells displayed a trend towards improved OS in primary tumor (HR 0.57, CI 0.29-1.09, p=0.089)) and stroma (HR 0.50, CI 0.16-1.62, p=0.249) across all pts investigated (n=52). When the pt cohort was divided into long-term responders (>5 year OS) and short-term responders (<5 years OS), the presence of tumor-infiltrating NK cells was significantly associated with better OS in long-term responders (HR 0.21, CI 0.05 – 0.86, p=0.03), but not in short-term responders (HR 0.59, CI 0.05 – 0.28, p=0.19).
Conclusions: Our results report a link between OS and tumor NK cell infiltrate in met HER2+ BC pts with long-term responses to trastuzumab. Further expansion of this dataset is warranted.

P5-05-22: ARX788 for patients with HER2-positive advanced breast cancer: characterization, time course, and monitoring and management of adverse event from the phase III ACE-Breast-02 study

Jian Zhang, Xichun Hu, Qingyuan Zhang, Leiping Wang, Biyun Wang, Quchang Ouyang, Xiaojia Wang, Wei Li, Weimin Xie, Zhongsheng Tong, Faliang Xu, Wei Liu, Shusen Wang, Tao Sun

Background: ARX788 is a non-natural amino acid site-specific conjugated ADC with high homogeneity and stability. In the ACE-Breast-02 primary analysis, ARX788 demonstrated significantly prolonged PFS by blinded independent central review in patients with HER2-positive advanced breast cancer (HR 0.64, 95%CI, 0.49-0.82; p=0.0006). Here we report the detailed safety analysis and management of ocular and interstitial lung disease (ILD).
Methods: 441 eligible patients were randomized 1:1 to receive ARX788 or lapatinib plus capecitabine. Safety analysis was conducted until the data cutoff (DCO) of Dec. 21, 2022. Patients who had history of eye disease, or ILD/pneumonitis not requiring steroids could enter this trial if otherwise qualified. Patients with grade 1-2 ILD could continue the study treatment when showing signs of improvement, and had to discontinue treatment only when it reached grade 3 as per protocol. Chest CT was done every 6 weeks during the treatment and afterwards. ARX788 was not pre-medicated with any prophylactic measures until evident AE of any grade as per protocol. AEs were summarized using descriptive statistics; and time-to-event analyses were conducted.
Results: Totally 220 patients who received ARX788 at lease one dose of ARX788 were included in safety analysis. The overall incidence and incidence of grade ≥3 of treatment-related adverse events was 98.6% and 41.4%, respectively, both of which were in the similar range to the control arm. Grade ≥ 3 of ALT and AST increased was 1.8% and 1.4%, respectively. Grade ≥3 of thrombocytopenia was reported at 2.7%, with 0.5% resulting in treatment discontinuation. Frequencies of any grade and grade ≥ 3 of gastrointestinal toxicity were 13.2% and 0.5% in nausea, 9.1% and 0 in vomiting, 5.0% and 0.5% in diarrhea, respectively. Ocular toxicity of any grade occurred in 74.5%, while grade 3 was 19.1% with 10.5% being self-care activity of daily life compromised. There was no grade 4 or 5, SAE, perforation, ulceration or blindness, or treatment discontinuation. Moreover, 59.1% of ocular toxicity patients had recovered or been recovering till DCO. 32.7% patients had ILD. Of the 13 patients (5.9%) experiencing grade ≥ 3 ILD, early adequate doses of steroids (≥ 360 mg/day of methylprednisolone) in 4 patients reversed ILDs with no death, while three deaths of the 9 patients with lower doses or delayed use of steroids were judged to be related to ILD (1.4%). 30 patients (13.6%) who had pulmonary diseases at baseline had a significantly higher chance of developing pulmonary toxicities than those who did not in terms of any grade and grade ≥ 3 (p=0.0365 and 0.0253, respectively). Ocular toxicity and ILD occurred with median days from starting ARX788 to onset (min-max) of any grade being 29.5 (1-264) and 129 (33-528), respectively. Overall, only 4.5% discontinued ARX788 due to AEs.
Conclusions: ARX788 demonstrated a unique safety profile with less hematological and GI toxicities. Liver enzyme elevations were commonest with predominantly low grades, while

ocular toxicity and ILD were common and manageable. The low rate of dose discontinuation suggested that, when with proactive monitoring and appropriately adequate management, these AEs did not pose a challenge to its clinical benefit.
Clinical trial information: CTR20201708.

P5-05-23: Metastatic HER2+ breast cancer across a decade – who does not receive treatment?

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Background: Over the last 25 years, targeted therapy for metastatic HER2+ breast cancer (BC) has dramatically improved prognosis. Survival benefit associated with monoclonal antibodies in combination with chemotherapy, and antibody-drug-conjugates have been clearly demonstrated in clinical trials, and represent current standard of care. Patients included in clinical trials represent a selected group in terms of age and general health, not necessarily representative of the average BC patient. Cancer registries represent a unique source of real-world treatment data that reflect national treatment practices across time. These data allows for in-depth analysis of treatment patterns and outcomes in an unfiltered patient cohort, including elderly and patients with comorbidity. Identification of patients who does not receive therapy is important in order to assess the quality of cancer care provided to vulnerable patient groups.

Methods: This is a population-based study based on standardized data from the Norwegian Cancer Registry (CRN), which is of high quality and close-to-complete. All women above 18 years at diagnosis of stage IV, HER2+ BC (both de novo metastatic disease and patients progressing from early stage BC) from 2011 to the end of 2021 were included. Data from the systemic anti-cancer treatment database of the CRN, which register cancer therapy given in Norwegian Hospitals, was supplemented with the National Patient Registry (NPR) and the Norwegian Medicines Registry in order to have as complete overview of HER2 therapy as possible. The Norwegian Cause of Death Registry supplied information on cause and place of death, and Statistics Norway provided information on socioeconomic variables. Information on comorbidity was provided from the NPR. Patient characterizations, HER2 therapy patterns and clinical outcomes are reported.

Results: In total, 715 patients with HER2+, metastatic BC were identified. The majority had progressed from early stage BC (66%). Median age at diagnosis was 60 years. Among patients who had progressed from early stage BC, 44% were above 70 years. The vast majority (91%) had relapsed within the first five years after primary disease. All evaluated comorbid conditions were more frequent among patients who had progressed from early stage BC. Median time from diagnosis to death was 11 months for progressed patients compared to 20 months in patients with de novo metastatic disease.
In total, 22% of the patients did not receive any antineoplastic therapy, including chemo-

and HER2 therapy, while 28% did not receive targeted HER2 therapy. Untreated patients were older than patients who received antineoplastic therapy (median age 54 versus 77 years). Heart disease, cerebrovascular disease, dementia and diabetes was more frequent among patients who did not receive therapy. Among the 333 patients who died, untreated patients had a median survival of 2 months compared to 21 months for the treated patients. However, 46% of untreated patients lived for more than three months after metastatic diagnosis. Among patients who had progressed to metastasis, 26% did not receive cancer antineoplastic therapy (compared to 15% of the de novo patients). Less than 10% of the total cohort received more than two lines of antineoplastic therapy, but patients who did receive therapy were treated according to national guidelines.

Conclusion: In this population-based cohort of HER2+ mBC patients, a clear risk profile for not receiving antineoplastic therapy is apparent. These patients are older, more comorbid and they primarily progress from early stage BC. Prognosis among these patients are poor, but a substantial proportion live for more than three months. Further studies are needed to assess if they are under-treated, or correctly deemed unfit for systemic antineoplastic therapy.

P5-05-24: RC48-ADC (Disitamab vedotin, a HER2-directed antibody-drug conjugate) in Combination with Pyrotinib for Trastuzumab-treated HER2-Positive Recurrent or Metastatic Breast Cancer: A Phase Ib/II Study

Xinrui Liang, Ningning Zhang, Xin Zhou

Background: RC48-ADC (an antibody-drug conjugate comprising a human epidermal growth factor receptor 2 (HER2)-directed antibody, linker and monomethyl auristatin E) exerts antitumor effects in various HER2-expressing solid tumors. Pyrotinib, as Tucatinib, belongs to the family of tyrosine kinase inhibitors (TKIs). Preclinical pharmacological studies have shown that RC48 in combination with Tucatinib enhances direct cytotoxicity by upregulating HER2 expression on cell surfaces. This study aims to preliminarily explore the efficacy and safety of RC48 combined with Pyrotinib in treating Trastuzumab-treated HER2-positive recurrent or metastatic breast cancer.

Methods: Patients were administered with RC48-ADC (2.0 mg/kg, intravenous infusion, every 2 weeks) and Pyrotinib tablets (400 mg, oral administration, once daily). Treatment cycles are administered every 2 weeks until disease progression or intolerable toxicity occurs. The primary endpoint are objective response rate (ORR) and safety. Secondary endpoints were progression-free survival (PFS) and overall survival (OS).

Results: Currently, a total of three patients have been enrolled. Patient 1 presented with initially diagnosed left breast cancer with lung and bone metastases (HR+HER2+). The first-

line treatment regimen was six cycles of THP following with 18 cycles of HP; the second-line regimen was RC48-ADC combined with Pyrotinib. The PFS for RC48 combined with Pyrotinib was 10 months, with stable disease (SD) as the best response. Patient 2 presented with initially diagnosed left breast cancer with lung metastasis (HR-HER2+). First-line treatment regimen was six cycles of TH following with 4 cycles of H, and the second-line regimen was RC48-ADC combined with Pyrotinib. PFS for RC48 combined with Pyrotinib was 20 months, achieving partial response (PR) at initial assessment, with PR as the best response. Patient 3 have completed one cycle of the combination regimen of RC48-ADC and Pyrotinib, efficacy has not yet been evaluated. The most common adverse reactions included diarrhea, leukopenia, neutropenia, and anemia (≤ grade II), which were manageable and preventable.

Conclusions: The study preliminarily demonstrates the efficacy and manageable toxicity of the novel ADC (RC48-ADC) combined with a small molecule TKI (Pyrotinib). This regimen may become a new second-line treatment option for HER2-positive advanced breast cancer previously treated with trastuzumab. However, validation with a larger sample size is necessary.

P5-05-25: Real-world Treatment for HER2-positive (HER2+) Metastatic Breast Cancer Patients and Compliance to Guideline Recommendations: HER2 REAL Brazil Cohort

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Introduction: The paucity of real-world data (RWD) imposes barriers to evidence-based decision making for managing patients (pts) with HER2+ unresectable (u)/metastatic (m) breast cancer (BC). We present current treatment practices and associated survival outcomes in HER2+ u/mBC pts in routine clinical care from the Brazil cohort of the HER2 REAL retrospective study (NCT04857619).

Method: Adult pts (≥18 years [yrs]) diagnosed with HER2+ u/mBC (index date) since Jan 2017 having ≥12 months (mo) follow-up data from diagnosis and completing ≥1-line of therapy (LOT) were enrolled based on medical chart review (data cut-off 31 Oct 2022). The primary endpoint was treatment patterns. Secondary endpoints included demographic and clinicopathologic characteristics, real-world median progression-free survival (mPFS), and median overall survival (mOS). Survival outcomes were calculated using Kaplan Meier method and presented as median (95% confidence interval [CI]). Data for the first 3 LOTs are presented.

Results: Of the 152 pts screened, 78.9% (120) were analyzed; some pts (21.0% [32]) were excluded from the final dataset due to insufficient proof of HER2 positivity or not completing ≥1 LOT in the metastatic setting. Median (range) age was 53 (25–85) yrs with 99.2% (119) females; 55.9% with available data (52/93) were postmenopausal. The median (range) time from initial BC diagnosis to index date was 18 (0–424) mo. Of those with available data at index date, 62.1% (41/66) had moderately differentiated tumors with 90.4% (66/73) having ductal histology; 29.2% (35/120) reported hormone receptor positivity (estrogen receptor: 65.7% [23/35]; progesterone receptor: 34.3% [12/35]) while data were unavailable for the rest. Of 86 pts with available data, 24 pts (27.9%) reported a family history of BC. Overall, 62.0% (62/100) with available data had visceral metastasis and 20.0% (20/100) had CNS metastases. LOT1 was received by 89.2% (107/120); 13 eligible pts were excluded because their LOT1 start date was >30 days before index date. LOT2 was received by 67.5% (81/120) pts, and LOT3 by 30.0% (36/120). Treatment attrition rates were 19.6% (21/107) after LOT1, 33.3% (27/81) after LOT2, and 11.1% (4/36) after LOT3. The top regimen in LOT1 was trastuzumab (TRA) + pertuzumab (PTZ) + chemotherapy (CT) reported in 35.5% (38/107), in LOT2 was trastuzumab emtansine (T-DM1) reported in 16.0% (13/81), and in LOT3 was TRA + CT reported in 27.8% (10/36). With a median follow-up of 35.1 (95% CI: 1.5–167.1) mo, the 2-yr survival rate was 52.0%; mOS was not estimable overall. For TRA+PTZ+CT in LOT1, real-world mPFS was 14.1 (95% CI: 9.2–21.7) mo, and for T-DM1 in LOT2, real-world mPFS was not estimable. The main reason for treatment discontinuation was disease progression (51.4% [55/107] in LOT1, 45.7% [37/81] in LOT2, and 36.1% [13/36] in LOT3); 7, 3, and 1 pt discontinued treatment due to toxicity in LOT1, LOT2, and LOT3, respectively.

Conclusion: This real-world analysis of HER2+ advanced or u/mBC showed that only a minority of pts followed the recommended guideline-directed medical therapy for the disease. These suboptimal treatment patterns are, at least partially, possibly due to access barriers and need to be further explored. These findings are in line with those reported in the HER2 REAL global dataset. This analysis highlights the need for a comprehensive study of real-world treatment patterns as they do not seem to reflect results observed in randomized clinical trials. Identification of the possible hindrances towards optimization of standard of care practices is an essential step for improving outcomes in this pt population.

P5-05-27: Multi-Institutional Quality Improvement on HER2-Positive and HER2-Low Metastatic Breast Cancer

Reshma Mahtani, Joseph Kim, Wendi Waugh, Linda Gracie-King, Marc David Viens, Victor Ocana, Jocelyn Timko

Introduction: Recent FDA approvals have expanded treatment options for patients with HER2-positive and HER2-low MBC, but these advancements also create a complex treatment landscape. Oncologists may lack experience with newer HER2-directed antibody-drug conjugates (ADCs) such as T-DXd, may need education on guideline-directed

approaches to sequencing therapies, and cancer care teams need to learn how to effectively monitor patients for unique treatment-related adverse (AEs) effects such as interstitial lung disease/pneumonitis.

Methods: Three cancer centers in the United States participated in a multi-institutional quality improvement (QI) initiative focused on HER2-positive and HER2-low MBC. The program included an online educational activity for a national audience of cancer clinicians and a QI intervention at each cancer centers. The initiative aimed to improve HER2 testing and reporting, treatment planning with HER2-directed ADCs, and management of unique treatment-related AEs. QI sites reviewed patient charts to assess their baseline practice and used Plan-Do-Study-Act (PDSA) cycles to improve care.

Results: The online educational activity was delivered to a national audience of 3,703 cancer clinicians during which 45% of participants reported they gained knowledge about second-line treatment of HER2-positive MBC, 38% learned how to manage suspected treatment-related pulmonary AEs, and 31% gained knowledge about treatment options for patients with HER2-low MBC.QI sites implemented clinical documentation changes to make it easier for oncologists to identify patients with HER2-low MBC and offer guideline-endorsed treatment with T-DXd following one line of chemotherapy. Sites observed a 36.8% increase in the use of T-DXd for patients with HER2-low MBC. Sites also achieved a 15% improvement in aligning HER2-directed therapy with NCCN guideline recommendations for HER2-positive MBC in the second-line setting. Sites revised treatment plans to incorporate a consistent approach for monitoring patients treated with T-DXd for pulmonary toxicity.

Conclusion: The program highlights the importance of ongoing education for oncologists in navigating the evolving treatment landscape for HER2-positive and HER2-low MBC. The QI program demonstrates the effectiveness of targeted education coupled with local implementation strategies to guide cancer care teams as they incorporate newer therapies into clinical practice. This project was supported by an educational grant from Daiichi Sankyo.

P5-05-28: Fully human internalizing anti-HER2 antibody with distinct epitope inhibits tumor formation of trastuzumab resistant breast cancer cells,

Ginette Serrero, Jianping Dong, Binbin Yue, Jun Hayashi

The human epidermal growth factor receptor (HER) family of receptors has been implicated in several human cancers. In 15-30% of breast cancers, HER2 overexpression and amplification are associated with shorter disease-free and overall survivals along with resistance to hormonal agents as well as increased risk of brain metastasis. Two types of

HER2 targeting have been developed: anti-HER2 monoclonal antibodies and small molecule tyrosine kinase inhibitors. Trastuzumab and Pertuzumab are humanized anti-HER2 monoclonal antibodies. Trastuzumab binds to domain IV of HER2 extracellular segment and blocks its signaling. It is the first therapeutic antibody targeting Her2 overexpression approved by the FDA. More recently, two antibody drug conjugates using trastuzumab have been approved: Kadcyla consisting of trastuzumab conjugated to the drug Mertansine DM1 and Enhertu to deliver the topoisomerase inhibitor deruxtecan. We are reporting here the development of fully human internalizing anti-Her2 antibodies that have distinct epitope to Trastuzumab and Pertuzumab. These antibodies have been developed by immunizing fully human (TC-Mab mouse) mice with recombinant Her2 protein. After production of hybridoma secreting fully human immunoglobulins, the screening process included inhibition of binding of Trastuzumab and Pertuzumab to Her2 by enzyme linked immunoassay and by Octet epitope binning as well as internalization assay. Several internalizing antibodies which did not compete with trastuzumab and pertuzumab with high affinity (Kd ranging from 10-9 M to 10-12 M) were selected. They were next investigated for their ability to deliver a cytotoxic payload duocarmycin as antibody drug conjugates (ADC) in several HER2 overexpressing breast cancer cells. Data related to these antibodies' biochemical characteristics as well as their dose-dependent ability to inhibit proliferation in vitro and in vivo in mouse xenografts studies will be presented. In particular, the effect of these antibodies in comparison to trastuzumab in trastuzumab resistant cells JIMT1 cells will be provided. These data will establish that these unique epitope antibodies were able to trigger tumor regression in these cells. In conclusion, our technology provides a powerful and attractive approach to develop fully human monoclonal antibodies against cancer targets allowing to by-pass the need for humanization and affinity maturation of antibodies.

P5-05-29: Real-world efficacy and safety of inetetamab-based therapy in patients with HER2- positive metastatic breast cancer with prior trastuzumab exposure: A prospective real-world study.

Qi Tang, Yiyin Tang, Hui Li, Yong Zhang, Ji Zhang, Shicong Tang, Xintong Zhao, Jiaqian Liao, Bingyu Ouyang, Xin Zhou, Rongkai Li, Dedian Chen

Background: Anti-HER2 monoclonal antibody works by both inhibiting HER2 pathway and inducing immune reactions such as antibody-dependent cellular cytotoxicity (ADCC) effect. Inetetamab (Cipterbin) is a neotype HER2-targeted monoclonal antibody with amino acids modified Fc segment which optimizes the ADCC effect. The combination therapy based on inetetamab can overcome the drug resistance of large-molecule monoclonal antibody drugs due to biological characteristics such as HER2 extracellular domain deletion and HER2 extracellular domain mutation leading to spatial conformational changes. This study aims to explore the efficacy and safety of inetetamab combination therapy for HER2-positive metastatic breast cancer treated with trastuzumab.

Method: This study is a single-center, prospective, observational, real-world study. It is planned to include 50 patients with HER2+ advanced breast cancer who have been treated with trastuzumab. The efficacy and safety of the inetetamab -based combination regimens that has received at least 2 cycles are evaluated. The primary endpoint is progression-free survival (PFS) estimated by the Kaplan-Meier method. Secondary endpoints are objective response rate (ORR), clinical benefit rate (CBR), and safety.

Results: From August 2021 to June 2024, a total of 30 patients with HER2+ advanced breast cancer who had been treated with trastuzumab were included in the study, with a median number of 2 lines of treatment. The combination regimens included: Inetetamab combined with patuzumab plus chemotherapy (I+P), Inetetamab combined with tyrosine kinase inhibitors plus chemotherapy (I+TKIs), and Inetetamab combined with chemotherapy (I). The median PFS of the overall patients was 17 months. The median PFS of patients who received Inetetamab combination therapy as first-line treatment was 18 months, second-line treatment was 6 months and third-line treatment or above was 23 months. Meanwhile, patients who received a combination treatment of I+TKIs achieved the most prolonged PFS of 17 months. Patients who received first-line treatment obtained better benefits. No new safety events were found in the safety analysis.

Conclusion: Inetetamab demonstrated promising efficacy and safety in HER2-positive metastatic breast cancer patients who had prior exposure to trastuzumab, particularly in combination with TKIs and chemotherapy. Further research and comparative analysis should be necessary for therapeutic benefits.
Research Sponsor: None.

rapeutic Potential of T-DXd (Enhertu): Mechanisms of Action in HER2-Low Cancers and Immunomodulatory Effects

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T-Dxd (Enhertu) is a novel antibody-drug-conjugate (ADC) targeting HER2, which has demonstrated profound clinical efficacy across breast cancers (BC) and other cancers exhibiting varying degrees of HER2 expression. However, the precise mechanism underlying its efficacy in HER2-low cancers and the role of Fc-mediated immune activation in its efficacy remain unclear. Using bystander killing studies and pharmacokinetic assessment of its payload, Deruxtecan (Dxd), we found that T-Dxd efficacy in HER2-low and HER2-negative cancers was mediated by extracellular cathepsin L proteases within the tumor microenvironment (TME). This unique mechanism enables broader Dxd cytotoxicity, bypassing traditional ADC-resistance mechanisms like HER2 downregulation and resistance to ADC-internalization. Significantly, our studies revealed that the Dxd cytotoxic payload

induces immunogenic cell death (ICD) with the secretion of specific Damage-Associated Molecular Patterns that activate myeloid cells through TLR4 and STING pathways. Additionally, the T-Dxd antibody backbone retains the capability to engage with Fcγ-receptors to stimulate Antibody-Dependent Cellular Phagocytosis (ADCP) that enhances tumor antigen uptake, which in concert with ICD responses, leads to augmented antigen presentation and expansion of tumor antigen-specific CD8+ T cells. Counteracting ADCP, we also found that T-Dxd stimulates tumor CD47 expression, which we subsequently blocked using an anti-CD47 antibody in combination with T-Dxd. This combination yielded a potent therapeutic synergy that enhanced anti-tumor adaptive immune responses, both systemically and within the TME. Critically, we found that combination therapy induces CD8+ T cell immune memory, preventing tumor recurrence post therapy discontinuation and suggests a critical therapeutic role for T-Dxd-mediated stimulation of adaptive immunity.

P5-06-01: Patient poverty burden influences the molecular biology of ER+ breast cancer

Jerry DeWitt, Elena Oropeza, Svasti Haricharan

Although the incidence of estrogen receptor positive (ER+) breast cancer is similar for Black and White women in the US, Black patients have significantly worse outcomes. Black women in the US have also have worse health care options, poorer educational opportunities, and face significantly higher rates of poverty burden. Collectively contributing to an overall lower socioeconomic status (SES) compared to White women. However, cancer health disparities research primarily exists in two forms: 1) The impact of socioeconomic factors on cancer outcomes, and 2) The molecular underpinnings associated with genetic ancestry on cancer biology. This siloing results in an incomplete picture of how systemic factors (i.e. racism, poverty burden) contribute to the biology of breast cancer in addition to, and along-with, ancestral genetics. Consequently, differences in the molecular biology between tumors from Black and White women remains a gap in knowledge in the field, because ancestral genetics cannot explain the breadth of disparities that patients face. Recent studies have found that Black cancer patients have a unique gene expression profile that may modulate treatment response. For instance, ER+ breast tumors in Black women have distinct signatures of defective DNA damage repair (DDR) that associate with more aggressive tumor growth. However, how factors of SES contribute to these differences in molecular biology and whether these differences alter treatment response remain unknown. One challenge to addressing these questions is that existing datasets significantly underrepresent Black women and are not sufficiently annotated to understand the complex interplay between molecular biology and factors of SES. Here, we present analysis of RNA expression datasets from 60 ER+ patient tumors (38 Black, 22 White), with corresponding SES and patient outcome annotations. Altogether, these data begin to define the complex interplay between factors of SES and changes in gene expression in addition to patient outcomes for women with ER+ breast cancer.

P5-06-02: Comparison of lobular vs NST hormone receptor-positive metastatic breast cancer patients in early lines of treatment via comprehensive genomic profiling and whole-genome sequencing of circulating tumor DNA

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Background: Invasive lobular carcinoma (ILC) represents the most common special subtype of breast cancer (BC), which demonstrates different clinical and histological features, particularly in the metastatic setting. As ILC is frequently hormone receptor-positive (HR+) and HER2-negative (HER2-), we compared circulating tumor DNA (ctDNA) profiles in patients with stage IV ILC to patients with stage IV HR+, HER2- BC with non-special type (NST).

Methods: From an initial cohort of 140 HR+/HER2- patients with metastatic breast cancer (mBC), we selected 116 patients diagnosed with NST (n=95) and ILC (n=21) to perform a comprehensive subtype comparison. We analyzed circulating tumor DNA (ctDNA) from plasma samples before initiating first or second-line endocrine-based therapy. Tumor content for each sample was initially estimated using mFAST-SeqS, an untargeted aneuploidy detection method which calculates genome-wide z-scores as surrogates of tumor fraction (TF). As an orthogonal approach, we employed ichorCNA from shallow whole genome sequencing (sWGS) data to more accurately quantify TF from plasma and infer genome-wide somatic copy number alterations (SCNAs). Additionally, we performed molecular profiling using the AVENIO ctDNA Expanded 77-gene panel, which includes clinically relevant genes, across histologies. For select samples where sWGS was available, we assessed focal events of genes affected by SCNAs. For select samples with suitably high TFs, we performed high-coverage whole-genome sequencing (~20-30x) of plasma DNA to be able to infer differences in transcription factor activity between histological subtypes using the LBFextract algorthim.

Results: Analysis revealed that all patients had detectable ctDNA alterations. Among metastatic NST cases, the most prevalent alterations were in PIK3CA, TP53, ESR1, PTEN and BRCA1, whereas in ILC, the predominant alterations were in PIK3CA, AR, EGFR, ESR1, and KDR. There was no statistical difference in median genome-wide z-scores between subtypes (ILC vs. NST: median 3.57 vs. 2.52, p=0.224). Across the entire cohort, regardless of subtype, genome-wide z-scores generally correlated with ichorCNA TFs (r=0.524, p=0.007). Patients with ILC had a significantly higher number of SNVs than those with NST (median 5.0 vs. 3.0; p= 0.023). After a comparative interim analysis between cohorts via GISTIC2.0, ILC samples harbored significant focal amplifications on 11q13.3 and 11q14.1 as well as deletions on 8p11.22, whereas NST samples harbored focal amplifications on 8p11.23 and deletions on 8p23.1 and 17q21.31. At the time of submission, analysis via LBFextract on 26 high-coverage WGS datasets (NST, n=13; ILC, n=13) is ongoing to be able to identify differential

transcription factor activity between NST and ILC, potentially elucidating novel features that can stratify these two cohorts, which currently lack distinctive genomic fingerprints.

Conclusions: Based on our CGP and WGS approach for ctDNA analyses of hormone receptor positive mBC, we can elucidate distinct features of NST vs ILC in early lines of treatment, including the mutational profile, a significantly higher number of SNVs in ILC and distinct focal chromosomal aberrations. Additional analyses may improve diagnoses, treatment monitoring and understanding the underlying biology and resistance evolution of these clinically and molecularly distinct diseases.

P5-06-03: Single-cell and spatial approach to study endocrine therapy sensitivity in breast cancer models with heterogeneous estrogen receptor expression

Svetlana Semina, Rosemary J. Huggins, Huiping Zhao, Debra Tonetti, Kent Hoskins, Geoffrey L. Greene, Jonna Frasor

Although most women with estrogen receptor (ER) positive breast tumors benefit from endocrine therapy (ET), up to 40% of these patients will experience relapse. The percentage of tumor cells expressing ER varies from 1 to 100%, and some studies suggest that higher expression of ER positively correlates with ET efficiency and a reduced rate of tumor relapse. Previously, we identified a cell population with active NFκB and integrated stress response pathways that overcome selective pressure of ET and contribute to tumor relapse. However, we and others routinely use models that are enriched for ER-expressing cells to study the ET effect. Less is known about ET efficacy in breast cancers with low to moderate ER expression, which are considered to be more aggressive. In this study, we utilized patient-derived xenograft organoids (PDxOs) and single-cell RNA sequencing (scRNAseq) approach to examine how breast cancer models with low to moderate ER expression respond to ET. Alongside established PDxOs (HCI003 and HCI017), we developed two new models (UIC013 and UIC020), where 22%, 30%, 12%, and 7% of cells express ERα, respectively. Using scRNAseq and various bioinformatic tools, we found several cell populations that are common among all models, but vary in terms of prevalence, as well as some populations that were largely model-specific, indicating the models' inter- and intra-tumors heterogeneity. To predict which cell populations are sensitive to ET and which may harbor aggressive features, we examined well-established gene signatures for ER activity, ET resistance, and metastatic relapse in each cell cluster using Functional Enrichment Analysis. However, the results were inconclusive because of inconsistency among gene signatures, suggesting that the variety of models, platforms, and experimental setups used to derive these gene signatures could account for such variability. Additionally, these gene signatures were generated from bulk-RNA-seq studies, which average the signals obtained from all cell populations within a tumor and may mask signals from smaller cell populations. To experimentally identify ET-sensitive and ET-resistant cell populations, we treated each PDxO with fulvestrant and 4-hydroxytamoxifen. scRNAseq revealed that some

ER-active cells were lost following ET, indicating responsiveness, while others remained unchanged or were enriched, indicating resistance to ET. Moreover, comparative analysis of all ET-resistant cell populations among the models showed their high degree of heterogeneity, suggesting that each cell population might use different mechanisms to overcome the selective pressure of ET. To find alternative therapies for the ET-resistant cells, we performed DREEP analysis (DRug Estimation from single-cell Expression Profile), a tool predicting drug responses for 450 compounds using scRNAseq data. Multiple compounds were predicted to be effective in combination with ET, including vandetanib, a VEGFR2 inhibitor currently in clinical trials with fulvestrant for metastatic breast cancer. ET also altered ER-resistant cell transcriptomes, predicting sensitivity to several additional compounds. Overall, our findings suggest that stratifying breast cancer cell populations based on ET response, rather than ER expression or activity alone, can identify new cell populations and therapeutic strategies, thereby improving outcomes for women with breast tumors with heterogeneous ER expression.

P5-06-04: Luminal subtype independent immune-enrichment in inflammatory breast cancer based on commercially available tumor portrait.

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Background: Inflammatory breast cancer (IBC) is a rare subtype of breast cancer which displays an aggressive clinical course compared to non-IBC cases. Comparative genomic analyses have thus far failed to identify unique driver mutations highlighting the role of the microenvironment in IBC. Commercially available tumor sequencing and bioinformatics now provide a standardized analysis of tumor and microenvironment features. Herein, we report results from BostonGene (BG) Tumor PortraitTM analysis from a cohort of patients with IBC.

Methods: Patients with Stage III or Stage IV IBC at diagnosis enrolled to the MD Anderson Cancer Center (MDACC) IBC Registry prospectively received BostonGene Tumor PortraitTM analysis inclusive of Tumor microenvironment (TME). Preliminary analysis of the first 44 patients including descriptive statistics are reported. Tissue was from breast or regional nodes in 34 cases, distant sites in 10.

Results: Along the total population of this study undergoing this testing on tumor tissue, 18(40.9%) were diagnosed with hormone receptor positive (HR+), HER2 low negative (HER2-) IBC, 19(43.2%) had triple negative IBC (TNBC), and 7(15.9%) had HER2+ IBC (HR+ and HR-). The distribution of PAM50 molecular subtypes from 37 samples from which RNA

was available was 1(2.7%) Luminal A, 12(32.4%) Luminal B, 12(32.4%) Basal-like, and 9(24.3%) HER2-enriched. Among luminal subtypes, 9 patients had immune-enriched, non-fibrotic TME, 1 had immune-enriched, fibrotic, 3 had fibrotic, and 3 had immune-desert TME subtypes. Basal-like tissues displayed a more balanced distribution of TME subtypes (2, 2, 3, and 4 of 11; respectively). HER2-enriched samples primarily had immune desert TME (7/9) and less frequently had immune-enriched, fibrotic (1/9) and fibrotic (1/9) subtypes. Only 4.76% displayed high tumor mutational burden (TMB), and 0% demonstrated microsatellite instability. Common somatic mutations were detected in TP53, PIK3CA, RB1, PTEN, and ARID1A genes with 2 cases harboring gene fusions (CASZ1-RAD51B and FANCL-PNPT1). CCNE was frequently amplified.

Conclusions: Over half of cases with luminal molecular phenotype demonstrate immune-enriched TME, suggesting a potentially significant role for immunotherapy in these patients and expanded efforts in this arena. Using the BG Tumor PortraitTM assay, low TMB and MSS was present in the majority of IBC samples evaluated in spite of numerous studies showing common DNA repair mutations. HER2 expressing cases demonstrated a predominantly immune-desert phenotype and further study of the clinical relevance and validation of this is warranted. Correlation to clinical demographics and outcomes is ongoing.

P5-06-05: Targeting Triple-Negative Breast Cancer with a Novel Adeno-Associated Virus-mediated Therapy

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Triple-negative breast cancer (TNBC) is a group of aggressive breast cancers with a higher mortality rate. TNBC poses a major clinical challenge for several reasons: 1) molecular heterogeneity among patients, 2) intra-tumoral heterogeneity, 3) phenotypic plasticity, and 4) failure of the immune system to eliminate malignant clones. Unfortunately, there are very limited therapies for TNBC, making it a critical medical need to develop a targeted therapy. Notch signaling has emerged as an important factor in TNBC. There is strong evidence for the involvement of Notch signaling in TNBC. Recent studies suggest that Cancer Stem cells (CSC) emerging after chemotherapy or targeted agents in TNBC are often Notch-dependent. Notch inhibitors, including Gamma Secretase Inhibitors (GSI), are quite effective in preclinical models of TNBC because they eliminate CSCs that are resistant to chemotherapy. Unfortunately, this has minimal success in clinical trials due to its intestinal toxicity and adverse effect on immune cells. Notch signaling is required for T-cell activation, including CD8 effector cells mediated tumor immunity. To avoid these toxicities, we aim to use a recombinant adeno-associated virus (rAAV) vector encoding a soluble Notch1 decoy (r-AAV-Notch1D) to block Notch signaling intratumorally in TNBC. r-AAVs are non-replicating viruses and, therefore, are a safe platform for in vivo gene therapy delivery. We hypothesize that blocking Notch with intra-tumoral rAAV-Notch1D will inhibit tumor cell growth by modulating tumor microenvironments and CSCs. We found that rAAV-GFP transduced both

human and mouse TNBC cells. Our rAAV constructs contain a C1QTNF3-derived collagen domain (CD) that facilitates the trimerization and stabilization of secreted fusion proteins. This allows local concentrations of therapeutic proteins to be maintained for prolonged periods without repeated injections. We purified the Notch1D proteins from the cellular supernatants of rAAV vector-transduced cells using His-Tag affinity chromatography. We found that Notch1D proteins significantly decreased human TNBC cell mammosphere growth and Notch expression. Intertumoral injection of the rAAV-Notch1D virus significantly reduced syngeneic mouse TNBC tumor (C0321) growth without affecting the mice's weight during the experimental period. Notably, rAAV-Notch1D increased the tumor-infiltrating lymphocytes (TILs), including CD3 and CD8, supporting their role in immunomodulation. Our study represents the foundation for novel r-AAV-based therapeutic approaches for TNBC that can be combined with immunotherapy and chemotherapy for translational application in the future.

P5-06-06: The Mechanistic Role of BRCA1 DNA and RAD51 Binding in DNA Double-Strand Break Repair

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Genomic instability is a hallmark of cancer, enabling the generation of mutations and gross chromosomal rearrangements to drive neoplastic cell transformation and oncogenesis. The BRCA1-BARD1 protein complex acts to eliminate highly toxic DNA double-strand breaks, to ensure the faithful propagation of our genetic blueprint and to suppress cancer development. BRCA1 is a well-described tumor suppressor protein associated with hereditary breast and ovarian cancers as well as sporadic breast cancers, with loss or mutation of BRCA1 leading to triple negative breast cancer and poor patient prognosis. The BRCA1-BARD1 complex promotes homologous recombination (HR), which is the major pathway for the accurate repair of double-strand breaks. However, there is little information regarding the intricate roles fulfilled by BRCA1-BARD1 in this process, or how loss of specific BRCA1-BARD1 functions leads to tumorigenesis. BRCA1 has been previously reported to physically interact with both DNA and RAD51, key factors in HR, but the contributions of the interaction attributes to DNA damage repair remain unknown. Here, we delineate major sites of DNA and RAD51 binding in BRCA1 and use a combination of biochemical and NMR methods to identify the specific residues mediating interactions with these ligands. This has allowed us to develop mutations to ablate BRCA1's ability to interact with these substrates without affecting BRCA1-BARD1's interaction with other key DNA repair substrates. Using these BRCA1 mutants impaired for either DNA or RAD51 binding, we have interrogated the contributions of these interaction attributes to BRCA1-BARD1's function by comparing the activity of WT to mutant BRCA1-BARD1 in biochemical assays to reconstitute various steps of HR. We have found that both DNA and RAD51 binding are indispensable for BRCA1-BARD1's ability to promote RAD51-mediated D-loop formation, thus helping to delineate the mechanism by which BRCA1 promotes HR. Our studies

provide the foundation to determine the functional consequences of cancer mutations in BRCA1-BARD1 and for the development of therapeutic strategies to target HR-deficient tumors.

P5-06-07: Luminal-to-basal subtype conversion is governed by TP53-GATA3 mutational status and is targetable by MAPK inhibition in patients with breast cancer

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Background: The molecular subtype largely governs therapeutic strategy in breast cancer patients, but the subtype is not static but frequently changes during treatment courses. The selective pressure on anti-tumor therapy is believed to drive lineage plasticity in luminal breast cancers, promoting a transition to basal-like phenotype and enhancing resistance against endocrine and targeted therapy. This study aims to elucidate the molecular mechanisms underlying luminal-to-basal (LB) subtype conversion through comprehensive genomic and molecular analyses.

Methods: We utilized the prospective metastatic breast cancer database to identify patients with LB subtype conversion and luminal-to-luminal (LL) subtype maintenance in matched primary and secondary tumor tissues. Whole-exome sequencing (WES) and RNA-seq were performed on available tumors of the patients (exploratory cohort). We also validated genomic alterations in a separate cohort of patients who received next-generation sequencing (NGS) for clinical practice (validation cohort). Molecular factors associated with LB conversion were manipulated in MCF7 and T47D cell lines to further explore changes driving lineage plasticitiy, including chromatin accessibility studies using ATAC-seq.

Results: Our analysis of metastatic breast cancer cohort with paired biopsy (n= 359) revealed overall 29.2% conversion rate of subtypes. LB conversion (from HR+HER2− to TNBC, 8.6%) was the most common subtype conversion and was associated with poorer survival compared to LL subtype maintenance. In the exploratory cohort, TP53 mutations were significantly more prevalent in patients undergoing LB conversion (64.3% vs. 15.4%), whereas GATA3 mutations appeared to be protective (0% vs. 23.1%). The validation NGS cohort (n=70) further confirmed the enrichment of TP53 mutation (85.7% vs. 23.2%) and the protective role of GATA3 (7.1% vs. 24.6%) in patients with LB conversion. The RNA-seq analysis indicated upregulation of KRAS-dependent signatures and decreased GATA3 expression in secondary tumors after LB conversion in TP53-mutant patients. TP53 depletion in MCF7 cells led to reduced ERα level and tamoxifen resistance, accompanied by increased accessibility in genes related to development regulation and AP-1 binding. Further combined TP53 and GATA3 depletion synergistically induced basal phenotype by activating the KRAS-MAPK pathway. MAPK inhibition restored luminal phenotype and

tamoxifen sensitivity in luminal breast cancer against LB conversion induced by TP53 and GATA3 knockdown. MAPK inhibition was also effective in overcoming endocrine therapy resistance in tamoxifen- and palbociclib-resistant breast cancer cells exhibiting the LB conversion phenotype.

Conclusion: Our findings establish TP53 mutations as a crucial predictor of LB subtype conversion in luminal breast cancers, with GATA3 mutations offering a protective effect. Combined TP53 and GATA3 loss synergistically promotes induction of basal phenotype through KRAS activation, and MAPK inhibition is a potential therapeutic strategy for luminal breast cancer patients with LB conversion.

P5-06-08: Molecular features of Pregnancy Associated Breast Cancer from a tertiary care cancer centre in India.

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Background: Pregnancy-associated breast cancer (PABC) encompassing breast cancer diagnosed during pregnancy or one-year post-partum is a rare and challenging entity wherein oncologists need to safeguard maternal oncological and foetal outcomes simultaneously. Little is known about genomic signatures which is worth exploring to throw light on aetiopathogeneses, prognosticators and might reveal potential targets with therapeutic relevance.
Aim: The study aimed to identify the molecular signatures to identify potential pathways implicated in pathogenesis and therapeutically relevant targets and independent prognosticators for PABC.

Methods: Patients with histologically confirmed breast cancer who were pregnant or diagnosed within one year of last pregnancy were included in study during the year 2013-2020 at our tertiary care cancer centre. Bio-specimens (tumour, tumour-adjacent normal, whole blood or plasma) were collected from the patients and the samples were subjected to DNA and RNA extraction and library preparations and then were sequenced at 200 X for tumour and 100 X for blood/tumour adjacent normal on an Illumina Platform and after through check for the quality control were analysed.

Results: There was a total of 104 patients enrolled in the registry from the year 2013-2020.Of these, 55 patients contributed at least one bio-specimen (tumour, tumour-adjacent normal, whole blood or plasma) to this study. Among those,7/55 samples were paired (Tumour and blood), with 9 were only plasma. Of the remaining 48 patients, 7 had paired (tumour and normal) samples, 38 had tumours alone and 3 had germlines alone (2 whole

blood, one tumour adjacent normal).
All the samples were subjected to DNA and RNA extraction.40/48 patients had at least one analyte (DNA or RNA) available. DNA from 29/38 tumour alone, and 6/7 with paired samples and 2/3 patients with germline alone were subjected to successful library preparation and sequencing at 200 X for tumour and 100 X for blood/tumour adjacent normal on an Illumina Platform. DNA Library prep for 1 patient tumour was unsuccessful due to low DNA quantity; however, that for the total RNA succeeded. RNA from 8 patient tumour samples that passed quality control (RIN>5.0) were also subjected to a total RNA library prep specific for fragmented RNA, followed by sequencing on an Illumina Platform to generate at least 50 million PE reads (100 million total reads). There was an overlap of 7 patients with both WES and RNA-Seq data for tumour samples.
Eight out of 38 patients were HER2 positive, with four being ER and/or PR positive and four being ER and/or PR negative. Eleven out of 38 patients were ER and/or PR positive and HER2 negative. Fifteen out of 38 patients were triple negative for ER, PR, and HER2 expression. Four patients were HER2 equivocal; among them, three were ER and PR positive, and one was ER and PR negative. The median age of patients was 30 years (22-45). 32 patients were postpartum at diagnosis while 6 were antepartum at diagnosis. Six patients were node-negative, while 32 were node-positive at diagnosis. Twelve patients presented with de novo metastatic disease at diagnosis, and 19 patients experienced metastatic progression post-diagnosis.
The median coverage for WES of Tumour samples was 182X and that for germline samples was 96X. Mutations were identified in PI3KCA (17%), TP53 (4%), and 2% in both. 6 patients harboured germline BRCA mutations. Additional mutations were identified in epigenetic modifiers. Pathways involved in PI3K signalling, mTOR signalling, ECM matrix related signalling and cell cycle related processes were de-regulated

Conclusion: The molecular profiling report for pregnancy-associated breast cancer patients presented here constitutes an invaluable, ultra-rare dataset. This will help in identifying factors associated with etiopathological and prognostic significance as well as relevant potential therapeutic targets for novel targeted therapeutics. This may contribute towards finding novel avenues which may enhance treatment armamentrium in this extremely challenging situation -an unmet need!

P5-06-09: Evaluating Functional Impacts of GATA3 Mutations in Breast Cancer Using Mesenchymal-to-Epithelial Transition Model

Aerica Nagornyuk, Mika Saotome, Nobuki Hida, Alexander Samardzic, Motoki Takaku

Breast cancer pathogenesis is closely linked to the mechanisms of cellular reprogramming, the process of converting differentiated cells into undifferentiated or different cell types. This reprogramming is achieved through the orchestrated expression of transcription factors which alter the cell's existing chromatin landscape and establish a transcriptome corresponding to the reprogrammed cell state. Pioneer factors, a type of transcription

factor, initiate cell development and cell fate transition by binding to specific DNA motifs within nucleosomes. This binding leads to the recruitment of co-factors, such as nucleosome remodelers, leading to chromatin opening and subsequent activation of specific genes essential for reprogrammed cell function. Improper engagement of these factors can lead to unanticipated or harmful gene expression, potentially contributing to various human diseases, including cancer.
GATA3, a pioneer factor, is essential for normal mammary gland development and differentiation. In breast cancer cells, GATA3 has been shown to suppress tumor growth by inducing mesenchymal-to-epithelial transition (MET), the reverse process of epithelial-to-mesenchymal transition (EMT). Interestingly, although mutations in GATA3 are frequent and considered driver mutations, breast cancer patients carrying GATA3 mutations are generally associated with better patient survival. The roles of these mutations remain largely unexplored. M294R and M294K are hot spot missense mutations found in luminal breast cancer patients. These mutations occur in the DNA-binding domain of GATA3, which is crucial for its function as a transcription factor.
For functional characterization of these mutants, we utilized MDA-MB-231 mesenchymal cells, a highly aggressive and invasive breast cancer cell line to model mesenchymal-to-epithelial transition (MET). Since ectopic expression of GATA3 induces MET in this cell line, these cells provide an excellent system to observe phenotypic and molecular alterations caused by its missense mutations M294R/K. To quantitatively measure changes in GATA3 mobility and its DNA binding dynamics in living cells, we utilized Fluorescence Recovery After Photobleaching (FRAP) assay. Additionally, to further elucidate the crucial role of GATA3, genomics analyses and phenotypic assays were conducted.
Functional characterization of M294R/K mutants in MDA-MB-231 breast cancer cells demonstrated distinct phenotypic and molecular alterations compared to wild-type GATA3. While wild-type GATA3 induced MET, mutant-expressing cells exhibited differential responses. Remarkably, mutants derived from breast cancer patients displayed increased chromatin binding, leading to a tighter chromatin structure and differential gene expression of mesenchymal or epithelial markers. Altered cellular phenotypes such as cell migration are also observed in M294R/K expressed cells.
Our finding underscores the importance of precise regulation of pioneer factor-chromatin interactions in cellular reprogramming processes. Understanding the molecular mechanisms underlying GATA3 mutations and their effects on cellular reprogramming in breast cancer provides valuable insights into the roles of GATA3 in breast cancer progression.

P5-06-10: Clinical and Transcriptomic Study of Matched Primary Breast Cancer and Brain Metastasis

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Background: Some studies suggest different genomic changes in brain metastasis (BM) compared to their primary tumors. There is a paucity of data comparing the genomic and transcriptomic features among matched pairs of primary breast cancer (PBC) and their BM. This single institution study aims to describe the clinical and transcriptomic data of matched triplets of normal tissue (NT), PBC and BMs.

Methods: Patients with archival formalin fixed paraffin embedded NT, PBC and BM tissues available at University of Rochester Medical Center (URMC) were included in this retrospective study. Survival analyses were done using Kaplan Meier method. Illumina Stranded Total RNAseq was used for library preparation, Illumina NovaSeq X Plus for sequencing. DESeq2-1.28.1 within R-4.0.2 was used to perform data normalization and differential expression analysis with an adjusted p-value threshold of 0.05 on each set of raw expression measures. Significant up-regulated and down-regulated genes based on p-adj <0.05 and abs(log2FoldChange) > 0 were submitted to Enrichr to identify significantly enriched pathways and transcription factors that could contribute to the observed phenotype.

Results: 7 patients (6 tissue triplets- NT, PBC, BM; 1 pair PBC, BM) were enrolled in the study. Median (IQR) age in years of patients was 63 (44-74). 2 patients had stage I, 3 patients stage III and 2 patients stage IV tumor at primary diagnosis (AJCC 8th, Anatomical Staging). 5 PBC out of 7 were grade 3. 3 PBCs were triple negative, while 4 were ER+/HER2-. Out of 5 patients with early stage PBC, 3 were treated with adjuvant chemotherapy and 3 out of 5 were treated with endocrine therapy. Median (IQR) days between PBC and MBC diagnosis and PBC to BM diagnosis were 730 (81-1665) and 730 (249-1665). 1 of the 2 cases with stage IV at PBC diagnosis had BM diagnosed with PBC. Out of the remaining 5 cases, 4 of them developed BM at the time of MBC diagnosis. Receptor subtypes were available on 4 BM- 2 TNBC, 2 ER+/HER2-, consistent with their original PBC subtypes. 3 out of 7 cases had lung or liver metastasis at BM diagnosis. Median overall survival (days) from PBC and BM diagnoses were 2072 and 541. Top 5 differentially expressed genes (most significant p-adjusted) with log2FoldChange in BM vs NT: FGB (-0.991), MFAP4 (-5.013), AQP7 (-5.825), ADIPOQ (-4.45), DPT (-5.769); in PBC vs NT: LINC01614 (6.493), CHP2 (-1.059), MUC4 (-5.904), MMP11 (4.734), GAS2L1P1 (0.547); in BM vs PBC: FGB (-1.815), IGHGP (-6.058), ENSG00000286034 (0.853), DPT (-4.211), HAND2 (-4.306). Top 3 most significantly upregulated pathways (based on adjusted p value) in BM vs NT: DNA metabolic process, DNA replication and extracellular structure organization pathways; PBC vs NT AND BM vs PBC: no enrichments found/insufficient genes. Top 3 most significantly down regulated pathways (based on adjusted p value) in BM vs NT: negative regulation of blood vessel morphogenesis, neuronal action potential, regulation of angiogenesis; PBC vs NT: Adipogenesis WP236, Triacylglyceride Synthesis WP325, BM vs PBC: extracellular matrix organization, extracellular structure organization, external encapsulating structure organization.

Conclusion: This small study has described the clinical and transcriptomic information

among matched normal tissue, primary breast cancer and brain metastasis. This study suggests decreased expression of genes related to extracellular matrix components- DPT and MFAP4 in BM compared to NT. Compared to PBC, BM has decreased expression of DPT (Dermatopontin) and FGB (Fibrinogen Beta chain) genes. Similarly, BM have downregulated pathways related to extracellular matrix/structure compared to NT or PBC. These results are exploratory and need validation in larger study.

P5-06-11: Serine Starvation Inhibits SRSF Protein Expression and Modulates RNA Splicing in Breast Cancer Cells

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Breast cancer is the most common cancer in women worldwide, and luminal/estrogen receptor positive (ER+) breast cancer accounts for ~50% of breast cancer deaths. Our lab has identified serine auxotrophy as a metabolic vulnerability of luminal/ER+ breast tumors due to low levels of the serine synthesis enzyme phosphoserine aminotransferase (PSAT1). Serine is critically important for cancer cell growth due to its multiple downstream functions in protein, nucleotide, and lipid biosynthesis. There is considerable interest in starving serine auxotrophic tumors of serine for therapy, including an ongoing clinical trial in pancreatic cancer. While the metabolic effects of serine starvation have been extensively investigated, our goal is to better understand how serine starvation affects protein synthesis and expression. We found that serine starvation inhibits global translation, while inducing adaptive translation of ATF4, and causes ribosome stalling and fall-off at serine TCC codons. Analysis of changes in the proteome upon serine starvation in auxotrophic luminal breast cancer cells shows that serine-rich proteins are particularly sensitive to serine starvation; this includes the serine/arginine rich splicing factor (SRSF) family that are important regulators of mRNA splicing. Accordingly, replicate multivariate analysis of transcript splicing (rMATS) on RNA-sequencing data suggests that serine starvation dramatically changes alternative mRNA splicing in multiple breast cancer cell lines, with many differential splicing events being conserved across the four cell lines tested. These results have led to our current hypothesis that serine starvation induces splicing changes due to reduced translation of serine-rich members of the SRSF family (such as SRSF6), which ultimately impacts DNA damage and cell survival. We believe that SRSF6 depletion and subsequent alteration of RNA splicing upon serine starvation may sensitize to DNA damaging agents (e.g., chemotherapy).

P5-06-12: BreastSubtypeR: Streamlining Intrinsic Molecular Subtyping in Breast Cancer Research with a User-Friendly R Package

Qiao Yang, Xinsong Chen, Johan Hartman, Emmanouil G. Sifakis

Breast cancer (BC) is characterized by significant transcriptomic heterogeneity, enabling the classification into various intrinsic molecular subtypes, each with important prognostic implications. The integration of intrinsic subtyping into clinical practice has been instrumental in guiding BC treatment decisions. However, in the research setting, the lack of standardization has led to the development of a multitude of intrinsic subtyping methods. These methods fall into two main categories: those based on the nearest-centroid (NC) approach and those employing a single-sample prediction (SSP) model. NC-based approaches face challenges stemming from the differences in the gene expression profiling (GEP) platform, preprocessing methods, and cohort composition. On the other hand, SSP model-based methods may encounter inherent limitations typical of a model-based approach. Therefore, the primary challenge for researchers lies in the careful selection of an optimal subtyping method that is tailored to their specific cohort. This selection process can be complex and tedious, especially for targeted patient cohorts. To mitigate this challenge, we have developed the R package, BreastSubtypeR. This package integrates six main intrinsic subtyping methods, along with their derivatives, enabling users to easily inspect the results. BreastSubtypeR employs standardized input and output and provides a unified framework that is highly compatible with other R packages in the GEP field. For users who are not proficient in R, we offer an R Shiny app. BreastSubtypeR is designed to streamline the process of intrinsic subtyping in BC research. We have also explored the selection criteria for different cohorts across methods, thereby simplifying the process of selecting the most suitable subtyping methods for a given cohort. Our ongoing analyses are focused on devising strategies to enhance the classification of intrinsic molecular subtypes in BC research. Given the limited availability of standardized tests in research settings, this work significantly contributes to enhancing our understanding of BC subgroups. This is crucial for advancing personalized treatment strategies in BC, emphasizing the importance of our work in this ongoing effort.

P5-06-13: ARRB1 cooperates with estrogen receptor to drive endocrine resistance in ER-positive breast cancer

Qian Xu, Afrin Sultana, Ananya Gupta, Sanjeev Gupta

Background: β-Arrestin1(ARRB1) is a member of arrestins protein family and act as a scaffold to regulate proteins through G-protein-coupled receptors signaling (1). Recent studies show that it plays an important role in different cancers. ARRB1 enhances progression of breast and prostate cancer via regulation of HIF1A activity (2, 3), promotes lung cancer by increasing E2F1 activity (4). ARRB1 associates with androgen receptor and augments its activity in prostate cancer (5) . However, a tumour-suppressor role of ARRB1 has also been observed. Overexpression of ARRB1 inhibited the growth of human neuroblastoma cells through regulating p27 transcription (6) and triple-negative breast cancer (7, 8). These reports suggest that ARRB1 exerts diverse roles in cancers.
Breast cancer is the most common cancer diagnosed in women. Around 70% of all breast cancers are estrogen receptor α (ER) positive(9). Despite advances in treatment of ER-

positive breast cancers, the development of resistance remains a major challenge necessitating the targeting novel mechanisms of endocrine resistance to improve clinical outcome (10). Indeed, Razavi et al., 2018 reported that 60% of endocrine-resistant metastatic breast cancers lacked known somatic drivers of resistance suggesting that there are yet unidentified factors that contribute to endocrine resistance (11). Here we have evaluated the role of ARRB1 in ER positive breast cancer.

Methods: ARRB1 overexpressed stable clone is generated through a lentiviral expression plasmid. ARRB1 knockout stable clone is generated using the CRISPR-Cas9 gene knockout strategy. These clones are validated using western blot. Colony formation assay and MTS assay are used to evaluate cell viability and cell proliferation. Luciferase reporter assay is used to evaluate the effect of ARRB1 on estrogen receptor. qRT-PCR and Western blot are used to identify the mRNA and protein level. CBioPortal is used to analyze genetic status. KM plotter is used for survival analysis.

Results: The expression of ARRB1 is increased in ER-positive breast cancer compared to ER-negative breast cancer. Depletion of ARRB1 decreases colony formation of ER-positive breast cancer cells. While overexpression of ARRB1 in MCF7 cells increases cell growth and amplifies the induction of estrogen-responsive genes. What's more, overexpress ARRB1 increases E2-dependent growth in MCF7 cells while absence of ARRB1 reduces E2-dependent growth in T47D cells. ARRB1 expression provides resistance against anti-estrogens. Combined with Spiperone treatment, which downregulates ARRB1, increases the effect of Tamoxifen on viability of MCF7 cells. Further, ARRB1 co-expression enhances the transactivation function of ERα WT and ER point mutation but not ESR1 fusion mutation. In contrast, estrogen downregulates mRNA and protein of ARRB1. In addition, KM Plotter analysis shows that increased expression of ARRB1 is associated with poor outcome in ER-positive breast cancer.

Conclusions: Our results suggest that ARRB1 functionally collaborates with ER to drive endocrine resistance and targeting of ARRB1 will provide a novel approach to overcome endocrine resistance.

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P5-06-14: PTBP1 is associated with tumor proliferation as an oncogene that regulates breast cancer metabolism.

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Background: We previously studied cancer cell metabolism. A critical difference exists between cancer and normal cells in terms of their energy-producing mechanisms. Polypyrimidine tract-binding protein 1 (PTBP1), which is predominantly expressed in tumor cells, plays a central role in supplying energy to cancer cells. Alternative splicing of pyruvate kinase muscle (PKM) by PTBP1 results in the formation of two isoforms: PKM1 and PKM2. Normal cells are PKM1-dominant and receive energy mainly from the TCA cycle through oxidative phosphorylation. In contrast, cancer cells are PKM2-dominant and receive more energy from the glycolytic system. This is considered to be the true nature of the energy supply caused by the Warburg effect. In our previous study on gastric cancer, PTBP1 suppression inhibited tumor growth and induced autophagy, which shifted the energy supply from PKM2 to PKM1. In this study, we investigated the significance of PTBP1 in breast cancer (BC) from both basic and clinical perspectives. (Objective) To evaluate the clinical significance of PTBP1 in breast cancer, we performed the following experiments.

Subjects and Methods: We conducted in silico, in vitro, and in vivo searches to clarify the relationship between BC and PTBP1. In an in silico search, we compared PTBP1 expression in BC between cancerous and normal tissues using the global public databases TCGA (n = 1074) and METABRIC (n = 1904). In addition, the relationship between clinical characteristics (tumor grade, stage, and prognosis) and PTBP1 expression was examined for each subtype. We then performed an in vitro search to confirm the expression of PTBP1 and its target genes, PKM1 and PKM2, in nine BC cell lines using western blotting. Among these cell lines, MCF7 (luminal type), SK-BR-3 (HER2 type), and MDA-MB-231 (triple negative) were used to knock down PTBP1 using two types of siRNA. Phenotypic changes were evaluated using cell proliferation and cancer metabolism-related assays, including ATP, NAD/NADH, and lactate assays. In addition, we performed RT-PCR on BC surgical specimens (luminal: five, HER2: five, and TNBC: five) to examine the expression of PTBP1 in vivo. (Results) In a study using paired samples from TCGA, PTBP1 was significantly more highly expressed in tumor tissues than in normal tissues ($p < 0.001$). Regarding tumor grade, PTBP1 significantly increased with advancing tumor grade in both the TCGA and METABRIC ($p<0.001$) datasets. In vitro analysis of nine BC cell lines showed that PTBP1 was highly expressed, and PKM2 expression was significantly upregulated compared to PKM1. PTBP1 using MCF7, MDA-MB-231, and SK-BR-3 cells significantly increased the PKM1/PKM2 ratio. PTBP1 knockdown significantly affected the proliferation of all cells. The ATP assay showed a significant increase in ATP production in MCF7 and SK-BR-3 cells, but not in MDA-MB-231 cells. We performed NAD/NADH and lactate assays using MCF7 and MDA-MB-231 cells and observed that NAD/NADH production was significantly increased and lactate production was significantly decreased by PTBP1 knockdown in both cell types. Thus, the expression of PTBP1 in BC cells suggested a PKM2-significant metabolic state.

Examination of PTBP1 expression using surgically resected paired samples revealed that tumor tissue showed higher PTBP1 expression in 14 of 15 cases (93.3%) than in normal tissue. Finally, an in silico search of the relationship between PTBP1 expression and prognosis revealed that in the METABRIC database, the high PTBP1 expression group had significantly worse DFS and OS than that in the low expression group ($p<0.001$, $p = 0.002$). This trend was particularly significant in the luminal group ($p<0.001$).

Conclusion: This study suggests that PTBP1 is involved in metabolism, cell proliferation, and prognosis in BC. PTBP1 can be a target molecule in BC and may lead to the development of therapies that regulate cancer cell metabolism.

P5-06-15: Nucleosome binding affinity of the pioneer factor GATA3 influences chromatin reprogramming in breast cancer

Jill Goodman, Mika Saotome, Motoki Takaku

Cellular reprogramming is a fundamental process in specific developmental stages and is also invaluable for disease modeling. It involves the conversion of differentiated cells into undifferentiated or specialized cell types. This transformation relies on coordinated action of transcription factors or chemical stimuli, which reshapes the cell's chromatin structure and establishes a different gene expression profile. Among these transcription factors, pioneer factors play a critical role, initiating cellular reprogramming and guiding cell fate transitions by selectively binding to specific DNA motifs within nucleosomes at inactive chromatin. The GATA3 protein serves as a pioneer transcription factor essential in mammary gland development and is involved in breast tumor development. GATA3 facilitates the cellular reprogramming of mesenchymal breast cancer cells into epithelial breast cancer cells. Despite the established role of pioneer factors in chromatin remodeling and cell fate regulation, the precise molecular mechanisms that govern their selective binding to chromatin are still not fully understood. Using the mesenchymal-to-epithelial transition (MET) model, we aimed to understand how GATA3 selectively binds to genes that are essential for successful cellular reprogramming. Utilizing a combination of machine learning and experimental approaches, we investigated GATA3's binding affinity to DNA and nucleosome substrates. Machine learning analysis identified two distinct zinc-finger domains which are required for effective GATA3 nucleosome binding. The results of this deep learning analysis were used to design a series of GATA3 mutant cell lines and DNA substrates. The experimental approach involved two strategies: first, analyzing the binding affinity of wild-type GATA3 to DNA substrates designed based on machine learning predictions, and second, examining the nucleosome binding affinity of GATA3 mutants, including those predicted by computational modeling and those derived from breast cancer patients. Electrophoretic mobility shift assays confirmed the differential binding affinities of GATA3 mutants, indicating the importance of specific motifs and zinc finger domains. Our findings highlight the significance of DNA sequence specificity in modulating pioneer transcription factor activity and chromatin accessibility. Mutational analysis revealed distinct roles for each zinc finger domain, with mutations affecting DNA binding affinity and

chromatin accessibility. This study provides insights into the molecular mechanisms governing GATA3-mediated chromatin remodeling during MET. By characterizing the interaction between DNA/nucleosome binding affinity and chromatin accessibility, we expand our understanding of pioneer transcription factor function in breast cancer progression.

P5-06-16: Preclinical Characterization of LAE118, a Novel Allosteric Pan-mutant Selective PI3Kα Inhibitor

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PIK3CA is one of the most frequently mutated oncogenes in cancer, found in approximately 13% human cancers. PI3Kα mutations are prevalent in patients with breast, colorectal, lung, endometrial, and numerous other cancers. Three hotspot mutations (H1047R, E545K, E542K) on the p110α subunit of PI3Kα are recognized activating mutations accounting for approximately 1/3 of all PI3Kα mutations. Targeting PI3Kα in cancer has been validated as a therapeutic strategy, as evidenced by the approved drug Alpelisib and the positive clincal trial result of Inavolisib, demonstrating clinical efficacy either alone or in combincation with other therapies. However, treament with non-mutant selective inhibitors raises tolerability concerns such as hyperglycimea, due to the inhibition of wild type PI3Kα. Moreover, the clinical use of PI3Kα orthosteric inhibitors, such as Alpelisib and Invaolvisib, has led to the emergency of secondary mutations (e.g. Q859K & W780R) in the ATP binding pocket resulting in resistance to these drugs. Therefore, there is a pressing need to develop novel PI3Kα-targeted therapies that can minimize on-target toxicities and overcome drug ressistance.
LAE118 is a novel allosteric pan-mutant selective PI3Kα inhibitor. LAE118 demonstrates excellent in vitro anti-proliferation activities in PI3Kα mutant cell lines and remains active against cells resistant to orthosteric PI3Kα inhibitors. LAE118 shows strong anti-tumor growth effect in Xenograft models at a dose that is much lower than other allosteric inhibitors and has less effect on glucose homeostasis compared to orthosteric inhibitors. In pre-clinical toxicology studies, LAE118 also demonstrated good tolerability. These data indicate that LAE118 offers improved efficacy and larger safety window. LAE118 is currently in IND enabling stage.

P5-06-17: The addition of ribociclib, palbociclib or abemaciclib to the next generation oral SERD, camizestrant, delivers greater anti-tumour efficacy in a range of ESR1wt and ESR1m breast PDX models

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The addition of a CDK4/6 inhibitor (CDK4/6i) to endocrine therapy (ET) in patients with hormone receptor positive (HR+) advanced breast cancer (ABC) showed significant

improvement in progression free survival (PFS) in pivotal trials. Three CDK4/6i; ribociclib, palbociclib and abemaciclib, have now been approved by global regulatory authorities, including the FDA, in this setting, leading to an improvement in patient outcomes over recent years. Unfortunately, tumours are resistant or develop resistance to these combinations, demonstrating a current unmet need for treatment regimens that can delay resistance and extend the time on 1st line therapy. This is particularly important due to shorter PFS in 2nd line.
Camizestrant is a next generation oral selective estrogen receptor degrader (ngSERD) and pure ER antagonist that has demonstrated superior activity to fulvestrant both pre-clinically and clinically. A major mechanism of resistance to current ETs is the emergence of ESR1 mutations (ESR1m) which occur in ~40% of patients after 1L therapy for metastatic disease. Camizestrant can degrade and antagonize both wild type and mutant forms of ER and delivers similar PFS in patients with or without detectable ESR1m at baseline. Therefore, camizestrant has the potential to become the backbone ET of choice.
We investigated the effect of combining camizestrant with the three globally approved CDK4/6i, palbociclib, abemaciclib or ribociclib in vitro and in vivo. Addition of camizestrant enhanced the growth inhibitory effects of all three CDK4/6i in the ER+ breast cancer cell lines, MCF7 and T47D, as well as in MCF7 cells engineered to express ESR1m (Y537S). Analysis of the response of downstream signalling biomarkers to the combination revealed that camizestrant limits the induction of cyclin D1 caused by CDK4/6i monotherapy treatment and enhances the reduction of pRB, a key regulator of the cell cycle. Consistent with this, the combination with camizestrant enhanced G1 arrest at lower doses of CDK4/6i. This combination benefit was also explored in vivo across a panel of 7 ER+ breast PDX models where the combination of camizestrant plus the CDK4/6i was well tolerated. In four of these models the addition of palbociclib, abemaciclib or ribociclib significantly enhanced the tumour growth inhibition (TGI) of camizestrant. For example, in ST1799PBR, monotherapy camizestrant delivered 60% TGI whereas the addition of palbociclib, abemaciclib or ribociclib drove 78, 89 and 76% TGI, respectively. Combination benefit was achieved in ESR1wt as well as ESR1m models and with no difference in anti-tumour activity between the CDK4/6i partners.
These preclinical data demonstrate consistent activity of camizestrant in combination with all three globally approved CDK4/6i. This, together with the efficacy of camizestrant in patients with ESR1wt and ESR1m tumours, suggests camizestrant in combination with CDK4/6i has the potential to provide extended benefit for patients with ER+/HER2 metastatic breast cancer in the first line setting, a concept under clinical investigation in the ongoing SERENA-6 (NCT04964934) and SERENA-4 (NCT04711252) studies.

P5-06-18: The Role of PARP1-mediated FOXA1 ADP-ribosylation in Breast Cancer Biology

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Breast cancer is the most common cancer diagnosed in women. At the time of diagnosis, approximately 70% of breast cancers express Estrogen Receptor alpha (ERα), a key signature of luminal subtype tumors. Equally important in these cases is the expression of FOXA1, a subtype-specific transcription factor (TF) essential for ERα-mediated cell proliferation. FOXA1 is a key pioneer TF that assists ERα with the assembly of enhancers and the transcription of estrogen (E2)-regulated target genes. Together, the FOXA1-ERα axis is a major driver of cancer cell growth. Our recently published work implicates poly(ADP-ribose) polymerase 1 (PARP1) and ADP-ribosylation (ADPRylation) in the regulation of E2-dependent transcriptional responses in ERα+ breast cancer cells.
To determine the underlying mechanisms, we identified FOXA1 as a substrate of PARP1-mediated ADPRylation. We hypothesize that (1) PARP1 elicits its effects on E2 signaling and ERα enhancer function, in part, by ADPRylating FOXA1 at specific residues and (2) Loss of these regulatory ADPRylation events alters FOXA1 activity, which in turn can alter the assembly of ERα enhancers and transform the breast cancer cell transcriptome. Indeed, the sites of FOXA1 ADPRylation are recurrently mutated in breast cancers and are highly enriched in metastatic cases. Using an integrated set of biochemical, molecular, cell based, genomic and proteomic approaches, we have validated the ADPRylation of FOXA1 by PARP1 in vitro and in vivo. We selected two FOXA1 ADPRylation sites, D226 and E255, for further analysis due to their positions in a mutation 'hot spot' region corresponding to the C-terminal forkhead DNA-binding domain. Mutation of these sites blocks FOXA1 ADPRylation and enhances cell proliferation in MCF-7 human breast cancer cells. Additionally, preliminary ChIP-seq analysis indicates that the binding of both ERα and FOXA1 are globally reduced at ERα enhancers in MCF-7 cells expressing the FOXA1 ADPRylation site mutants compared to wild-type but may also increase both at selected enhancers. While there is a global reduction in mutant FoxA1 binding near the canonical estrogen-regulated genes, interestingly, we observe an overall enhanced binding of FOXA1 D226N compared to wild-type outside of this canonical program. Collectively, these studies will (1) reveal new molecular endpoints modulated by PARP1, providing new insights about the use of PARP inhibitors to treat ERα+ breast cancers, and (2) suggest new therapeutic strategies to disrupt the aberrant signaling pathways associated with mutant FOXA1 luminal breast cancers, and more importantly metastatic cases, which is of utmost clinical significance.

P5-06-19: Single-cell transcriptional features of human breast tumors and model systems

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A deeper understanding of the complicated transcriptional landscape of human breast cancers are vital to identifying more efficacious treatments. Investigating genetic variations among breast cancer subtypes at a single-cell level holds the potential to enhance our

understanding of cancer formation, progression, and elucidate actionable targets. In this study, we compile single-cell RNA sequencing data from patient tumors and matched lymph metastasis, reduction mammoplasties, breast cancer patient-derived xenografts (PDXs), PDX-derived organoids (PDXOs), and cell lines, forming a diverse dataset consisting of 117 samples with a total of 506,719 cells. These samples include hormone receptor-positive (HR+), human epidermal growth factor receptor 2 positive (HER2+), and triple-negative breast cancer (TNBC) subtypes, along with isogenic model pairs. Within this study, we outline the similarities and differences across these models and patient samples, and assess therapeutic drug efficacy based on subtype proportions. Within the TNBC subtype, PDX models exhibited a closer resemblance to patient samples in terms of tumor heterogeneity and cell cycle features compared to cell lines. Acquired drug resistance in TNBC PDX tumors was linked to an increase in basal-like cell proportions, as defined by SCSubtype and TNBCtype cell typing predictors. Cell-wise subtyping revealed all patient samples comprised a mix of subtypes; notably, HR+ lymph node metastases had a lower proportion of HER2-Enriched cells compared to matched primary tumors. PDXOs displayed differences in metabolic-related transcripts relative to matched PDX tumors. Correlative analyses of cytotoxic drugs on PDX cells demonstrated that therapeutic efficacy was dependent on subtype proportions. Herein we provide an extensive multi-model dataset, a dynamic approach to cell-wise sample annotation, and a thorough examination of models within human breast cancer systems. This analysis and reference resource will support informed decision-making in preclinical research and therapeutic development by establishing model limitations, subtype-specific insights, and novel targetable pathways.

P5-06-20: Advances in Molecular Characterization and Targeted Therapeutics for Lobular Breast Cancer: Enhancing Personalized Treatment Strategies

Mohammed Imad Malki, Ibrahim Elmakaty

Lobular breast cancer (LBC), accounting for 10-15% of all breast cancer cases, is distinct in its histopathological and clinical presentation. Despite its prevalence, LBC has been historically under-researched compared to ductal breast cancer, leading to a gap in optimized therapeutic strategies. This study aims to elucidate the unique molecular and genetic landscape of LBC, offering insights into potential targeted therapies. We conducted a comprehensive analysis of 150 LBC tissue samples, employing next-generation sequencing, transcriptomic profiling, and immunohistochemical methods to identify key mutations and pathways driving LBC pathogenesis. Our findings highlight recurrent mutations in CDH1 (50% of cases), PIK3CA (35% of cases), and GATA3 (20% of cases), alongside alterations in the estrogen receptor pathway, which were prevalent in 70% of LBC cases. Additionally, we observed significant variations in the tumor microenvironment of LBC, characterized by a unique immune cell infiltration pattern, with higher levels of tumor-associated macrophages and regulatory T cells compared to ductal carcinomas. In vitro and in vivo studies were conducted to evaluate the efficacy of targeted therapies against identified

molecular aberrations. PI3K inhibitors demonstrated potent anti-tumor activity in LBC models harboring PIK3CA mutations, reducing tumor growth by 60% in murine models. CDH1-deficient models responded favorably to E-cadherin targeted approaches, showing a 50% reduction in metastatic spread. Furthermore, our data revealed that patients with GATA3 mutations exhibited increased sensitivity to estrogen receptor modulators, with a 40% improvement in progression-free survival. Our research underscores the necessity for tailored therapeutic strategies in LBC, advocating for the incorporation of molecular diagnostics in clinical practice to personalize treatment. These findings not only advance our understanding of LBC but also pave the way for developing precision medicine approaches that improve patient outcomes.

P5-06-21: Longitudinal profiling of cell-cell interactions in estrogen receptor positive breast cancer patients treated with aromatase inhibitors: insights from single cell RNA sequencing analysis

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Introduction: Postmenopausal breast cancer patients with estrogen receptor positive (ER+) tumors are frequently treated with aromatase inhibitors (AIs) to effectively reduce estrogen levels, suppress the ER signaling pathway, and inhibit tumor growth and recurrence. The NEOLETEXE trial aimed to treat postmenopausal ER+ patients with locally advanced breast cancer in the neoadjuvant setting. In this randomized, open-label, intra-patient, cross-over, phase II clinical trial, patients received either letrozole (2.5 mg daily) or exemestane (25 mg daily) for 3 months before crossing over to the alternative aromatase inhibitors for an additional 3 months.

Methods and aims: To understand the mechanisms of resistance and sensitivity to aromatase inhibitors in ER+ breast cancer, we profiled 23 patients in the NEOLETEXE trial using single-cell RNA sequencing (scRNA-seq) at baseline, after 3 months (prior to the crossover), and at the end of neoadjuvant therapy (after 6 months).
Our aim was to analyze the dynamic changes of cell type interactions under treatment pressure using two algorithms, CellPhoneDB and ContacTracing, which reliably and complementarily identify cell-cell interactions and their functional effects from scRNA-seq data.

Results: Unsupervised clustering of approximately 400,000 cells obtained from the three longitudinal biopsies of 23 patients revealed thirteen distinct cell types. CellPhoneDB was utilized to identify the significant cell type-cell type interactions for each biopsy, providing an atlas of cell type interactions in ER+ breast cancer. Further analysis of the robust interactions across longitudinal biopsies identified which cell types significantly changed

their interactive network during treatment. Additionally, we used spatial transcriptomics to investigate the importance of cell type proximity in the commitment of cell type-cell type interactions. Finally, using ContacTracing, we aimed to understand the importance of specific ligand-receptor interactions in shaping cell phenotypes and the tumor microenvironment in relation to sensitivity or resistance to aromatase inhibitors.

Conclusions: Through scRNA-seq, we uncovered the cell-cell interaction landscape of ER+ breast cancer and evaluated the significance of cell-cell spatial proximity. Furthermore, we assessed which cell types significantly changed their interactions under treatment pressure. Our goal is to gain a deeper understanding of the role of cell type interactions in breast cancer physiopathology and under treatment pressure with different classes of aromatase inhibitors.

P5-06-22: Harnessing the Power of the Variant: Breast Cancer's Strategic Incorporation of the Histone H2A.J

Rana Gbyli, Tyler Jensen, Joseph Balowski, Yao Li, Xiang Zhang, Qin Yan, Andrew Xiao

Breast cancer (BC) is the leading cause of cancer-related deaths in women. It is molecularly and clinically heterogeneous, driven by distinct genetic and epigenetic alterations, making prognosis prediction and treatment selection challenging. Identifying novel markers for BC progression and therapeutic targets remains an urgent need. Although growing research has highlighted the role of epigenetic mechanisms, particularly cancer-associated histone mutations (onco-histone), in regulating BC pathogenesis and its hallmarks, including stemness and drug resistance, the role of histone variants—the naturally derived sequence variants—remains largely unknown, with the exception of H3.3. The newly identified, mammalian-specific histone variant H2A.J has been shown to be highly enriched in human luminal BC and implicated in a stem cell signature correlated with metastatic risk in primary BC. However, it is unclear what role H2A.J plays in BC and how it may function in other BC subtypes. Using cell lines of multiple BC subtypes and patient-derived xenograft (PDX) tumor microarrays, our studies have discovered that H2A.J is present in human triple-negative BC (TNBC), with significantly elevated levels in metastatic TNBC compared to the primary counterpart.
Our unpublished results show that H2A.J is the only histone variant that strongly and specifically interacts with the tumor suppressor, retinoblastoma 1 (RB1) in metastatic TNBC cells and luminal BC. Our data also demonstrate that RB1 protein level is significantly correlated with H2A.J in estrogen receptor-positive (ER+) luminal BC PDX samples. Our preliminary results suggest that H2A.J chromatin occupancy affects RB1 recruitment to chromatin, RB1-bound chromatin targets, and ultimately RB1-dependent phenotypes. We will discuss how H2A.J regulates RB1-bound chromatin loci, and hence, the canonical and non-canonical functions of RB1 in tumorigenesis. Our bulk RNA-sequencing and western blotting data show that knocking down (KD) H2A.J interferes with G1/S cell cycle progression and results in the upregulation of estrogen response, apoptosis, and mTOR

signaling. Notably, the H2A.J KD-transcriptome signature strongly correlates with that of CDK4/6 inhibitor treatment. Our data also suggest H2A.J's role in maintaining cell lineage fidelity.

Overall, our results suggest that H2A.J regulates cellular heterogeneity, stemness, and metastasis in BC. The outcome of our research will not only advance our understanding of the underlying mechanisms leading to BC progression and metastasis but also offer new opportunities for utilizing H2A.J as a target for diagnostic and therapeutic interventions in BC.

P5-06-23: NDRG1-AKT signaling promotes tumor stemness in inflammatory breast cancer

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Background: Inflammatory breast cancer (IBC) is a rare, aggressive form of breast cancer but accounts for 10% of breast cancer-related deaths. Cancer stem cells (CSCs; tumor stemness) play a key role in tumor dormancy, progression, and treatment resistance, yet mechanisms that drive CSCs remain poorly defined. We recently identified that NDRG1 promotes tumor growth and progression in IBC mouse models, and that its depletion inhibits AKT phosphorylation. We hypothesized that NDRG1 is a key regulator of tumor stemness in IBC via activating AKT signaling.

Methods: CSCs were assessed using surrogate markers including CD44+/CD24-, mammospheres, and in vivo limiting dilution assay experiments. To identify which AKT isoforms (AKT1, AKT2, or AKT3) or upstream kinases (SGK1, GSK3β) mediate NDRG1-induced CSCs, NDRG1-depleted cells were transfected with NDRG1 WT, AKT plasmids or SGK1/GSK3β phospho-site mutants or siRNA for SGK1/GSK3β.

Results: NDRG1 depletion significantly reduced CD44+/CD24- subpopulation and mammosphere formation ($p<0.001$). Limiting dilution experiments demonstrated a significant reduction in tumor incidence and stemness frequency in mice transplanted with NDRG1 knockdown cells ($p= 1 \times 10-12$). Each of the three AKT isoforms partially rescued tumor stemness in the NDRG1 knockdown IBC cells [CD44+/CD24-: NDRG1 KD vs AKT1 OE, $p=0.008$; vs AKT2 OE, $p=0.005$; vs AKT3 OE, $p=0.001$)]. Overexpression of known inactive SGK1 phospho-site mutants of NDRG1 restored tumor stemness in NDRG1-depleted cells ($p\leq0.005$). While silencing GSK3β reduced CSCs in IBC cells SGK1 did not affect this subpopulation, indicating that the tumor stemness effect of NDRG1 is independent of SGK1 and its kinase activity and is regulated by GSK3β.

Conclusions: Our findings underscore the critical role of NDRG1 as a regulator of IBC tumor stemness. We observed all three isoforms of AKT restored CSCs in NDRG1-depleted breast cancer cells, highlighting the significance of the NDRG1-AKT axis in governing stemness and tumor progression in IBC.

P5-06-25: Dose-dependent Effects of Estrogen on Genomic Regulation in Breast Cancer Cells

Hyung Bum Kim, Tulip Nandu, W. Lee Kraus

Estrogens are steroid hormones that play a key role in a wide range of physiological and pathological processes. Notably, estrogens act as the primary mitogen in estrogen receptor positive breast cancers (ER+ BC). The primary mode of action of these hormones is through estrogen receptor-alpha (ERa), which functions primarily as a nuclear transcription factor (TF) through ligand-dependent activation by 17β-estradiol (E2), the major naturally-occurring estrogen. The physiological levels of E2 are regulated by production, sequestration, and clearance, which can limit the amount of bioavailable E2. However, many studies examining the genomic effects of E2-regulated signaling in cell-based assays used supraphysiological levels of hormone. While this has allowed us to understand the extremes of E2 signaling, it has limited our ability to examine cellular functions which operate at lower hormone concentrations. In particular, the genomic actions of ligand-bound ERa through transcriptional enhancer formation and function are not understood at physiological E2 doses. Here, we present studies examining two largely overlooked aspects of E2 signaling: (1) genomic actions at physiological concentrations of E2 and (2) cellular accumulation and retention of E2. Using an integrated genomic approach with RNA-seq, PRO-seq, ChIP-seq, and ATAC-seq, we identified gene sets that express preferentially at either picomolar or nanomolar concentrations of E2. Expression of these genes is regulated mainly at the transcriptional level and is associated with distinct enhancer features, including chromatin accessibility and H3K27ac enrichment, that may play a role in determining hormone responsiveness. These have implications of functional differences between response at low and high doses of E2. Furthermore, we found that ERa positive breast cancer (ER+ BC) cells accumulate E2 from the culture medium and retain the hormone for an extended duration. Accumulated intracellular E2 supports sustained ERa chromatin binding and E2-dependent gene expression, as well as cell proliferation. We are exploring the link between intracellular steroid-binding proteins and E2 cellular retention. Taken together, our results are expanding the role of E2 dose in hormone signaling in ER+ BC.

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P5-06-26: APOBEC3B And DNA Damage Repair As Synthetic Lethal Pairs

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APOBEC3 (A3) enzymes play a pivotal role in mutagenesis across various cancer types. These enzymes, which are single-stranded DNA cytosine deaminases, convert cytosine to uracil (C-to-U) as part of innate antiviral immune responses. Aberrant expression of A3 enzymes in several cancers leads to DNA damage, mutagenesis, and genomic instability. In breast cancer, in particular, APOBEC3B (A3B) is the major endogenous mutator that contributes to tumor evolution and resistance to treatment (1–4). DNA C-to-U deamination events result in characteristic C-to-T transitions and C-to-G transversions, and genomic uracils can also be processed into single- or double-stranded DNA breaks and larger chromosomal aberrations. Consequently, tumors with elevated A3B levels endure chronic genotoxic stress and may exhibit increased sensitivity to inhibitors targeting specific DNA repair pathways.

Previous research from our laboratory identified DNA uracil glycosylase 2 (UNG2), a critical initiator of the base excision repair (BER) pathway, as a synthetic lethal partner with A3B (5). Genetic disruption of UNG2, combined with high A3B expression, led to cell death (5). This finding suggests that other DNA repair proteins could potentially serve as synthetic lethal partners with A3B under conditions of elevated A3B-induced DNA damage.

To test this hypothesis, we used pharmacological and genetic inhibition of DNA damage repair proteins in isogenic cell line models for A3B. Specifically, we quantified cancer cell viability following treatment with various commercially available DNA damage response inhibitors, in the presence or absence of A3B. Additionally, we performed CRISPR screens using a guide RNA library targeting 237 DNA damage repair and response genes in a doxycycline-inducible TREX-293-A3Bi-eGFP cell line. Cells expressing or lacking A3B were harvested for DNA extraction and sequencing at different time points. By comparing guide RNA abundance between doxycycline-treated cells and untreated cells, we identified dropout guides that disrupt genes, creating potential synthetic lethal combinations with A3B. The results from both pharmacological and genetic inhibition approaches will be presented.

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P5-06-27: OXTR inhibits breast cancer cell invasion and metastasis by regulating the post-translational modification of the COL1A1 protein to accelerate its degradation through the lysosomal pathway.

Qing Shao

Background: The oxytocin receptor (OXTR) is a hormone receptor that is highly expressed in the breast, and its expression is significantly reduced when breast tissue becomes cancerous. In addition to serving as a receptor for receiving stimulation from hormones, neurotransmitters, and growth factors to mediate their effects, the biological function and molecular mechanism of OXTR itself in breast cancer (BrCa) are still unclear.
Methods: Using quantitative PCR (qRT-PCR), Western blot, immunohistochemistry (IHC), and public database analysis, the expression of OXTR in BrCa adjacent normal tissues, BrCa tissues, and metastatic tissues, as well as its correlation with prognosis were evaluated. The migration and invasion functions of OXTR were analyzed by wound-healing and transwell assays in vitro. Lung metastatic tumor models were used to study the invasion and metastasis of OXTR in vivo. RNA-seq, Western blot, immunofluorescence (IF), immunoprecipitation (IP) and Co-IP were further applied to determine the detailed mechanism.
Results: OXTR is downregulated in BrCa tissues, especially in distant metastases. Patients with lower OXTR expression have worse overall survival (OS) and distant metastasis-free survival (DMFS). In functional experiments, overexpression of OXTR suppresses BrCa cell migration, invasion and metastasis in vitro and in vivo. Conversely, knockdown of OXTR increased BrCa cell spreading. OXTR functions by inhibiting the expression of the oncoprotein collagen type I alpha 1 chain (COL1A1), which is closely associated with epithelial-mesenchymal transition (EMT). The mechanism is mainly that OXTR causes the decrease of COL1A1 protein phosphorylation and the increase of O-GlcNAcylation by down-regulating CDK1. However, O-GlcNAcylated COL1A1 is unstable and can be easily degraded by the lysosomal pathway.
Conclusions: This study extends the ligand-independent function of OXTR in BrCa and highlights the role of OXTR in suppressing invasion and metastasis of BrCa cells, as well as predicting prognosis. This study elucidates the specific mechanism by which OXTR accelerates the degradation of COL1A1 protein by regulating its post-translational modifications (PTMs), thereby affecting the malignant progression of cancer cell metastasis. These findings suggest that OXTR may serve as a marker to predict the metastatic potential or prognosis of BrCa.
Keywords: Breast cancer, OXTR, COL1A1, metastasis, PTMs

P5-06-28: Characterization of cell type specific distal cis-regulatory elements from 5’ scRNA-seq in estrogen receptor positive breast cancer patients treated with aromatase inhibitors

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Introduction: Aromatase inhibitors (AIs) are widely used in the treatment of estrogen receptor-positive (ER+) breast cancer to inhibit estrogen production and suppress the ER signaling pathway. Target genes regulated by estrogen stimulation of ER+ malignant cells lead to uncontrolled proliferation.
The NEOLETEXE trial evaluated the sequential use of letrozole (2.5 mg daily) and exemestane (25 mg daily) in the neoadjuvant setting. We performed single-cell RNA sequencing (scRNA-seq) on 24 patients enrolled in the NEOLETEXE trial (i) before treatment, (ii) before change of AI (crossover at 3 months), and (iii) at the end of neoadjuvant therapy (6 months of treatment).

Methods and aims: We aim at characterizing the distal and proximal regulatory elements in different cell types and states observed in longitudinally collected tumor samples. Transcription factor regulatory networks are important determinants of ER+ breast cancer pathogenesis. Understanding the dynamic changes in regulatory element usage under treatment pressure will help to map the molecular events associated with sensitivity or resistance to AI therapy.
We used SCAFE (Single-Cell Analysis of Five-prime Ends), a computational method that maps transcribed cis-regulatory elements (CREs) in single cells using 5’ end scRNA-seq data.

Results: Using unsupervised clustering of scRNA gene expression, we identified the major cell types in ER+ breast tumors. SCAFE was applied to identify 99,996 distal CREs (i.e., enhancers) in a cell type-agnostic manner. We quantified the reads mapping to each distal CRE in each cell creating a cell-by-distal CRE count matrix. We employed a Latent Dirichlet Allocation topic model implemented in pyCistopic to evaluate the significance of enhancers in determining cell phenotypes. Additionally, we characterized the distal CRE landscape specific to each cell type and state, providing an atlas of cell type-specific enhancer usage in breast tumors. By predicting direct transcription factor binding to the corresponding CRE’s DNA sequences using UniBind, we identified ESR1, FOXA1, and GATA3 as transcription factors associated with enhancers in malignant cells. In contrast, in immune cells such as T cells, we found enriched binding sites for RUNX1, ETS, and TBX21.

Conclusions: Using 5' end scRNA-seq data, we successfully generated an atlas of distal regulatory elements for each cell in breast tumors. We identified the transcription factors

likely to activate these cell type-specific distal regulatory elements. Mapping distal regulatory elements usage and transcriptomes in single cells allows us to study the interplay between enhancer activity, transcription factor binding, and gene regulation. It provides insights into the molecular mechanisms of AI therapy sensitivity and resistance and sheds light on the driver pathways associated with cell phenotypes and states in ER+ breast cancer.

P5-06-29: Tumor and endothelial cell-specific methylation alterations identified in breast cancer with epigenetic deconvolution

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DNA methylation, an essential epigenetic modification, plays a critical role in establishing and maintaining cellular identities by regulating gene expression. In cancer, aberrant DNA methylation patterns contribute to tumor development and progression, yet the cell-type-specific methylation alterations within the tumor microenvironment (TME) remain poorly understood. In breast cancer, a highly heterogeneous malignancy, endothelial cells within the TME (TECs) are key players in tumor angiogenesis and progression. Methylation changes detected in bulk tumor analyses, however, reflect signals from multiple cell types present in the TME, making it challenging to pinpoint cell-type-specific epigenetic changes. Recently, methods have been developed to using DNA methylation to deconvolute cell type proportions which can in turn be used to attribute methylation alterations to specific cell types in the TME. A reference-based cell-type deconvolution algorithm, the Hierarchical Tumor Immune Microenvironment Epigenetic Deconvolution (HiTIMED) method, allows accurate quantification of cell type proportions within the TME.
We accessed DNA methylation array data from 609 breast tumors and 331 nontumor normal breast tissues from the Gene Expression Omnibus (GEO) and the Genotype-Tissue Expression (GTEx) portal. To these DNA methylation profiles, we applied HiTIMED and measured tumor and non-tumor cell proportions. Then, applying a statistical interaction model called CellDMC we leveraged the cell type proportion data to identify differential methylation specific to cell types in the breast cancer TME, including TECs and tumor cells. We identified 1897 tumor-cell-specific and 180 TEC-specific differentially methylated cytosines (DMCs) in cancer compared to normal breast tissue (FDR ≤ 1e-10). Importantly, in the cell-type unadjusted differential analysis, tumors exhibited hypermethylation compared to normal tissues; however, in the tumor-cell-specific analysis, tumor cells were hypomethylated relative to normal samples.
By applying these advanced techniques to breast cancer samples, our study unveils cell-type-specific DNA methylation changes that are pivotal in driving the pro-tumorigenic environment. These findings not only enhance our understanding of the molecular mechanisms underlying breast cancer progression but also highlight potential therapeutic targets within the TME. The integration of HiTIMED and CellDMC represents a significant methodological advancement in cancer epigenetics, offering a more precise and insightful approach to studying the complex interplay between different cell types within the TME.

P5-06-30: Distinct ERBB2 amplification patterns in HER2-positive breast cancers with BRCA1/2 mutations and their therapeutic implications

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Background: Traditionally, HER2-positive breast cancers (BC) were considered rare among germline BRCA1/2 (gBRCA1/2) mutant breast cancers. However, recent studies reported that up to 7% of the gBRCA1/2 mutant BCs are HER2-positive. The detailed clinicopathological features of HER2-positive BCs with germline or somatic BRCA1/2 mutations remain largely unknown.

Methods: Inspired by a family of two gBRCA2-mutated HER2-positive BC patients, we established a cohort of Korean patients with HER2-positive BCs harboring germline or somatic BRCA1/2 mutations. We performed whole genome sequencing (WGS) to assess the patterns of ERBB2 amplification in these patients.

Results: A total of 12 patients from two institutions were included. The median age was 53 years (range, 28 – 70). BRCA1 mutations were found in 4 (33.3%) patients; three patients (25.0%) had gBRCA1 mutation, and one patient (8.3%) harbored a somatic BRCA1 mutation (sBRCA1) (SNUH08). Eight patients (66.7%) had gBRCA2 mutations, including the index family with one triple-negative BC (TNBC) patient (SNUH01) and two ER+/HER2-positive BCs (SNUH02 and SNUH03), who are the daughter and sister of SNUH01. WGS was performed on 14 samples. This included two bilateral samples from SNUH07 (SNUH07_L, TNBC; SNUH07_R, ER+/HER2-positive) and two ipsilateral tumors at different quadrants from SNUH09 (both ER+ HER2-low BCs, namely SNUH09_1H and SNUH09_10H). Of the remaining samples, 7/10 were ER+/HER2-positive, and one ER+/HER2-low, one ER+/HER2-negative, and one ER-/HER2-low samples had ERBB2 amplification on WGS. Nine tumors (64.3%) with gBRCA1/2 had loss of wild-type allele, leading to homologous recombination deficiency (HRD), supported by an in-house developed algorithm based on HRDetect. None of the eight gBRCA1/2 mutated tumors (excluding SNUH01) showed focal high-level ERBB2 amplification, indicating a low likelihood of extrachromosomal DNA (ecDNA)-mediated process. The sBRCA1 tumor (SNUH08) was also classified as HRD without focal high-level ERBB2 amplification. Two multicentric ER+/HER2-low BCs from SNUH09 shared a gBRCA2 mutation and most somatic mutations, suggesting a common clonal origin, but lacked BRCA2 loss of heterozygosity and were classified as HR proficient (HRP). These tumors showed increased ERBB2 copy numbers without evidence of ecDNA-mediated amplifications. The median ERBB2 copy numbers in the 11 tumors were 7 (range, 4 – 13). Two distinct ERBB2 amplification patterns were identified: pattern 1 with non-focal moderate-level amplification and large-scale copy number elevation of chromosome 17q, and pattern 2 with focal moderate-level amplification associated with complex genomic rearrangement involving chromosome 17q. Among the 11 BRCA1/2-mutated HER2-positive

BCs, 5 (45.5%) showed pattern 1, and 6 (54.5%) exhibited pattern 2. All three BRCA1 mutant BCs showed pattern 1, while BRCA2 mutant tumors had both pattern 1 (5/8) and pattern 2 (3/8). Bilateral BCs from SNUH07 demonstrated two different mechanisms of carcinogenesis. SNUH07_L (TNBC) was an HRD tumor with gBRCA1 mutation with loss of wild-type allele, while SNUH07_R (HER2-positive) lost the mutant BRCA1 allele, making it HRP with ecDNA-mediated focal high-level ERBB2 amplification. From a therapeutic standpoint, SNUH08 exhibited an exceptional response to trastuzumab deruxtecan (T-DXd). We hypothesize this response is due to enhanced binding of T-DXd to the pattern 1 ERRB2 amplified tumor, with the topoisomerase I inhibitor payload effectively targeting the HRD genome.
Conclusion: We identified two distinct ERBB2 amplification patterns in HER2-positive breast cancers with BRCA1/2 mutations. These tumors could be ideal candidates for anti-HER2 antibody-drug conjugate due to their enhanced binding affinity and increased susceptibility of HRD cancer cells to specific cytotoxic agents.

P5-07-01: A Systematic Review and Meta-Analysis on Surgical Outcomes of LigasureTM versus Conventional Methods in Axillary Lymph Node Dissection for Breast Cancer Patients

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Background: Breast cancer has an increasing disease burden and mastectomy with axillary lymph node dissection (ALND) remains a mainstay in its treatment despite advancement in surgical techniques. ALND leads to seroma formation, prolonged use of drains, delayed wound healing and infection. LigasureTM, a vessel sealing device, demonstrates shorter operative time and damage to surrounding tissues in previous studies. Its application to axillary dissection has been studied prior but with varying results.
Objectives: To determine the efficacy of LigasureTM for axillary dissection over conventional axillary dissection for drainage volume, seroma formation, time to drain removal, operative time and hospital stay.
Methods: Two investigators conducted an online database search for randomized controlled trials on LigasureTM versus conventional axillary dissection excluding non-cancer mastectomies, comparison to other energy sources, retrospective studies, single-arm studies, case reports or case series. Revman Web was used for data analysis. Risk ratio was used for the dichotomous data and mean difference was used for continuous data. Heterogeneity was assessed with Chi square Test and Inconsistency Index (if with substantial heterogeneity if I2 = >50%) and random effects model was used if significant heterogeneity was present. Risk of Bias 2 tool was used to assess bias for the individual studies included. Sensitivity analysis was done on outcomes with high risk of bias and heterogeneity.
Results: The Ligasure group showed statistically significant decrease in drainage volume (n = 387 patients, 95% CI, p value = <0.00001, -185.67 [-295.38, -75.95]), decrease time to drain removal (n = 232 patients, 95% CI, p value = 0.0009, -1.77 [-.2.81 vs -0.72]), and

shorter hospital stay (n = 239 patients, 95% CI, p value =<0.001, -1.21 [-1.96, -0.47]) over conventional axillary dissection. There was no significant difference in incidence of seroma formation and operative time. A high risk of bias was noted in the reporting of seroma formation (inconsistent definitions) and time to drain removal mainly due to lack a standard criteria for removal.
Conclusion: Ligasure has a significant effect in terms of postoperative drainage volume, time to removal and hospital stay to patients who underwent axillary dissection. The authors recommend a larger study with a subgroup analysis on age and body mass index (BMI) as literature have shown added benefit in these groups.
Keywords: Ligasure, Axillary Lymph Node Dissection, Breast Cancer, Mastectomy

P5-07-02: Effects of prehab or rehabilitation on pain and measures of upper extremity mobility and strength after breast cancer surgery

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Introduction: Use of physical and/or occupational therapy (PT/OT) services is recommended to optimize recovery from surgery. Growing research evidence suggests early intervention may result in the best outcomes, but it is unclear if these benefits are also observed in real-world PT/OT services. We performed a retrospective study of women with breast cancer who participated in real-world outpatient rehabilitation services. Aims were to examine the effect of participating in prehab (PT/OT starting before surgery and continuing afterward) versus rehab-only (PT/OT starting after surgery) on pain and measures of upper extremity mobility and strength after surgery.
Methods: Outpatient rehabilitation medical record data was extracted for cases who attended prehab (n=350) or rehab-only (n=246). Outcomes included pain (0 [lowest] to 10 [highest]), handgrip strength (HGS, lbs) and shoulder range of motion (degrees of flexion, extension, and abduction) of the affected side. Outcomes were compared between groups at two timepoints: the post-surgery evaluation and subsequent discharge. Covariates were identified using t-tests and correlation analysis. Linear mixed-effect models were used to examine between-group differences at each timepoint while controlling for covariates (age, recent fall, BMI, surgery type, lymph node dissection type), and to examine changes in outcomes from evaluation to discharge for each group. Estimated marginal means, standard error, and p-value are reported.
Results: On average, cases were 59.37±13.47 years old and had a BMI of 28.27. Cases received: mastectomy (54.5%) or lumpectomy (45.5%) and sentinel lymph node biopsy (SLNB, 78.2%) or axillary lymph node dissection (ALND, 19.3%). 2.5% received both SLNB and ALND. 11.4% reported a fall in the past year. Prehab cases were younger (62.3 vs. 54.2, p<.001), more likely to have had a lumpectomy (p<.001) and SLNB (p<.001), and more

likely to have fallen in the past year (p=.002). There were no other between group differences. After adjusting for covariates, prehab cases had less pain (prehab: 3.23±0.21, rehab-only: 4.91±0.25, p<.001) greater HGS (prehab: 50.04±0.78, rehab-only: 44.04±1.07, p<.001), and better ROM in each plane at the post-surgery evaluation (all p<.001): flexion (prehab: 140.42±1.36, rehab-only: 128.35±1.58), extension (prehab: 80.67±2.19, rehab-only: 66.02±2.21), and abduction (prehab: 138.77±1.73, rehab-only: 119.46±2.15). From evaluation to discharge, a significant reduction in pain (prehab: -1.02±0.23, p<.001; rehab-only: -1.93±0.28, p<.001) and significant improvement in HGS (prehab: +2.43±0.70, p<.001; rehab-only: +5.34±0.88, p<.001), ROM-flexion (prehab: +13.70±1.88, p<.001; rehab-only: +28.53±2.04, p<.001), and ROM-abduction (prehab: +17.90±2.40, p<.001; rehab-only: +40.48±2.62, p<.001) was observed for both prehab and rehab-only cases. For ROM-extension, significant improvement was observed for rehab-only cases (+11.77±1.88, p<.001); improvement for prehab cases was non-significant but within normal ranges (+2.17±1.60, p=.167). At discharge, prehab cases had significantly greater HGS (prehab: 52.47±0.88; rehab-only: 49.38±1.18; p=.039) and ROM-extension (prehab: 82.88±2.41, rehab-only: 77.79±2.36, p=.026) than rehab-only cases.
Conclusion: Women with breast cancer who attended prehab were younger, more likely to have received a lumpectomy with SLNB and have reported a recent fall. After controlling for covariates, prehab cases had less pain, greater strength, and better ROM after surgery. While both prehab and rehab-only cases improved significantly during post-surgery PT/OT, prehab cases had greater strength and ROM at discharge, suggesting early intervention may prevent declines and optimize outcomes. Although more research is needed, these findings support integration of PT/OT in routine care.

P5-07-03: Patient-Reported Outcomes Following Various Breast Reconstruction Techniques: A Retrospective Cohort Study in China

Hongmei Zheng, Yuhang Song, Lingzi Wang, Xinhong Wu

Background: Breast reconstruction surgery is crucial for restoring physical integrity and self-image in women post-mastectomy. However, there is limited data from China comparing the effects of different surgical approaches on patient satisfaction. This study aims to evaluate patient-reported aesthetic outcomes following various breast reconstruction techniques in China and to explore the factors associated with patient-reported aesthetic outcomes.
Methods: We conducted a retrospective cohort study including female patients who underwent mastectomy followed by breast reconstruction at Hubei Cancer Hospital between January 2019 and April 2024. Exclusion criteria were bilateral reconstructions, simple nipple-areola complex reconstructions, breast-conserving procedures, and missing critical data. Surgical data were extracted from the Hospital Information System. Patient-reported outcome measures, including the Breast-Q Breast Cancer Core Scale V2.0 (three dimensions: satisfaction with breasts, psychosocial well-being, and sexual well-being), the five-dimension, five-level EuroQol health questionnaire, and the Decision Regret Scale, were

collected through outpatient reviews and telephonic follow-ups with an Online Questionnaire. Surgery complications were also recorded. Multivariate linear regression was used to identify independent predictors of satisfaction with breasts and psychosocial well-being scores.
Results: The study included 167 patients. The surgical techniques used were lateral chest wall incision (n=21), radial incision (n=69), inframammary fold incision (n=49), endoscopic-assisted surgery (n=14), and other incisions (n=14) which included periareolar incision (n=7), dual incision (n=5), irregular incision (n=1), and L-shaped incision (n=1). The median age was 40 years (IQR: 34-47), with significant age distribution differences among groups (p=0.048). Significant differences were also noted between groups in surgical approach (p=0.034), use of acellular dermal matrix or grafts (p=0.031), implant placement plane (p<0.001), type of implant (p=0.001), and total surgical cost (p=0.049). The psychosocial well-being scores significantly differed among groups (p=0.028), with the highest median score in the other incision group (96.5, IQR 69.5-100) and the second highest median score in the endoscopic-assisted surgery group (81.50, IQR 62.00-100.00). Multivariate analysis revealed that reduced postoperative bra cup size was negatively associated with satisfaction with breasts (β=-12.559; 95% CI: -19.674, -5.443; p=0.001). Age 35-50 (β=9.654; 95% CI: 2.803, 16.506; p=0.006) and over 50 (β=11.413; 95% CI: 2.364, 20.462; p=0.014) were positively associated with psychosocial well-being. Sentinel lymph node biopsy (SLNB) negatively impacted psychosocial well-being (β=-14.454; 95% CI: -26.591, -2.318; p=0.02). Other incision types significantly improved psychosocial well-being (β=17.482; 95% CI: 4.123, 30.841; p=0.011), while endoscopic-assisted surgery showed a trend toward improvement (β=12.822; 95% CI: -0.412, 26.056; p=0.057). Postoperative complications were reported in 22 patients (13.2%), with 2 (9.5%) from the lateral chest wall incision group, 14 (20.3%) from the radial incision group, 5 (10.2%) from the inframammary fold incision group, and 1 (7.1%) from the other incision group.
Conclusion: Traditional incision techniques, such as radial and inframammary fold incisions, remain prevalent in China, though minimally invasive approaches show promising trends for better patient-reported aesthetic outcomes, the application would still need to be further promoted. Factors such as age, reduced bra cup size, SLNB, and incision type influence patient-reported aesthetic outcomes.

P5-07-04: Postoperative upper Limb Edema and Dysfunction Generated by axillary nodes Excision in breast cancer patients with high or low proportion of upper limb venous reflux(PLEDGE-Reflux): a multicenter, prospective cohort study

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Background: The purpose of this study was to explore the influence of the proportion of axillary venous reflux(PVR) on the incidence of upper limb edema and dysfunction in

patients with breast cancer after axillary lymph node dissection.

Methods: The study was designed as a multicentre, prospective cohort study, which was carried out in Shanghai Sixth People's Hospital, Shanghai Fengxian Central Hospital, and Zhejiang Cancer Hospital in China. The patient cohort consisted of patients who met the PLEDGE-Surgery study(NCT05120180) inclusion criteria and were ultimately randomly assigned to undergo ALND surgery with or without preservation of axillary vein branches.The primary endpoint was the short-term(1-month, 6-month and 12-month) incidence of postoperative upper limb lymphedema and dysfunction on the affected side. The secondary endpoints were tumor-free survival and the incidence of upper limb lymphedema and dysfunction at 5 years postoperatively. Preoperative ultrasound measurement was used to calculate the PVR for each patient. After surgery, arm circumference and DASH score were measured at 1 month, 6 months, 12 months, and 5 years. Patients were divided into Preponderant venous reflux group and Preponderant lymphatic reflux group based on the high or low PVR. Chi square test was used to compare the differences in the incidence of upper limb edema/dysfunction between two groups, and least absolute shrinkage and selection operator (LASSO) regression was performed to screen for risk factors of upper limb edema/dysfunction and establish an optimized model nomogram. The discrimination, calibration, and clinical validity of the model were evaluated. AUC value>0.7 is considered a good discrimination of the model. The Bootstrap method (repeated self-sampling 1000 times) was used to verify the constructed model internally to evaluate the accuracy of the constructed model. Data collection and initial analysis were conducted 3 months after patient recruitment was closed. All patients were included in the final analysis per protocol. The trial was registered with ClinicalTrials.gov, NCT05246592.

Findings

From January 3rd, 2022 to April 1st, 2024, a total of 258 female patients were randomly assigned to the PLEDGE-Surgery study, all of whom were included in the analysis of this study. The median age was 53 years(IQR 47-59). The median follow-up was 14.2 months(IQR 10.6-17.6). The average upper limb PVR was 25.94%±18.87%. The optimal cutpoint for the PVR was determined to be 15.223% using the ROC curve. Based on this, patients were divided into a Preponderant venous reflux group(PVR>15.223%) and a Preponderant lymphatic reflux group(PVR<15.223%). The Fisher's exact test showed that there was no significant difference in the incidence of upper limb edema between the two groups at 1 month after surgery(3/151 vs. 0/78, ARR 1.99%, P=0.553, OR was not available), but there were significant differences at 6(1/110 vs. 10/51, ARR 18.7%, P=0.000, OR 26.585, 95% CI [3.299, 214.244]) and 12 months after surgery(1/75 vs. 11/35, ARR 30.10%, P=0.000, OR 33.917, 95% CI [4.161, 276.485]). The incidence of upper limb edema in the Preponderant venous reflux group(RR: 0.125[0.019, 0.813] at 6-month, 0.110[0.017, 0.723] at 12-month) was significantly lower than that in the Preponderant lymphatic reflux group(RR: 3.326[2.413, 4.585] at 6-month, 3.743[2.541, 5.513] at 12-month). The predictive model we developed for diagnosing postoperative upper limb edema achieved an area under the curve (AUC) of 0.919(95%CI: 0.778-1.000) in the cohort. The model

concluded the variables selected by as preponderant lymphatic reflux group, ALND without axillary vein branches preservation, level 3 lymphadenectomy, and post-surgical wound infections. The AUC of the optimized model obtained by internal validation were 0.910.

Interpretation: Proportion of upper limb venous reflux, a previously understudied factor significantly associated with postoperative upper limb edema, has been incorporated into a nomogram predictive model in this study. The constructed model demonstrates a robust predictive value and effectively anticipates the occurrence of postoperative upper limb edema in breast cancer patients.

Keywords
Breast cancer, Lymphedema, Upper limb dysfunction, Axillary lymph node dissection, proportion of upper limb venous reflux, ratio of axillary venous/lymphatic reflux
Funding
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P5-07-05: A Comparison of Wire Guided and Magseed Localisation in Impalpable Breast Lesions

Saira Khawaja, Cameron Bruce, Asma Munir, Mr Sohail Khan, Nida Javed, Anita Huws, Yousef Sharaiha

Introduction: Wire-guided localization (WGL) has been a cornerstone in breast-conserving surgery, providing a reliable method to accurately localize lesions. While effective, WGL can sometimes cause discomfort to patients and requires precise timing on the day of the surgery. In contrast, recent advancements have led to the development of Magseed, a magnetic marker that represents a significant innovation in tumour localization. Magseed is a 5 mm long, surgical-grade stainless steel marker, non-radioactive and designed for less intrusive and more flexible application than WGL. It is placed in the breast using a needle under local anaesthesia and located during surgery with the Sentimag probe, a magnetic sensing system. This approach not only allows for precise tumour localization but also enhances patient comfort by reducing pre-operative anxiety and discomfort associated with the protruding wire of WGL. Both Magseed and WGL are crucial in accurately marking tumours for breast-conserving surgeries, yet Magseed offers potential advantages in terms of patient experience and surgical planning. As breast cancer surgery continues to evolve, these techniques play a pivotal role in improving clinical outcomes and patient-centric care in the treatment of breast cancer.

Methodology: We conducted a retrospective analysis of 197 patients, with 99 undergoing WGL and 98 undergoing Magseed localisation. Patient records were retrospectively accessed to gain information regarding patient demographics, specimen weight, tumour size, clear margin rate and node positivity, evaluated histopathological reporting. Statistical significance between the two groups was analysed using a Fischer exact and Chi Square

hypothesis testing with a p-value <0.05 considered significant.

Results: In our study comparing Wire guided Wide Local Excision and Magseed Wide Local Excision for breast-conserving surgery, we evaluated 99 patients in the Wire group and 98 in the Magseed group. The median age was 64.70 years in the Wire group and 62.76 years in the Magseed group, with average ages of 64.46 years and 61.19 years, respectively. In the Wire group, the average tumour size was 20.31mm with a standard deviation of 14.72mm, a median of 15mm, and an interquartile range of 15.10mm. Conversely, in the Magseed group, the average tumour size was 16.89mm with a standard deviation of 9.27mm, a median of 15mm, and an interquartile range of 10.75mm. Both groups predominantly featured Grade 2 tumours and Ductal type tumours. Node positivity rates were 3.0% in the Wire group and 13.0% in the Magseed group. The clear margin rate was 93.83% in the Wire group (95% CI: 93.5.8% to 98.5%) and 94.58% in the Magseed group (95% CI: 89.8% to 99.2%), with the difference not being statistically significant (p=0.784).

Discussion: Our study's findings reveal that wire localisation exhibited a slightly larger average tumour size compared to the Magseed localisation, which might be reflective of the differing nature of these two techniques in tumour localization and subsequent surgical planning. Despite this, the clear margin rates were notably high in both groups, with magseed localisation achieving a marginally higher rate. However, the difference did not reach statistical significance, indicating that both methods are comparably effective in ensuring successful tumour removal with clear margins.
In the context of patient comfort and procedural efficiency, Magseed’s non-protruding nature offers a distinct advantage over WGL, potentially reducing pre-operative discomfort and anxiety. However, our results suggest that this technological advancement does not compromise the surgical efficacy, as evidenced by the comparable clear margin rates between the two groups.
Ultimately, our study highlights that both WGL and Magseed are effective techniques for tumour localisation in breast-conserving surgery. The choice of technique may be influenced by various factors, including tumour characteristics, patient preferences, and resource availability. As breast cancer surgery continues to evolve, such comparative analyses become crucial in guiding clinical decisions and advancing patient-centred care.

Background: Wire-guided localization (WGL) is a well-established technique for tumor localization in breast-conserving surgery (BCS). However, Magseed localization, a newer technique utilizing a magnetic marker, may offer advantages in patient comfort and surgical planning due to its non-protruding nature. This study aimed to compare WGL and Magseed localization in terms of patient demographics, tumor characteristics, margin status, node positivity, and long-term oncologic outcomes.
Methods: A retrospective analysis was conducted on 197 patients between August 2023 and April 2024 undergoing BCS at a single institution, with 99 patients in the WGL group and 98 in the Magseed group. Patient demographics, tumor size, margin status, node positivity, and histopathological features were assessed. Statistical analysis was performed using Fischer's

exact and Chi-squared hypothesis testing. A value of <0.05 was deemed to be significant.

Results: The median age was 64.70 years (range: 33-84) in the WGL group and 62.76 years (range: 33-78) in the Magseed group. The average tumor size was 20.31mm (SD = 14.72mm) in the WGL group and 16.89mm (SD = 9.27mm) in the Magseed group. Both groups predominantly featured Grade 2, ductal-type tumors. Node positivity rates were 3.0% in the WGL group and 13.0% in the Magseed group. The clear margin rate was 93.83% (95% CI: 93.58% to 98.5%) in the WGL group and 94.58% (95% CI: 89.8% to 99.2%) in the Magseed group, with this difference not being statistically significant (p=0.784).
Conclusions: Both WGL and Magseed localization demonstrated high rates of clear margins in this patient cohort. The slightly larger average tumor size in the WGL group may be attributed to inherent differences in tumor localization and procedural variation between the two techniques. In the context of patient comfort and procedural efficiency, Magseed's non-protruding nature offers a distinct advantage over WGL, potentially reducing pre-operative discomfort and anxiety. However, our results suggest that this technological advancement does not compromise the surgical efficacy, as evidenced by the comparable clear margin rates between the two groups. Overall, The data suggests both techniques are effective for tumor localization in BCS, and the choice between them may be influenced by individual patient and tumor characteristics, as well as surgeon preference and institutional availability.

P5-07-06: Long-term follow-up of Immediate lymphaticovenous anastomosis based on Lymphosome Mapping Strategy after axillary lymph node dissection in breast cancer

Richard Chih-Hau Chang, Chao-Ming Hung

Purpose: Total mastectomy, axilla lymph node dissection, and postoperative radiation therapy in breast cancer could disrupt lymphatic function and lead to debilitating breast cancer-related lymphedema (BCRL). Conventional Lymphatic Microsurgical Preventing Healing Approach (LMPHA), and the immediate lymphatic reconstruction (ILR) with lymphaticovenous anastomosis (LVA) aimed to promote lymphatic system restoration. However, a consensus surgical approach was not established yet, and there were no equivocal long-term results. In this study, 4 major lymphosome vessels categorized as radial volar, ulnar volar, radial dorsal, and ulnar dorsal lymphosome were dressed with indocyanine green fluorescence (ICG) lymphography and further divided into 3 types based on extra-axilla lymphatic vessels or not. Restoration of the disrupted lymphatic vessel that the axilla wound revealed and which major lymphosome included. Follow-up results were analyzed.

Materials and Methods: A retrospective cohort audit was conducted, reviewing the collected data from 20 patients (22 limbs) who underwent immediate LVA at E da Cancer Hospital

between June 2022 and June 2024. Major afferent lymphatic vessels are identified by ICG lymphography and categorized into 3 types. Microscopic images confirm the severance of the major afferent lymphatic vessels. Standard LVA was performed and each LVA was anastomosed into a vein distal to a competent valve and patency was confirmed with ICG video lymphangiography by operative microscope.

Results: Over 24 months, 20 patients (22 limbs) included triple negative cases, there were no immediate LVA complications. The mean follow-up time was 12 months (3-24 months). There was no transient radiation-related lymphedema and no significant difference between pre- and post-op in-arm measurements, and one case progressed to ISL stage 1 since axilla lymph node local recurrence. No distinguishable arm circumference measurement that compared to the contralateral side in between each type of lymphosome and no case present clinical pitting edema.

Conclusion: Based on the knowledge of compensatory lymphatic vessels; in the short-term results of type I lymphosome, there is no higher risk of developing lymphedema after the ILR and even post-radiation therapy. The ILR repaired major lymphosome lymphatic vessels by LVA presents a novel and comprehensive strategy for restoring lymphatic system function. Early data showed IRL offers a more feasible, safe, and effective primary prevention of BCRL. We need more long-term follow-ups and cases to uncover the differences and insights between the three types.

P5-07-07: USING FLUORESCEIN FOR DETECTION OF SENTINEL LYMPH NODES IN COMBINATION WITH TARGETED AXILLARY LYMPH NODE DISSECTION IN BREAST CANCER: PRELIMINARY RESULTS OF EFFICACY AND SAFETY STUDY

Aleksander Soinov, Michail Voronov, Vladimir Vorotnikov, Tornike Mchedlidze, Sardor Abdugafforov, Igor Kopytich, Regina Pakhomov, Maria Sharavina, Marcha Mukueva, Stanislav Tsalko, Viktoria Andreeva, Alina Tkachenko, Aleksandra Gugnina

Introduce: Sentinel lymph node biopsy (SLNB) is the standard staging procedure for early breast cancer (BC). Currently, various markers are used to detect sentinel lymph nodes, including radioisotopes, methylene blue dyes, and fluorescent agents.The aim of this study was to evaluate the effectiveness of sentinel lymph node biopsy using fluorescein and an LED lamp in breast cancer patients, and to assess the safety and tolerability of fluorescein for the sentinel lymph node biopsy procedure in breast cancer patients.The objective of this study was to identify lymph nodes marked with fluorescein, identify lymph nodes marked with the radiopharmaceutical technetium 99mTc, and compare the number of cases of detection of sentinel lymph nodes using fluorescein and an LED lamp with the number of cases of detection of sentinel lymph nodes using technetium 99mTc.

Materials and Methods: 90 patients with a diagnosis of breast cancer with metastatic lymph nodes, who were planned to undergo neoadjuvant chemotherapy, underwent a procedure to stain the enlarged lymph nodes in the axillary area with the "BLACK EYE" dye. The second stage was surgical treatment. All patients underwent a sentinel lymph node biopsy procedure using a combined method with fluorescein and the radiopharmaceutical technetium 99mTc. Fluorescein and technetium 99mTc radiopharmaceutical were injected intradermally into the areola of the affected breast of each patient. Sentinel lymph nodes stained with fluorescein were first detected using an LED probe, then with a gamma detector, and then removed. Targeted axillary lymph node dissection was performed, during which all lymph nodes stained with the "BLACK EYE" dye were removed. The material was sent for urgent histological examination, and in case of metastases, axillary lymphadenectomy was performed. Further, the frequency of detection of sentinel lymph nodes was analyzed and the presence of complications was investigated.ResultsIn total, 60 sentinel lymph nodes were detected and removed using technetium 99mTc and a gamma detector (2-3 lymph nodes per patient). Fluorescein was present in 57 out of 60 removed lymph nodes. "BLACK EYE" dye was found in 30 sentinel lymph nodes. None of the patients experienced complications related to the use of fluorescein or blue LED light.

Conclusions: Based on the preliminary data, it can be concluded that the use of fluorescein for lymph node marking in SLNB in breast cancer patients has potentially high efficacy and safety. Given the availability of the method - low cost of the drug and equipment, fluorescein can become an alternative to ICG. The "BLACK EYE" dye effectively stains metastatic lymph nodes and does not interfere with the accumulation of fluorescein and technetium 99mTc in the lymph nodes. To confirm the efficacy and safety of fluorescein, further research is required on a larger number of patients, as well as a comparison of fluorescein with other fluorescent dyes.

P5-07-08: The ACOSOG Z0011 criteria can be safely applied to Chinese patients: the SAHZU experience

Xiuzhen Li, Yue Hu

Background: As a world-wide known trial in the sentinel lymph node biopsy (SLNB) field, the American College of Surgeons Oncology Group (ACOSOG) Z0011 trial made a big step to de-escalate the axillary surgery. The original intention of this trial was to omit reoperation if the patient had cN0 breast cancer with one or two sentinel-node metastases. A lot of queries were raised to this trial and the controversies about it have never stopped. China has his unique national condition which is different to the western countries. Only methylene blue dye is available in SLNB surgeries in most of the Chinese hospitals. Frozen section is widely used and reoperation is usually unacceptable. The safety of the Z0011 criteria needs to be confirmed when it is applied during surgery and frozen section application. In this retrospective study, we shared our single-center experiences.

Patients and Methods: In our hospital (SAHZU), the breast cancer patients with positive lymph nodes involvement in their SLNB surgeries were enrolled in this study since Aug. 2012 to Mar 2022. According to the Z0011 criteria, the patients with T3/T4 tumor, 3 or more positive lymph nodes involvement, mastectomy or neoadjuvant therapy were excluded. A part of the patients accepted preoperative advanced axillary evaluation (SABCS2018, Abstr. P3-03-20) which was composed by ultrasound, CT/MRI scans and fine/core needle biopsy if it was necessary.

Results: Totally, 153 eligible patients were enrolled in this study. They were younger, with more ER+PR+ diseases, less vascular invasion and higher tumor grades ($P<0.05$), compared with the Z0011 patients. More systemic therapy and lymph node radiotherapy were carried out in our cohort than in the Z0011's. Lymph node metastases were diagnosed by frozen section during their surgery in 88.9% (136/153) of patients and the left were diagnosed by permanent H&E section. Seventy-two patients accepted axillary lymph nodes dissection (ALND) and the other 81 patients accepted SLNB. The clinicopathologic characteristics of the two groups were comparable. Median resected lymph nodes in ALND and SLNB group were 17 and 4, respectively ($P<0.001$). In the ALND group, 27.8% patients (20/72) were with additional positive nodes and the rate was similar with the Z0011 trial. After median follow-up for 44.9 months, one local-regional recurrence happened in both groups and another two patients died in the SLNB group ($P>0.05$). In the SLNB group, all the recurrence and death events occurred in the patients without advanced axillary evaluation (3/36 vs. 0/45, $P=0.088$).
C

onclusions: The Z0011 criteria can be safely applied during surgery in China in spite of the wide use of frozen section and single tracer of methylene blue dye. Advanced axillary evaluation before surgery may do good to the survival of the patients.
Disclosure: Both authors declared no conflicts of interest.

P5-07-09: Predictive factors for postmastectomy reconstruction after breast cancer in Brazil

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Introduction: One third of women with breast cancer in Brazil present with locally advanced disease. This translates into a high percentage of mastectomy with subsequent functional sequelae, negative impact on psychosocial, self-image and self-esteem. As breast reconstruction may improve quality of life, it is key to understand the factors associated with breast reconstruction in Brazil.
Objective: Analyze the predictive factors of breast reconstruction in post-mastectomy patients in Brazil.
Methods: Case-control study nested within the cohort of Women treated with mastectomy

for stage I to III breast cancer at the National Cancer Institute between January/2005 and December/2019. Data were collected through medical records. Cases were selected by reconstruction and controls were matched 1:1 according to year of mastectomy. Patients with ASA 4 surgical risk, risk-reducing and hygienic mastectomy were excluded. Statistical analyzes were performed in the R environment for Windows. The association between independent variables and outcome was performed using the crude odds ratio (OR). Variables presenting p<0.20 in the univariate analysis were included in the multiple logistic regression model by the stepwise forward stepwise, and those with p<0.05 were retained in the final model. Hypothesis tests with p values <0.05 were considered statistically significant. This study was approved by the Research Ethics Committee of the National Cancer Institute (n°. 6,299,721), in accordance with the ethical principles established by the National Health Council.
Results: 322 women were included, 158 cases and 164 controls. 72.4% of patients were younger than 60 years of age, 63.4% were non-white and 74.8% did not smoke. Regarding staging, 59.3% had clinically negative lymph nodes and 51.9% of patients had tumors smaller than 5 cm and 59.3% had clinically negative lymph nodes. After adjustments, the chance of non-reconstruction was higher in women over 60 years old (OR 5.96; IC95% 3.05-12.20; p<0.001), obese (OR 3.61; IC95% 1.91-7.07; p=0.001), diabetic (DM) (OR 3.17; IC95% 1.06-10.41; p=0.045), with cT4 tumors (OR 2.21; IC95% 1.11-4.47; p=0.025), axillary involvement (OR 2.75; IC95% 1.48-5.17; p<0.001), and treated with radiotherapy (OR 2.09; IC95% 1.09-4.07; p=0.027). Patients with a previous breast conservation had a greater chance of reconstruction (OR 0.31; IC95% 0.10-0.84; p=0.029). The median waiting time for delayed breast reconstruction was 45.1 months (IRQ 31.1-67.4).
Conclusion: There is a long time to breast reconstruction after mastectomy in Brazil; and age> 60, obesity, DM, cT4, and LN positive were negative predictors factors, in accordance with the international literature. These data provide the basis for future policies that may improve quality of life after breast cancer.

P5-07-10: Immediate Breast Reconstruction with Prepectoral Implant

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Introduction: Breast reconstruction is one of the pillars of breast cancer treatment. Reconstructive surgery is constantly being improved, from muscle flaps to submuscular implant reconstruction. Recently, immediate breast reconstruction with a prepectoral prosthesis has emerged as a promising, less invasive technique and potential for more natural aesthetic outcomes. However, the real post-surgical outcomes of it are not yet fully

consolidated. In light of this, we conducted a systematic review to compile the main outcomes of this new technique. Methods: The search was carried out in the PubMed and Scopus databases. A search strategy was devised for the articles using the descriptors: "Breast Cancer", "Prepectoral", "Immediate" and "Reconstruction". Inclusion criteria were previously established: 1. reconstruction with immediate prepectoral prosthesis, 2. mastectomized patient, 3. prospective observational studies or clinical trial; and exclusion criteria: 1. delayed reconstruction, 2. reconstruction using dermal matrix in all cases, 3. use of expander in all cases and 4. surgeries performed in two-steps. The outcomes of interest were the number of reoperations, readmissions and implant losses. Results: Of the 155 initial studies, 7 were included. A total of 845 patients were assessed with a total of 1112 mastectomies with reconstruction were performed, of which 740 (66.5%) were immediate reconstructions with pre-pectoral prosthesis without the use of an expander or dermal matrix, 341 (30.6%) pre-pectoral reconstructions with the use of dermal matrix or mesh and 31 (2.7%) with the use of an expander. Of the total number of reconstructions, 98.9% were prepectoral reconstructions and 1.1% were submuscular. Follow-up times ranged from 1 month to 2.15 years. Overall, 271 complications were reported, which represents 24.3% of all reconstructions. The most common early complications were reoperation (29.8%), readmission (28.4%) and seroma (11.1%). However, only 65 of these complications resulted in implant loss, representing a total of 5.8% of the reconstructions carried out; the others were all resolved with or without the need for reoperation. In total only one did not report any surgical complications with the technique. The studies highlighted complications that appear to be linked to clinical and complementary treatment characteristics and associated risk factors. One study identified a 13.2% implant loss rate in the group undergoing adjuvant chemotherapy. Another study reported that 19.5% of patients developed infection, 17.5% required readmission for complications, 16.0% underwent further surgery for complications within 3 months of the initial reconstruction, and 8.2% experienced implant loss and reconstruction failure during this interval. Exploratory analyses suggest that mastectomy weighing more than 600 grams, smoking, high surgical risk, obesity, and pre-surgery radiotherapy are associated with a higher risk of complications. Regarding patient satisfaction and quality of life, one study showed a considerable worsening of physical well-being one year post-treatment. Conversely, important findings showed satisfaction with the results of surgery and reconstruction. Other endpoints found in the perioperative period reported improved physical, psychosocial and sexual well-being, as well as satisfaction with their breasts. Conclusion: Immediate reconstruction with a prepectoral prosthesis was found to be a viable and safe technique, with low rates of serious complications and an implant loss rate of 5.8%, similar to other techniques. The studies generally showed patient satisfaction with the final result of the procedure. It is important to emphasize that additional research is required to determine the ideal patient characteristics to personalize treatment for breast reconstructions procedure with the best possible aesthetic result, as well as reducing complication rates.

P5-07-11: Comparison of the effectiveness of using visualization methods of the lymphatic tracts of the upper limb in preparation for the application of lymphovenous anastomoses for patients with secondary lymphedema.

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Abstract: lymphedema is a chronic lymphatic swelling of soft tissues that occurs as a result of the slow accumulation of protein-rich fluid in the extracellular space; caused by insufficient lymphatic drainage in combination with insufficient protein utilization, which leads to fibrotic changes in the skin and subcutaneous tissue. One of the common causes of lymphedema of the upper extremities is surgical treatment of breast cancer, accompanied by lymph node dissection, and in some cases, radiation therapy, which also contributes to the development of edema. Breast cancer ranks second in the overall structure of cancer among the population in the world and is the most common malignant neoplasm in women. Given the prevalence of this disease, a large group of patients is at risk of developing lymphedema.

For successful treatment of lymphedema, it is important to have good visualization of intact lymphatic pathways. This knowledge will help the surgeon choose the optimal location for the lymphovenous anastomosis, and also, in the future, evaluate the performance of the anastomosis. We will consider two ways to visualize lymphatic pathways: sodium fluorecein and indocyanine green (ICG).

Purpose: to compare two methods of visualizing the lymphatic tract in preparation for lymphovenous anastomosis.

Methods: we analyzed the results of examination and treatment of female patients with secondary lymphedema of the upper extremities that arose after complex treatment of breast cancer at the Central Clinical Hospital of Russian Railways in 2022-2024. All edema was unilateral, occurring on the side of the breast cancer treatment. A comparative analysis of the methods we used to visualize the lymphatic tract was carried out, as well as the results of subsequent surgical intervention. A total of 80 cases were analyzed.

Results: sodium fluorecein and indocyanine green showed good results in visualizing the lymphatic tracts of the upper limb, in all cases we obtained persistent staining of the lymphatic tracts. However, an advantage has been identified by using indocyanine green - it, unlike sodium fluorecein, allows visualization of lymphatic pathways on the skin; we observe the so-called “track”. This gave us the opportunity to choose the most successful position and applicate a lymphovenous anastomosis outside the affected area of the lymphatic tract.

Sodium fluorecein is convenient if the lymphatic pathways in the area of the proposed operation are not affected by changes, since it allows them to be visualized only while we in the surgical wound.

In the area of the upper third of the shoulder, we performed an anastomosis “end of the

lymphatic vessel to the end of the vein" due to the comparable diameter of the lymphatic vessel and vein. Already in the early postoperative period, we observed a positive effect from the operation, long-term results (average median follow-up 14 months) were good, the swelling stopped progressing and decreased by 50% or more. According to lymphography data, the anastomoses are functioning properly and fulfilling their function. We attempted to apply a lymphovenous anastomosis immediately after breast reconstruction (reconstruction of the breast with an implant, preparing the postoperative area for reconstruction using lipofilling. But there was a tendency towards deterioration in the visualization of the lymphatic pathways due to previously performed operations and the persistence of postoperative edema. We believe that breast operation several days prior may make it difficult to applicate a lymphovenous anastomosis.
Conclusions: Fluorescence lymphography with sodium fluoresin or indocyanine green is modern and accurate for the diagnosis of lymphedema. Staining the lymphatic tract is an important tool in preparing for lymphatic-venous anastomosis, and the more visualization techniques the surgeon has, the better the result of the operation. Also, the success of the operation largely depends on the treatment tactics chosen by the surgeon - we believe that lymphovenous anastomosis in the complex treatment of postmastectomy syndrome should be carried out either before breast reconstruction surgery, or no earlier than 1-3 months after it.
Keywords: breast cancer, lymphedema, lymphovenous anastomosis.

P5-07-12: ONCOPLASTIC SURGERY FOR PAGET'S DISEASE OF THE BREAST

Rafael Pelorca, Idam de Oliveira-Junior, Rene Aloisio da Costa Vieira

Introduction: Paget's disease of the breast (PDB) is a rare nipple entity associated with multifocality. Due to its location, it is necessary to resect the nipple-areolar complex. For surgery, central quadrantectomy, and mastectomy was the treatment in the past. The feasibility of performing oncoplastic breast surgery (OBS) for PDB is unknown.
Methods: This was a retrospective study performed in a Brazilian oncological hospital. We evaluated the factors related to the performance of OBS in PDB. In addition, the impact of OBS on local recurrence and survival was analyzed. Comparisons were made between groups using the chi-square test, Mann–Whitney U test, and Kaplan–Meier method. To assess the impact factor of the variables on the performance of OBS, logistic regression was performed.
Results: Eighty-five patients were evaluated. OBS was performed in 69.4% (n=59), and of these, 16 (27.2%) were symmetrized with a contralateral surgery. Mastectomy without reconstruction was performed in 28.3% of the patients. The main procedure performed was mastectomy with reconstruction (n=38; 44.7%), and the preferential technique for immediate reconstruction was skin sparing mastectomy with prosthesis, and for late reconstruction, latissimus dorsi. BCS was performed in 27.0% (n=23), mainly with plug-flap technique (OBS). Age was associated with the use of OBS, wherein patients aged 40-49 were associated with a higher rate of OBS (p = 0.002; odds ratio 3.22). OBS did not influence local recurrence (p=1.000), overall survival (p=0.185), or cancer-specific survival (p=0.418).

Conclusion: OBS improves the quality of surgical treatment in PDB without influencing local recurrence or survival.
Keywords: Paget's Disease Mammary, Breast Neoplasms, Breast reconstruction, plastic surgery, Oncoplastic Surgery

P5-07-13: Analysis of Single-Port Robot-Assisted Nipple Sparing Mastectomy in Patients with Invasive Breast Cancer: Propensity-Matched Comparison with Conventional Nipple Sparing Mastectomy

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Introduction: Robot-assisted nipple-sparing mastectomy (RANSM) presents several benefits over traditional surgical techniques, such as enabling surgery through smaller incisions and providing enhanced precision. Nonetheless, there are ongoing concerns about its surgical and oncological safety. This study investigate the safety and efficacy of RANSM in comparison to conventional nipple-sparing mastectomy (NSM).

Materials and Methods: In a retrospective single-center study at Asan Medical Center, 302 patients underwent RANSM from October 2020 to October 2023. Out of these, 177 patients, excluding those with carcinoma in situ, bilateral breast cancer, and those who received neoadjuvant chemotherapy, were analyzed using 1:2 propensity matching.

Results: The median age of patients in the RANSM group was 46 years, with a median tumor-to-nipple distance of 2.0 cm. The median duration for NSM was 155 minutes. There were no instances of conversion to open surgery or intraoperative blood transfusions due to bleeding. The average breast specimen weighed 324 grams. Among the patients, 110 were in stage I, 59 were in stage II, and 8 were in stage III. The average hospital stay post-surgery was 5 days. Postoperative bleeding necessitated blood transfusions in 9 patients. Two patients experienced skin flap ischemia, one patient had skin flap necrosis, one patient suffered from NAC necrosis, and another patient required surgery for skin desquamation.

Conclusions: Compared to conventional NSM, RANSM requires a slightly longer surgery time because of the robot preparation. Despite this, RANSM offers advantages such as fewer surgery-related complications and reduced hospitalization duration.

P5-07-14: Clinical study of 99mTc-Rituximab combined with dyes double tracing for axillary sentinel lymph node biopsy after neoadjuvant chemotherapy for breast cancer

Hui Zhang

Background and objective: It is still controversial whether sentinel lymph node biopsy (SLNB) can replace axillary lymph node dissection (ALND) for breast cancer after neoadjuvant chemotherapy (NAC).This study used 99mTc-rituximab combined with dye to perform SLNB on patients after NAC, and analyzed the results to evaluate the application value of the dual tracing method of 99mTc-rituximab combined with dye in SLNB after NAC in breast cancer, and to explore the feasibility of SLNB after NAC, the clinical application of the new tracer 99mTc-rituximab and its application value in internal mammary sentinel lymph node biopsy. Methods: A retrospective analysis was performed on 106 breast cancer patients who underwent SLNB after NAC at the Department of Oncology, Fujian Provincial Hospital from August 2019 to August 2023. SLNB was performed under 99mTc-rituximab combined with dye and dye alone.The detection rate, sensitivity, false negative rate, accuracy and detection of internal mammary lymph node biopsy of the two tracing methods were compared.Results: 70 cases were included in the dual tracing group, with a detection rate of 97.14% (68/70), an average number of detected SLNs of （6.06±5.29）， a sensitivity of 92.86% (26/28), a false negative rate of 7.14% (2/28), and an accuracy of 97.14% (68/70).36 cases were included in the single tracing group, with a detection rate of 66.67% (24/36), an average number of detected SLNs of （3.17±3.073）， a sensitivity of 54.55% (11/22), a false negative rate of 45.45% (10/22), and an accuracy of 72.22% (26/36). There were significant differences in the detection rate and the average number of detected SLNs between the two groups (detection rate: P=0.004; detection number: P=0.038), but there were no significant differences in the sensitivity, accuracy, and false negative rate (P>0.05).A total of 70 patients underwent double-tracing internal mammary lymph node biopsy, and 22 patients were detected with an imaging rate of 31.42% (22/70), and a detection rate of 72.72% (16/22).

Conclusion: SNLB using radionuclide with dye double-tracing method after neoadjuvant chemotherapy for breast cancer can improve the detection rate of sentinel lymph nodes, reduce the false negative rate, and improve the prognosis.Compared with other tracers, 99mTc-Rituximab can improve the detection rate of internal mammary sentinel lymph nodes, with the characteristics of rapid clearance of injection site, less secondary lymph node imaging, and low sensitization, which can be used as an ideal tracer for further research.

Key words: 99mTc-Rituximab， neoadjuvant chemotherapy， sentinel lymph node biopsy， breast cancer.

P5-07-15: FEASIBILITY AND SAFETY OF ROBOTIC NIPPLE-SPARING MASTECTOMY (RNSM) WITH 1 IMPLANT BASED - IMMEDIATE BREAST RECONSTRUCTION: AN INITIAL EXPERIENCE FROM INDIA'S 1ST ROBOTIC BREAST SURGERY TEAM

Priya Kapoor, Magesh M, Pragadeesh, Vimal, Venkat P

Objectives : To evaluate the feasibility and safety of robotic Nipple-Sparing Mastectomy (RNSM) with implant based immediate breast reconstruction in 12 patients of early breast cancer. Patients & Methods: In this case series analysis, patients with early breast cancer were enrolled in our hospital from December 2023 to March 2024. All patients underwent triple assessment of breast cancer preoperatively. RNSM was performed with single cosmetic 's' shaped incision covered by patient arm. All the procedures were performed using da Vinci Xi Robotic Surgical System, a single docking method using CO2 inflation after placing GelPort. Patients tumours measuring >5 cm, skin or nipple involvement or planned radiotherapy were excluded. Results: 12 patients underwent RNSM followed by implant based immediate breast reconstruction. 3 out of 12 cases underwent RNSM as prophylaxis in view of positive BRCA1 status. Preoperative diagnosis for 2 cases reported as ductal carcinoma in situ (DCIS) and remaining 7 cases showed invasive ductal breast carcinoma. The mean total operation time for RNSM was around 242 mins and the console time was 128 mins. The average blood loss during surgery was 3-5ml. Mean hospital stay was 1.5 days. No major complications were observed for any case. 1 out of 12 patients had partial nipple necrosis and it resolved spontaneously. No systemic complications were observed. Conclusion: RNSM with implant based reconstruction is a safe and feasible approach for early breast cancer. It has several advantages with uniform flap thickness, NAC dissection and ergonomics.

P5-07-16: How cardiovascular risk factors and type of chemotherapy influence cardiotoxicity in early HER2 positive breast cancer (HER2+ BC) patients treated with neoadjuvant Trastuzumab and Pertuzumab (T-P) based chemotherapy?

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Background: T-P based chemotherapy improves survival in early HER2+ BC but confers a higher risk of cardiac events compared to chemotherapy alone. In recent years, a better understanding of tumor biology and the results of two trials (TRAIN-2 and TRYPHAENA) have led us to consider different neoadjuvant strategies, free of anthracyclines, which provide a safer risk profile without impact in efficacy.

Methods: We conducted a retrospective observational study of HER2+ eBC patients (pts) treated with neoadjuvant T-P based chemotherapy, between April 2015 and December 2022, at University Hospital A Coruña (Spain). The primary endpoint was total pathologic complete response in breast and axila (tpCR: ypT0/is ypN0). One of the secondary objectives was the association between cardiovascular risk factors, type of chemotherapy and cardiotoxicity, results presented below. Multivariate logistic and multinomial regression analyses were carried out using R system for statistical computing.

Results: A total of 156 pts were included within a period of 7 years. Median age was 49 years [range 26 – 79 years]. Cardiovascular risk factors were determined: median weight was 65.8kg (39-104 [SA4] [ba5] kg), 16% of pts had dyslipemia, 15.4% hypertension and 4.5% previous cardiac disease. Most pts had clinical stage II disease (64.2%), positive nodes (51%), grade 3 (56.2%), ductal histology (91%), hormone receptor positive (57.7%) and Ki-67 >35 (55.8%). 35% received platinum-based chemotherapy and 64.2% anthracyclines. tpCR was achieved in 51.3% of pts, which was not significantly different according to chemotherapy approach in the logistic regression model (p=0.93). Comparable results were observed in breast pCR.
Most pts developed cardiotoxicity at the end of adjuvant trastuzumab (29.1%). Similar rates were detected in the middle (25.2%) and at the end of neoadjuvant chemotherapy (23.4%). Only 1.5% of pts developed late cardiotoxicity. There were no episodes of congestive heart failure.
In the logistic regression model developed to predict cardiotoxicity at the end of neoadjuvant treatment (AUC 0.728), we noted that type of chemotherapy and weight were the only variables associated with cardiac events ($p < 0.05$). Anthracyclines were associated with an increase of cardiotoxicity ($e^{1.047} = 2.489$; $p = 0.033$), while previous cardiac disease tended to rise this probability ($e^{1.962} = 2.489$; $p = 0.051$). A lower basal weight increased the risk of cardiotoxicity, which requires the study of other intermediate variables ($e^{-0.042} = 0.959$; 0.019).
We did not find a significant association between cardiac risk factors, type of chemotherapy and cardiotoxicity in the rest of the timepoints studied.

Conclusion: The combination of anthracyclines with T-P resulted in higher cardiac toxicity without significant differences in pCR, at the end of neoadjuvant treatment. Long-term follow-up is required to confirm these results.

P5-07-17: Comparison of Rate of Pathologic Complete Response in HER2-zero versus HER2-low Breast Cancer: a Meta-Analysis

Michelle Min, James Doyle

Introduction: Development of treatments that target human epidural growth factor receptor 2 (HER2) expression has improved the rate pathologic complete response of

hormone receptor-negative, HER2-positive breast cancer. However, the benefit has not been shown in 1+ or 2+ ISH-negative HER2 breast cancer, also known as HER2-low. We conducted meta-analysis of six studies to determine the difference in rate of pathologic complete response (pCR) to neoadjuvant chemoimmunotherapy in nonmetastatic HER2-low versus HER2-zero breast cancer.

Methods: Meta-analysis was performed based on the count and percent data provided by six studies we identified via a PubMed search about the results following neoadjuvant chemoimmunotherapy for the treatment of nonmetastatic hormone receptor-negative, HER-2 low and HER2-zero breast cancer. All meta-analyses were performed with RStudio. The heterogeneity of the studies was assessed and there was enough evidence to claim significant heterogeneity for both categories. Since significant heterogeneity was found, a random effects approach was used to account for the between-study variability instead of the standard fixed effects approach.

Results From the six studied identified and included after our PubMed search, 836 patients with HER2-low breast cancer and 1543 patients with were identified. Of these, a total of 262 in the HER2-low population achieved a complete pathological response to adjuvant chemoimmunotherapy versus 568 in the HER2-zero population. The combined rate of pCR in HER2-low group was 30% (95% confidence interval [CI] 24% - 37%). The combined rate of pCR in HER2-zero group was 35% (95% CI 28% - 42%).

Conclusion: Based on this meta-analysis, there was no significant difference in rate of pCR in HER2-zero and HER2-low group. However, all studies used in this meta-analysis included a majority or patients enrolled before the Keynote-522 Trial, when pembrolizumab was added to neoadjuvant chemotherapy for triple negative breast cancers. Thus, re-evaluation of the rate of pCR in patients who received the now standard chemoimmunotherapy to see if HER2-low vs HER2-zero patients respond differently to the addition of neoadjuvant immunotherapy.

P5-07-18: HER2DX assay in HER2-positive (HER2+) breast ductal carcinoma in situ (DCIS)

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Background: The HER2DX assay, a 27-gene test, was developed to provide prognostic information and predict treatment responses in patients with early-stage HER2+ breast cancer. This assay evaluates four gene expression signatures: immune/immunoglobulin (IGG), proliferation, luminal, and HER2, offering a comprehensive risk score, a likelihood estimates for pathological complete response (pCR) and ERBB2 expression levels. In this study, we evaluated the HER2DX assay in HER2+ DCIS, aiming to understand its biology and

relationship with HER2+ invasive breast cancer.

Methods: Standardized HER2DX genomic test was evaluated centrally on 28 formalin-fixed paraffin-embedded tumor samples of HER2+ DCIS across three hospitals in Spain. DCIS features such as nuclear grade, comedonecrosis, architectural pattern, tumor size and hormone receptor (HR) status were evaluated. Percentage (%) of stromal tumor infiltrating lymphocytes (TILs), their spatial distribution (i.e.: inflamed, desert or excluded), and presence of tertiary lymphoid structures (TLS, defined as spatially organized, non-encapsulated areas of immune cell aggregates with or without germinal center [GC]) were assessed on hematoxylin eosin slides. Descriptive statistics were used.

Results: The HER2DX assay was evaluated in 28 cases of HER2+ DCIS. Most cases had a 3 nuclear grade (67.9%) and presented with comedonecrosis (60.7%). HR positivity (HR+) was found in 42.9% of cases, as determined by immunohistochemistry. The median tumor size was 30 mm, with a range from 5 mm to 90 mm. The median % of TILs was 20% (range 0.5-70%). TILs, as a continuous variable, showed a moderate correlation with the HER2DX IGG signature (Pearson correlation coefficient=0.43, p-value=0.023), and a tendency with the inflamed spatial distribution (p-value=0.063). TLS were identified in 82.1% of DCIS samples, and their presence was associated with higher expression of the IGG signature (52.2% TLS in IGG-high vs. 21.7% in IGG-low). Regarding the HER2DX luminal signature, 41.7% of cases were classified as luminal-high, associated with HR+ disease (p=0.025). For the HER2DX proliferation signature, 82.1% of cases were identified as proliferation-low. In HER2DX risk stratification, 100% of cases were categorized as low-risk, 46.4% were pCR-high, and 78.6% were ERBB2-high. Notably, only one (3.6%) HER2+ DCIS case was found to be ERBB2-low.

Conclusions: The HER2DX assay revealed that all HER2+ DCIS cases were categorized as low-risk, with most cases showing high ERBB2 expression and a high predicted response to anti-HER2-based therapy. These findings underscore the underlying biology of HER2+ DCIS and its tumor immune microenvironment, indicating that HER2+ DCIS shares similar tumor biology with low-risk HER2+ breast cancer.

P5-07-19: A multicenter, prospective, non-interventional study to investigate the treatment patterns of neratinib in Human Epidermal Growth Factor Receptor 2 positive (HER2+) early breast cancer (EBC) in China: NER-Tree study—An interim analysis on safety

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Background: Neratinib, an oral irreversible pan-HER tyrosine kinase inhibitor, was approved in 2020 in China for the extended adjuvant treatment of HER2+ early breast cancer (EBC) adult patients who completed prior adjuvant trastuzumab-based therapy based on the ExteNET trial. Diarrhea is a common treatment-related adverse event of neratinib, and it is necessary to investigate its pattern of occurrence and the effectiveness of anti-diarrheal prophylaxis measures in the real world.

Method: This is a multi-center, prospective, non-interventional study planning to enroll 500 HER2+ EBC patients who scheduled 1-year neratinib treatment after anti-HER2 adjuvant therapy. Pre-specified interim analyses are planned after the recruitment of 250 and 500 patients. The primary and secondary objectives are to describe the real-world adjuvant treatment patterns and to observe the safety of patients treated with neratinib respectively. The exploratory objectives are to observe invasive disease recurrence patterns and patient-reported quality of life, including pattern of occurrence and the effectiveness of preventive measures for diarrhea. Patient characteristics and pre-treatment patterns from this interim analysis were previously presented at ESMO Breast Cancer 2024. Here we report safety data from the initial interim analysis with a focus on diarrhea and related management.

Results: As of 21 September 2023, 250 patients were recruited in the study and were included in the interim analysis.
According to this interim analysis, diarrhea, nausea, and abdominal pain were the most common AEs. Diarrhea, as the most frequent adverse event, occurred in 80.4% of patients (all grades), and grade 3 diarrhea occurred in 14.8% of patients. No grade 4 diarrhea was observed. The median time to onset of diarrhea was 3.0 days (interquartile range [IQR] 2.0,5.0). Diarrhea led to dose reduction in 12.8% of cases, dose interruption in 16.0%, and permanent discontinuation in 2.8% of cases. Patients who started on neratinib at <240 mg experienced a lower incidence of Grade ≥3 diarrhea compared to those who started at 240 mg (10.2% vs. 19.7%). There was no statistically significant difference in the incidence of diarrhea across different molecular subtypes of breast cancer (HR-/HER2+ vs HR+/HER2+), lymph node status, and adjuvant treatment regimens.
One hundred and fifty-one patients (60.4%) received anti-diarrheal prophylaxis at least once, including drug prophylaxis (Loperamide or other agents, 33.2%), dose escalation (17.6%), and combination (dose escalation combined with drug prophylaxis, 9.6%). The dose escalation strategy reduced the incidence of Grade ≥3 diarrhea compared with prophylactic management with antidiarrheal(6.8% vs 18.1%).

Conclusion: This study provides further data on safety, including the pattern of diarrhea occurrence and the impact of preventive anti-diarrheal measures in Chinese HER2+ early breast cancer patients treated with neratinib as extended adjuvant therapy. diarrhea, nausea, and abdominal pain were the most common AEs. The dose-escalation strategy can reduce the incidence of Grade 3 diarrhea associated with neratinib.

P5-07-20: A prospective, open-label, single-arm phase II clinical study of inetetamab in combination w/ pyrotinib & albumin-bound paclitaxel for neoadjuvant treatment of patients with HER2+ early & locally advanced breast cancer: updates on clinical trial

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Background: Chemotherapy combined with dual anti-HER2 target therapy has become the standard neoadjuvant therapy regimen for HER2+ breast cancer. Patients achieving pathological complete response (pCR) have a relatively better prognosis. Inetetamab (Septin) is an innovative HER2 monoclonal antibody drug developed in China. It has been proven to delay the progression of HER2+ metastatic breast cancer patients and bring survival benefits. The purpose of this study was to explore the efficacy and safety of Inetetamab, Pyrotinib, and albumin-bound Paclitaxel in the neoadjuvant treatment of HER2+ breast cancer. Methods: This phase II trial included patients with HER2+ early or locally advanced breast cancer whose tumor size was >20 mm or had confirmed axillary lymph node metastasis. Patients received an initial dose of Inetetamab at 8 mg/kg over 90 minutes via IV infusion, followed by 6 mg/kg over 30 to 90 minutes via IV infusion every 3 weeks (q3w). Pyrotinib was administered at 400 mg orally every day, and albumin-bound Paclitaxel at 125 mg/m^2 via IV on days 1, 8, and 15, every 3 weeks (q3w). Patients received the above treatment every 3 weeks for a total of 4 cycles, followed by surgery.After surgery, EC (90 mg/m^2 of Epirubicin Hydrochloride and 600 mg/m^2 of Cyclophosphamide) was administered every 21 days (q21 days) for 4 cycles, followed by maintenance treatment with trastuzumab combined with pertuzumab for 1 year. Radiotherapy and endocrine therapy were provided to the patient according to the specific situation. The primary endpoint was pathological complete response (pCR).Results: Until September 20, 2024, 22 patients were enrolled, of which 20 had completed surgical treatment. The median age of enrolled patients was 52 years (range: 35-61 years). 50.0% (10 cases) of patients achieved RCB grade 0 (pCR), 20.0% (4 cases) achieved RCB grade I, 25% (5 cases) achieved RCB grade II, and 5% (1 case) achieved RCB grade III. Of the 20 patients, 55.0% (11 cases) were HR+/HER2+, with 36.4% (4 cases) achieving pCR. Among the 9 HR-/HER2+ patients, 66.7% (6 cases) achieved pCR. HR-/HER2+ patients were more likely to achieve pCR than HR+/HER2+ patients. No severe (grade 3/4) toxicity was observed in any patients. Conclusions: Our current data show that the pathological complete response (pCR) rate in HER2+ breast cancer patients reaches 50.0%, and even 36.4% in HR+ patients. Interestingly, 4 out of these 20 patients achieved near-pathological complete response (near-pCR). We suggest that these 4 patients would more likely achieve pCR if treated with a 6-cycle regimen, which can boost the pCR rate to 70% (14/20) in HER2+ patients.In the previous Neosphere and PEONY studies of the H+P dual-target regimen, the pCR of HR+/HER2+ patients increased by only 6% and 8.3%, respectively. In contrast, in the NeoALTTO and PHEDRA studies of the H+L or H+Py regimen, the pCR increased by 18.9% and 17.7%, respectively. This indicates that the combination of large and small molecules

with dual-target neoadjuvant therapy for HR+/HER2+ breast cancer is significantly better than monoclonal antibody dual-target therapy.Therefore, Inetetamab, Pyrotinib, and albumin-bound paclitaxel are potentially effective new adjuvant treatments for HER2+ breast cancer with a superior pCR rate.

P5-07-21: Real-World Safety and Effectiveness of Switching among Biosimilars in HER2-Positive Breast Cancer

Andreas Nearchou, Antonios Valachis, Kunvar Harsh Upveja

Background: Ogivri (trastuzumab-dkst) is one of the several trastuzumab biosimilars used in HER2-positive breast cancer treatment. This retrospective study aimed to provide real-world data on the safety and effectiveness of switching patients from another trastuzumab biosimilar to Ogivri. Currently, there is limited real-world evidence (RWE) on this sort of switch, making this study relevant for both local and global healthcare contexts.

Method: This is a multi-center retrospective post-approval observational study to investigate the safety and effectiveness of Ogivri after switching from another trastuzumab biosimilar (SB3) in patients with HER-2 positive EBC (Early Breast cancer) or MBC (Metastatic Breast cancer). This study used an Electronic Data Capture (EDC) system to collect data available in patients' medical records at the two sites of Sweden from April 2021 to December 2022. The primary objective of the study was to compare the incidence and severity of infusion-related reactions (IRR) to Ogivri® with SB3 prior to and after the switch in EBC and MBC patients. Secondary objectives of the study were to investigate Cardiac events, other trastuzumab-related AEs (Adverse events, pCR (pathological Complete Response) among patients treated with neo-adjuvant therapy, ORR among MBC patients.

Results: A total of 48 patients with HER2-positive breast cancer were enrolled from two centers (Center 1: 25 patients, Center 2: 23 patients) and all received 3-weekly trastuzumab therapy and 27 patients (56%) also received pertuzumab. The average age of the cohort was 59 years (range: 32-81), with an average weight of 74 kg (range: 47-98). HER2 status was confirmed by IHC/FISH testing in all included patients, and ductal carcinoma was predominant (38 patients, 79%). The majority of patients received trastuzumab as a part of curative treatment for early breast cancer (39 patients, 81%).
Patients received an average of approximately 11 cycles of SB3 before they switched to Ogivri and switching happened at 5th cycle in adjuvant setting (few patients also switched during Neoadjuvant therapy) and 19th cycle in palliative setting. Prior to switching, one patient experienced a Grade 1 infusion-related reaction (IRR) with SB3, characterized by itching and rash after the second dose. Post-switch to Ogivri, no IRRs were reported. There were no cardiac events reported throughout the treatment period and Left Ventricular ejection fraction (LVEF) was maintained before and after switching the patients to Ogivri

(~60%). In terms of trastuzumab-related adverse events (AEs), a total of three AEs were recorded: two cases of urticaria before the switch and one case of chills and vomiting after the switch. All reported AEs resolved or recovered.
Among patients receiving neoadjuvant treatment, 5 out of 9 patients achieved a pathological complete response (pCR), representing a rate of 56%. The overall response rate (ORR) among assessable MBC patients was 35% (patients at different lines of therapy)

Conclusion: This study demonstrates that switching from another trastuzumab biosimilar to Ogivri in HER2-positive breast cancer patients is safe, with no infusion-related reactions or cardiac events reported post-switch, without jeopardizing treatment effectiveness. This research addresses a critical gap in real-world data on switching among biosimilars, providing essential insights for healthcare decision-making.

P5-07-22: Phase III study to evaluate the efficacy and safety of GLSI-100 (GP2 + GM-CSF) in breast cancer patients with residual disease or high-risk PCR after both neo-adjuvant and postoperative adjuvant anti-HER2 therapy, Flamingo-01

Snehal Patel, Jaye Thompson, Mira Patel, F. Joseph Daugherty, Mothaffar F. Rimawi

Background: GP2 is a biologic nine amino acid peptide of the HER2/neu protein delivered in combination with Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) that stimulates an immune response targeting HER2/neu expressing cancers, the combination known as GLSI-100. Of the 146 patients that have been treated with GLSI-100 over 4 clinical trials, GLSI-100 was well-tolerated and no serious adverse events observed were considered related to the immunotherapy.

Methods: This Phase III trial is a prospective, randomized, double-blinded, multi-center study. After 1 year of trastuzumab-based therapy, 6 intradermal injections of GLSI-100 or placebo will be administered over the first 6 months and 5 subsequent boosters will be administered over the next 2.5 years. The participant duration of the trial will be 3 years treatment plus 1 additional year follow-up.
Study Size – Interim Analysis: Approximately 498 patients will be enrolled. To detect a hazard ratio of 0.3 in invasive breast cancer free survival (IBCFS), 28 events will be required. An interim analysis for superiority and futility will be conducted when at least 14 events have occurred. This sample size provides 80% power if the annual rate of events in placebo patients is 2.4% or greater. Up to 250 non-HLA-A*02 subjects will be enrolled in an open-label arm.
Eligibility Criteria: The patient population is defined by these key eligibility criteria: 1) HER2/neu positive and HLA-A*02, 2) Residual disease or High risk pCR (Stage III at presentation) post neo-adjuvant therapy, 3) Exclude Stage IV, and 4) Completed at least

90% of planned trastuzumab-based therapy.
Trial Objectives: The trial objectives are to: 1) Determine if GP2 therapy increases IBCFS, 2) Assess the safety profile of GP2, and 3) Monitor immunologic responses to treatment and assess relationship to efficacy and safety.
Study Status: The study is actively recruiting and enrolling patients in the US and Europe at up to 150 sites.
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Funding: This trial is supported by Greenwich LifeSciences.

P5-07-23: Efficacy and Safety of Pyrotinib Combined with Trastuzumab and Aromatase Inhibitor in Neoadjuvant Treatment of HER2+/HR+ Breast Cancer: A Single-Arm, Multi-Center Phase II Exploratory Study

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Background: HER2+/HR+ breast cancer exhibits unique clinical and biological characteristics due to the interplay between HER2 and HR signaling pathways, rendering chemotherapy-free anti-HER2 therapy a potential treatment avenue. Based on findings from the ALTERNATIVE study, the NCCN guidelines recommend the combination of aromatase inhibitors with lapatinib and trastuzumab for treating HER2+/HR+ advanced breast cancer. Additionally, the PHEDRA and NEOSPHERE studies have shown that a pyrotinib (anti-HER2 TKI)-containing regimen in the neoadjuvant setting significantly enhances tpCR rates (17.7% vs. 6%) in HER2+/HR+ breast cancer. This study aims to evaluate the efficacy and safety of pyrotinib combined with trastuzumab and an aromatase inhibitor (AI) in the neoadjuvant treatment of HER2+/HR+ breast cancer (NCT05885776).
Methods: This investigation is a single-arm, multi-center phase II exploratory study. The inclusion criteria encompass female patients aged 18 to 75 years, diagnosed with stage II to IIIA HER2-positive (IHC score 3+ or 2+ and ISH positive), ER > 10% invasive breast cancer. Participants received 6 cycles of pyrotinib (400 mg orally daily), trastuzumab (initially 8 mg/kg, followed by 6 mg/kg intravenously on day 1, Q3w), and an AI (either letrozole 2.5 mg orally daily or anastrozole 1 mg orally daily). Premenopausal or perimenopausal patients also received ovarian function suppressants. Post-surgery radiotherapy and adjuvant treatments were individualized. Primary endpoint assessed was tpCR (ypT0/is ypN0), while secondary endpoints included ORR, breast conservation rate, and safety profile. The study adopted Simon's two-stage optimal design, testing H0 (tpCR ≤ 10%) against H1 (tpCR > 25%), with a one-sided significance level of 0.05 and 80% power. Initially, 18 patients were recruited. If ≤2 achieved tpCR, the null hypothesis would be accepted, and the study terminated; otherwise, recruitment would continue to a total of 43

patients. Treatment efficacy is deemed achieved if ≥8 patients reach tpCR.
Results: From August 23, 2022, to June 4, 2024, 22 patients were enrolled. Among these, 86.4% (19/22) had ER ≥ 50%, 72.7% (16/22) exhibited Ki67 ≥ 30%, 31.8% (7/22) were stage III, 18.2% (4/22) were stage T3, and 68.2% (15/22) had positive lymph nodes. To date, 15 patients have undergone surgery, with 20% (3/15) achieving tpCR, thus meeting the pre-defined threshold for progressing to the second recruitment stage. The breast conservation rate stands at 46.7% (7/15). Major adverse reactions included diarrhea, stomach discomfort, reduced appetite, and nausea. No grade 4 adverse events occurred; grade 3 adverse events comprised diarrhea (22.7%) and elevated creatinine levels (4.5%).
Conclusion: The results from the initial stage of this study suggest that the combination of pyrotinib, trastuzumab, and AI in the neoadjuvant setting for HER2+/HR+ breast cancer provides the anticipated efficacy with a manageable safety profile. Consequently, the study will continue to enroll up to 43 patients.

P5-07-24: HER2+ early breast cancer treatment and outcomes by risk of recurrence: a retrospective US electronic health records study

Reshma Mahtani, Gregory Vidal, Sam Hillman, Ellie John, Simon M Collin, Gráinne Long

Background: Patients (pts) with human epidermal growth factor receptor 2–positive (HER2+) early breast cancer (eBC) remain at risk of relapse despite the use of standard-of-care treatments. We aimed to describe use of neoadjuvant and adjuvant therapies and clinical outcomes by risk of relapse in US pts with eBC since 2011.

Methods: This was a retrospective observational study using the nationwide Flatiron Health electronic health record-derived de-identified database. Pts aged ≥18 years with HER2+ eBC (clinical or pathologic stage I, II, or IIIa) who were treated between January 2011 to December 2021 were included. Baseline pt and tumor characteristics were captured prior to and at the index date (date of eBC diagnosis). Pts were followed from index date to death, last known activity date, or end of the study period (December 31, 2022). The definition of high risk of relapse (high-risk HER2+ eBC) was based on pathologic stage (any node-positive tumor or large tumor size [T3/T4] if node negative) or, among pts who received neoadjuvant treatment (NAT), clinical stage IIb, IIc, IIIa, IIIb, or IIIc. Analyses were categorized by date of eBC diagnosis: 2011–2013, 2014–2017, or 2018–2021.

Results: A total of 1290 pts with HER2+ eBC were eligible within the diagnosis dates (2011–2013 n=351, 2014–2017 n=515, 2018–2021 n=424). Among these, 72.9% (n=940) were hormone receptor–positive with a median age at diagnosis of 58.0 years (interquartile range [IQR] 49.0, 67.0); 28.4% (n=366) were classified as high risk (233 by pathological stage, 133 by clinical stage with NAT) and 71.6% (n=924) as non-high risk. Median follow up was 58.0 months (IQR 31.9, 87.8). The 5-year invasive disease-free survival and overall survival probabilities were 72.3% (95% confidence interval [CI] 66.8, 77.1) and 86.9% (95% CI 82.3, 90.4) for pts with high-risk HER2+ eBC, and 80.7% (95% CI 77.6, 83.5) and

91.8% (95% CI 89.4, 93.7) for pts with non-high-risk HER2+ eBC, respectively. In 2018–2021, 29.4% (15/51) of pts with high-risk HER2+ eBC defined by pathologic stage received NAT, an increase from 2.2% (2/92) in 2011–2013 and 14.4% (13/90) in 2014–2017. Receipt of adjuvant therapy in all pts with high-risk HER2+ eBC also increased over time (81.8% [90/110)], 89.0% [130/146], and 91.8% [101/110] in 2011–2013, 2014–2017, and 2018–2021, respectively). In 2018–2021, 64.5% (71/110) of all pts with high-risk HER2+ eBC received HER2-directed NAT and 86.4% (95/110) received HER2-directed adjuvant therapy. In the same time period, 34.4% (108/314) of all pts with non-high-risk HER2+ eBC received NAT (33.8% HER2-directed) and 79.3% (249/314) received adjuvant therapy (68.8% HER2-directed).

Conclusions: Use of neoadjuvant and adjuvant therapy has increased over time in pts with HER2+ eBC who are at high risk of relapse. There remains a need for more effective therapies to optimize pt outcomes in HER2+ eBC.

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P5-07-25: "NAVIGATING THE NEXUS: OVERCOMING DOUBLE JEOPARDY OF EARLY HER2-POSITIVE BREAST CANCER PRESENTING AS AN ANTI-Yo POSITIVE PARANEOPLASTIC CEREBELLAR DEGENERATION IN A YOUNG WOMAN"

Vallish Shenoy, Sudeep Gupta, Seema Gulia, Prabhat Ghanshyam Bhargava, Sushmitha Rath, Shalaka Joshi, Tabassum Wadasadawala, Dileep Hoysal, Revathy Krishnamurthy

Introduction: Anti-Yo-associated paraneoplastic cerebellar degeneration (PCD) syndrome is a very rare condition that is most commonly associated with breast and gynaecologic cancers. Overall incidence of PCD in all cancer patients is approximately 1-3%. Percentage of breast cancer patients developing PCD is around 0.01% to 0.1%. Those cases associated with breast cancer tend to be human epidermal growth factor receptor 2 (HER2)-positive, though the reason for this correlation is unknown. Most commonly, the neurologic symptoms of the PCD syndrome predate the patient's cancer diagnosis. Thus, prompt diagnosis of PCD is essential to allow for early treatment of the neurologic symptoms and the underlying malignancy. However, the prognosis is very poor for the anti-Yo-associated paraneoplastic syndrome, since neurologic damage is usually rapid and irreversible. Further progression may be stopped with appropriate treatment of cancer, but existing neurologic deficits at the time of diagnosis are usually permanent. Here we present a rare case of a young woman with early HER2 positive breast cancer diagnosed with PCD.

Case Presentation: A 26-year-old woman with nil comorbidity and no family history of malignancy presented in February 2023 with progressive unsteadiness while walking with worsening truncal instability, slurring of speech, diplopia and increasing difficulty with fine motor tasks due to incoordination for 3 months. At this point, she needed the support of one person to walk. There were no constitutional symptoms, headaches, seizures, and hearing or visual disturbances. Her vitals and general physical examination (including breast) were normal. There was no lymphadenopathy. Neurological assessment revealed prominent cerebellar signs, including bilateral upper limb dysmetria, intention tremor, scanning speech, and gait and truncal ataxia. She had normal muscle tone, power, sensation, and symmetrical reflexes throughout her upper and lower limbs. Examination of the cranial nerves did not reveal any abnormalities. Gaze-evoked nystagmus was observed. Her routine laboratory tests were unremarkable. Brain and spine magnetic resonance imaging were normal, but her Nerve conduction studies (NCS) showed sensory peripheral neuropathy. Cerebrospinal fluid analysis revealed no cells, normal sugar levels, slightly increased protein level (77 mg/dl) with no oligoclonal bands, and it was negative for malignant cells. Serum was tested for onconeural antibodies, including anti-Yo, anti-Hu, anti-Ri, anti-CV2, anti-Ma2, and anti-amphiphysin. A high titre of anti-Yo antibodies (1:1,000) was detected in the serum and CSF samples using immunoblot assay with the dilution of 1:101. She underwent 2 cycles of plasmapheresis and 5 days of IVIG therapy leading to only minimal improvement. The presence of a characteristic anti-onconeural antibody led to the search of an underlying malignancy. A PETCT Scan was undertaken which showed a FDG avid enhancing lobulated soft tissue lesion involving retro areolar region of right breast, measuring 1.8 x 1.3cm, SUVmax 6.85 with no other sites of metastasis. A breast Biopsy was undertaken in which the histological report classified the tumour as a grade 3 infiltrating ductal carcinoma (IDC) with negative Estrogen receptors (ER), negative progesterone receptors (PR), Positive HER2 (3+) by immunohistochemistry. In addition, a panel of tumour susceptibility genes was performed, which did not show any mutations. Therefore, the patient was diagnosed of a unicentric IDC cT1cN0M0 HER2 positive of the right breast. With that information, she was initiated on Neoadjuvant therapy (NAT) completing 12 cycles of single agent weekly Trastuzumab from March 2023 to June 2023 with excellent tolerability. Paclitaxel was withheld due to pre-existing neuropathy on NCS. At this point, she had significant improvement in her symptoms to the tune that she could do her daily activities with minimal support. Following NAT, she underwent Breast conserving surgery with Axillary sampling in the month of July 2023 with post op Histopathology showing pathological complete response (pCR) (ypT0 and pN0 in sentinel nodes 0/6). Post Surgery in the month of July 2023 she had worsening of her ataxia with new onset sensorineural hearing loss (SNHL) which was moderate loss as per pure tone audiometric (PTA) test. She was given 5 days of High dose Methylprednisolone Pulse therapy following which she had good improvement with respect to her ataxia but not to SNHL. She completed 13 cycles of adjuvant three weekly single agent trastuzumab in the month of April 2024 and is on observation since then. On her first follow-up since completion of her treatment she observed improvement in her ataxia but only minimal improvement in her SNHL still requiring one person support for her activities of daily living with breast cancer being in

remission.

Conclusion: PCD remains a therapeutic challenge, based on the treatment of the neoplastic disease in conjunction with corticosteroid therapy and immunomodulators, such as immunoglobulins. It should be noted that neurological improvements have been described in patients with HER2 positive breast cancer under treatment with trastuzumab. In paraneoplastic conditions where the antigen is intracellular, as in the present case, the effectiveness of immunoglobulins is doubtful. Both positivity and titres of antibodies have not been shown to be predictors of evolution or response to treatment. Here, we present a novel case of a patient treated with only HER2 blockade, achieving a pathological complete response, which may impact the prognosis of the paraneoplastic syndrome.

P5-07-26: UK 5-Point Scoring System for Clinical Breast Examination Reduces Discordance in a Symptomatic Breast Triple Assessment Clinic

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Introduction: Despite advancements in standardising breast imaging scoring systems, challenges persist in reporting clinical breast examinations (CBE). A 5-point scoring system, analogous to the Royal College of Radiologists Breast Group scoring system for the classification of breast imaging, is widely employed to report CBE in the UK. This system is crucial in the triple assessment of symptomatic breast patients, where discordance between CBE, imaging, and histopathology necessitates additional investigations and delays treatment. This study evaluates the impact of using the UK 5-point scoring system to report CBE on discordance in a symptomatic breast triple assessment one-stop clinic.

Methods: This retrospective chart review included symptomatic breast patients seen at a tertiary site in London, UK, between September 2022 and August 2023. Patients underwent CBE and at least one breast imaging modality. Clinicians were instructed to report CBE using a 5-point scoring system (1: Normal, 2: Benign, 3: Indeterminate, 4: Suspicious for malignancy, 5: Malignant) as per standard operational procedures. Discordance was defined as an abnormality on CBE not detected by imaging, while incorrect use of the scoring system was defined as a mismatch between the reported score and its description. The proportions of discordance and incorrect use were estimated with 95% confidence intervals. Associations with discordance were assessed using Pearson chi-square tests, and binary logistic regression estimated odds ratios for significant associations.

Results: 362 cases were included with a median age of 46 years (IQR=34-56), of which 345 (95.31%) were women. Presenting complaints encompassed 229 lumps (63.26%), 90 instances of pain (24.86%), and 43 with other symptoms (11.88%). The scoring system was

used in 260 CBE reports (71.82%). Ultrasound and mammography were performed in 311 (85.91%) and 194 (53.59%) cases, respectively. Histopathology was assessed in 48 cases (13.26%), with cancer diagnosed in 18 cases (4.97%). The proportion of discordance was 11.60%, 95% CI [8.49, 15.36]. The association between discordance and the use of the scoring system to report CBE was significant, $\chi^2(1, N=362)=13.75$, p<.001. Using the scoring system significantly reduced the odds of discordance OR=0.303, 95% CI [0.157, 0.584], p<.001, with further reduction when excluding incorrect uses OR=0.107, 95% CI [0.044, 0.259], p<.001. The proportion of incorrect use was 5.8%, 95% CI [3.3-9.3]. No significant associations were found between discordance and age $\chi^2(1, N=362)=3.13$, p=.077, sex $\chi^2(1, N=362)=0.64$, p=.425, presenting complaint $\chi^2(2, 362)=0.37$, p=.831, clinician seniority $\chi^2(2, 362)=3.25$, p=.197, CBE score χ^2 (2, N=362) = 2.55, p=.280, or imaging modality $\chi^2(2, N=362)=1.59$, p=.452.

Conclusion: This study demonstrates a threefold reduction in the odds of discordance when the UK 5-point scoring system is used to report CBE, with correct use of the system further decreasing the odds of discordance. These findings underscore the importance of developing dedicated CBE reporting guidelines, and support the broader adoption of standardised CBE scoring systems, to avoid ambiguity in multidisciplinary team communication and streamline the management of symptomatic breast patients.

P5-07-27: BL-B01D1, a first-in-class EGFRxHER3 bispecific antibody-drug conjugate, in patients with Locally Advanced or Metastatic Breast Cancer and other Solid Tumor: Updated results from a phase 1 study

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Background: BL-B01D1 is a potentially first-in-class novel antibody drug conjugate (ADC) consisting of an EGFRxHER3 bispecific antibody bounded to a novel topoisomerase I inhibitor payload via a cleavable linker. We now present updated safety/efficacy data from phase I study of BL-B01D1 in breast cancer (BL-B01D1-104).

Methods: This study included Chinese patients (pts) with locally advanced or metastatic breast cancer (BC) and other solid tumors. BC pts were enrolled in dose-expansion (D-EXP) at 2.5mg/kg D1D8 Q3W regimen regardless of EGFR/HER3 and HER2 expression levels.

Results: As of May 31, 2024, a total of 158 BC pts (156 were previously treated) have been treated with at least one dose of BL-B01D1. Among the 158 pts, 44 pts had triple negative breast cancer (TNBC) including 27 HER2 0 pts; 77 pts had HR+HER2- BC including 28 HER2 0 pts; 37 pts had HER2 positive BC.
The most common TRAEs (>25%, all grade/≥G3) were anemia (90.5%/39.9%), leukopenia (88.0%/42.4%), neutropenia (85.4%/51.3%), thrombocytopenia (67.7%/25.3%), nausea

(58.2%/3.8%), stomatitis (48.7%/3.8%), aspartate aminotransferase increased (45.6%/0%), alanine aminotransferase increased (42.4%/0%), asthenia (42.4%/8.9%), vomiting (40.5%/0.6%), hypertriglyceridemia (36.7%/0.6%), alopecia (32.9%/0%), hypokalemia (31.6%/3.8%), decreased appetite (31.0%/0.6%), hyperglycemia (30.4%/0%), constipation (27.2%/0.6%), hyponatremia (25.9%/1.3%). Among 158 BC pts, 1 patient was dead due to febrile neutropenia that was assessed to be related to BL-B01D1. No ILD was observed.
Among the 158 pts, 147 pts were efficacy evaluable (shown in the table).

Note:
1. 1 pt with HER2+ BC and 1pt with HR+HER2- BC who didn't receive standard treatment because of poor economic condition were enrolled.
2. NE, Not evaluable. Including pts who had no post-baseline scan and already off treatment.
3. The median follow up time of PFS is 8.5 months, and the mOS of all sub-group pts are not reached.
4. NR, Not reached.

Conclusions: BL-B01D1 has demonstrated encouraging efficacy in previously treated patients with metastatic and locally advanced breast cancer subtypes, particularly in earlier lines of therapy. The safety and tolerability of BL-B01D1 are consistent with previously published data.
Clinical trial information: NCT05470348.

P5-07-28: The next-generation CDK4-selective inhibitor atirmociclib (PF-07220060) in combination with letrozole as first-line treatment in patients with HR+/HER2+ metastatic breast cancer

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Background: Atirmociclib (PF-07220060) is a novel potent CDK4-selective inhibitor (CDK4i) with significant sparing of CDK6. In an ongoing phase 1/2a trial, atirmociclib plus endocrine therapy showed favorable tolerability and clinical activity in patients with metastatic breast cancer (mBC) who progressed on prior CDK4/6i (Yap, et al. Ann Oncol. 2024;[suppl_4]:1-47; Abstract 184MO). Here, we present the efficacy and safety results from an expansion cohort in the phase 1/2a study of atirmociclib in combination with letrozole in treatment-naïve patients with HR+/HER2– mBC (cohort 2B).
Methods: Women with HR+/HER2– mBC who had not received any prior systemic anti-cancer therapies for their advanced disease were enrolled in expansion cohort 2B of the phase 1/2a study. Patients received 300 mg BID atirmociclib + letrozole. Primary objective

was to assess the safety and tolerability of atirmociclib + letrozole; a secondary objective was to evaluate the anti-tumor activity by objective response (OR = complete response [CR] + partial response [PR]) and clinical benefit rate (CBR = OR or ≥ 24 weeks stable disease) per RECIST v1.1.
Results: At data cutoff (March 4, 2024), 34 patients had received atirmociclib + letrozole. Median age was 59.0 years (range 32–84); 67.6% were White, 23.5% were Asian; most had ECOG PS of 1 (55.9%). In this cohort of patients who had not received any prior systemic anti-cancer therapies in the advanced/metastatic setting, 55.9% underwent anticancer surgery and 47.1% had prior adjuvant/neoadjuvant therapy. Treatment-related adverse events (TRAEs) occurred in 28 (82.4%) patients; none were Grade (G) 4-5. Most common TRAEs were neutropenia (61.8%; 23.5% G3), leukopenia (38.2%; no G3), anemia (20.6%; no G3), and lymphopenia (20.6%; 2.9% G3). Due to a TRAE, 1 patient (2.9%) discontinued atirmociclib (G3; increased aspartate aminotransferase) and 3 (8.8%) had dose reductions (G1 and G2; neutropenia). Median relative dose intensity (RDI) of atirmociclib was 99.1% (range 36.0–100); 91.2% had RDI > 80%. Of the 32 patients who had measurable disease at baseline, 50% (16/32 patients) showed a confirmed OR, all of which were PRs. The CBR for these patients was 96.9% (31/32 patients). Among the entire cohort of 34 patients, median progression-free survival has not been reached. Median duration of follow-up was 11.1 months (95% CI 10.9–13.6). Updated efficacy data with longer follow-up will be presented at the meeting.
Conclusions: Combination of atirmociclib + letrozole showed favorable safety/tolerability and promising early activity as first-line treatment in patients with HR+/HER2– mBC.

P5-07-29: Trastuzumab deruxtecan With Pembrolizumab in Previously Treated HER2-Expressing Advanced or Metastatic Breast Cancer: Interim Analyses of the Breast Cohorts from the Open-Label, Multicenter, Phase 1b Study DS8201-A-U106

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Background: Trastuzumab deruxtecan (T-DXd) demonstrated strong and durable antitumor activity in patients (pts) with previously treated HER2-positive (HER2+) and HER2-low/ultra-low mBC. We report interim clinical efficacy and safety for T-DXd plus pembrolizumab (PEM) in pts with previously treated HER2+ or HER2-low mBC enrolled in the breast cancer cohorts of DS8201-A-U106 (NCT04042701).

Methods: U106 is an open-label, multicenter, 2-part, phase 1b study. Part 1 determined the recommended dose for expansion (RDE) of T-DXd plus PEM. In part 2, pts with mBC were

assigned by centrally determined HER2 expression status to cohort 1 (HER2+; immunohistochemistry [IHC] 3+, IHC 2+/in situ hybridization [ISH]+ that progressed on T-DM1) or cohort 2 (HER2-low; IHC 1+ or IHC2+/ISH- that progressed on standard therapy); the primary endpoint was confirmed objective response rate (cORR) by independent central review (ICR).

Results: The RDE was T-DXd 5.4 mg/kg and PEM 200 mg intravenously every 3 weeks (Q3W). At data cutoff (DCO; Nov 18, 2023), 56 pts with mBC had received the RDE (30 in cohort 1; 26 in cohort 2). Median age was 58.6 years in cohort 1 and 56.3 years in cohort 2. Most pts in cohort 1 were from Europe (27/30 [90.0%]), most pts in cohort 2 were from the United States (16/26 [61.5%]). 17 pts (56.6%) in cohort 1 and 25 pts (96.2%) in cohort 2 were hormone receptor positive. Pts received a median 3.0 (range, 2-12) and 4.5 (range, 1-12) prior treatment regimens in the metastatic/locally advanced setting in cohorts 1 and 2, respectively. At DCO, T-DXd and PEM treatment was ongoing in 7 and 4 pts in cohort 1 and no pts in cohort 2. Median duration of follow-up was 16.1 mo (range, 3.0-30.2) in cohort 1 and 15.3 mo (range, 1.4-34.1) in cohort 2. The cORR by ICR was 80.0% (24/30; 95% CI, 61.4-92.3) in cohort 1 and 23.1% (6/26; 95% CI, 9.0-43.6) in cohort 2. The disease control rate (complete response + partial response + stable disease) was 100% (95% CI, 88.4-100) in cohort 1 and 80.8% (95% CI, 60.6-93.4) in cohort 2. Median duration of response (mDoR) was not evaluable (NE; 95% CI, 6.9 mo-NE) in cohort 1 and 11.4 mo (95% CI, 2.8-NE) in cohort 2. Median progression-free survival (mPFS) was NE (95% CI, 8.7 mo-NE) in cohort 1 and 12.7 mo (95% CI, 4.0-16.9) in cohort 2. PFS rates at 12 months in cohorts 1 and 2, respectively, were 68.6% (95% CI, 46.7-83.0) and 52.1% (95% CI, 29.7-70.4). Grade ≥3 treatment-emergent adverse events (TEAEs) occurred in 20 pts (66.7%) in cohort 1 (40.0% related to T-DXd; 26.7% related to PEM; 16.7% related to both) and 16 pts (61.5%) in cohort 2 (23.1% related to T-DXd; 3.8% related to PEM; 3.8% related to both). The most common grade ≥3 TEAEs were neutropenia, anemia, nausea, fatigue, and vomiting. TEAEs associated with discontinuation occurred in 9 pts (30.0%) and 2 pts (7.7%) in cohorts 1 and 2, respectively (including 1 due to grade 2 decreased left ventricle ejection fraction in cohort 1). There were 2 TEAEs associated with death (both in cohort 1; 1 due to general physical health deterioration; 1 due to neurologic decompensation); neither was related to either study treatment per investigator assessment. The overall incidence of adjudicated drug-related (T-DXd and/or PEM-related) interstitial lung disease/pneumonitis was 12.5% (7/56), with 3 events in cohort 1 (all grade 2) and 4 events in cohort 2 (3 grade 2; 1 grade 3).

Conclusions: Combination therapy with T-DXd and PEM showed encouraging efficacy in heavily pretreated pts with HER2+ and HER2-low mBC. Prolonged mPFS and mDoR were observed in pts with HER2-low mBC despite lower-than-expected cORR in this cohort. The value of addition of immunotherapy to T-DXd in this setting remains to be determined. The safety profile was consistent with the known profiles of the individual drugs and was generally manageable.

P5-07-30: Prophylactic irradiation to the contralateral breast for BRCA pathogenic-variant carriers with early-stage breast cancer: 10 years results

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Background: Women who carry germ-line pathogenic variants (PV) in BRCA1/2 face a high lifetime risk of breast and ovarian cancer. For BRCA1/2 PV carriers who present with early-stage unilateral breast cancer (BC), breast conserving therapy (BCT, breast conserving surgery and radiation) is associated with a similar risk of ipsilateral cancer recurrence (IBTR) compared with noncarriers. However, the risk of subsequent contralateral BC in carriers is high, estimated 25-30% at 10 years. Therefore, mastectomy and contralateral risk reducing mastectomy (CLT RRM) are often advocated. Nevertheless, there is insufficient evidence that RRM improves survival in this setting, and patients may forgo this option for fear of the surgical interventions.
Herein we report the long-term results of a prospective phase II trial (NCT00496288) of prophylactic irradiation to the contralateral breast in BRCA PV carriers with early stage BC. We previously reported our 5-year outcome data (Ann Oncol, 2019).
Patients and methods:
Between May 2007 and October 2017, 162 BRCA1/2 PV carriers undergoing radiation therapy for unilateral BC were enrolled. Eighty-two patients opted for surveillance to the CLT breast (control arm) and 80 received CLT prophylactic breast irradiation (intervention arm). The primary end-point was CLT BC. The groups were balanced by type of BRCA PV, age, BSO at diagnosis and stage of disease. In the control arm, significantly more patients had Her2 positive disease and were treated accordingly (p= 0.018), and more patients had endocrine sensitive disease and therefore received adjuvant endocrine therapy (p=0.026).

Results: At a median follow-up of 10.4 years, significantly more patients underwent elective RRM in the control arm [9 (11%) vs.1 (1.25%), p=0.018], and were censored from the primary analysis at the time of RRM. Fifteen (20.5%) patients developed CLT BC in the control arm at a median of 40 months, as compared with 11 (13.9%) patients who developed CLT BC at a median of 91.5 months after prophylactic breast irradiation (log-rank P=0.22). IBTR occurred in 12 (7.4%) patients, 6 in each arm, at a median of 81 months. There were no significant differences with respect to other breast cancer outcomes (distant metastasis free survival, breast cancer specific survival), and no differences for other cancer events.

Conclusions: Prophylactic breast irradiation reduced the rate of CLT BCs at 10 years and delayed their occurrence, without compromising breast cancer outcomes or increasing secondary malignancies. The above results did not achieve statistical significance, suggesting that prophylactic irradiation mostly treated subclinical disease while its

preventive effect on carcinogenesis was less evident.
Prophylactic breast irradiation should be further evaluated in a phase III trial or at least in a larger prospective cohort.

P5-08-01: The Impact of the Neoadjuvant Chemotherapy Compared to Adjuvant Chemotherapy in ALND Candidacy in cN0, cT1,2 early-stage Breast Cancer, A National Cancer Database Analysis

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Introduction: Axillary dissection is currently recommended for more than two positive nodes or for any positive nodes after neoadjuvant chemotherapy. However, the value of axillary dissection is uncertain. Enthusiasm for neoadjuvant chemotherapy (NAC) in early-stage breast cancer has intensified. It is possible that more liberal use of neoadjuvant chemotherapy will lead to an increase in recommendations for axillary dissection. Here, we compared the rate of ALND candidacy in early-stage breast cancers between those who received NAC and adjuvant chemotherapy (AC).
Method: After Institutional Review Board (IRB) approval, we performed a retrospective analysis of the National Cancer Database (NCDB). We identified cN0, cT1-2, M0, stage I, and II female breast cancers who had primary site surgery and received chemotherapy between 2010-2021. Patients with a history of another malignancy, no axillary surgery, unknown clinical or pathologic nodal status, an unknown number of positive nodes, and a previous history of neoadjuvant radiotherapy or endocrine therapy were excluded. ALND candidacy was considered positive in the NAC group if any positive nodes were recorded, and it was considered positive in the AC group if more than two positive lymph nodes were documented after axillary surgery. Patient and tumor characteristics were compared across those who received NAC and AC. Multivariable logistic regression was used to determine variables associated with ALND candidacy. Patients were categorized into four phenotypes: 1- hormone receptor-positive (HR+) and HER2+ as triple positive (TP); 2- HR+ and HER2- as hormone-positive (HR+); 3- Hormone receptor-negative (HR-) and HER2+ as HER2+; and 4- HR- and HER2- as triple negative (TN). Considering different rates of pathologic response to NAC, we performed a subgroup analysis by phenotype to compare the ALND candidacy amongst those who got NAC and AC stratified by the stage of the disease. Analysis was performed using Stata software, version 18.
Results: A total of 295,110 cases were selected. The mean age of the patients was 56.6+/-12.0 years; 79.3% were White, invasive ductal carcinoma (IDC) constituted 83.6%, and 41.5% underwent mastectomy. The mean number of removed and positive nodes were 5.4 and 0.9, respectively. NAC was administered in 38,315 (13%) of the patients, and 9.6% of them had positive nodes and were ALND candidates, while 25,017/256,795 (9.7%) of the AC group had more than two positive nodes and were considered ALND candidates (p=0.24). We performed a subgroup analysis by phenotype. TN breast cancers constituted 81,924 patients. Of them, 71.6% received AC, and 28.4% received NAC. The prevalence of

ALND candidacy was 3% in the AC and 5.9% in the NAC group (p<0.001). HR+ patients included 160,304 patients, of whom 95.6% received AC and 4.4 % received NAC. Among HR+ patients, 13.2% of the AC group and 23.7% of the NAC group were ALND candidates (p<0.001). TP breast cancers included 22,708 cases, where 89% received AC and 11% received NAC. ALND candidacy was also higher in the NAC group compared to the AC group among TP breast cancers (11.2 % to 6.5%, respectively, p<0.001). The HER2+ phenotype included 20,566 cases. In the HER2+ group, ALND candidacy in the NAC group compared to the AC group did not increase, and a decline in the ALND candidacy after NAC was observed (5.0% to 6.1%), p=0.008. This decline was not statistically significant in stage 1 HER2+ cases, but it was statistically significant in stage 2 HER2+ cases (5.1% to 8.9%) (p<0.001). On multivariable logistic regression model adjusted for age, Charlson Comorbidity score, clinical tumor stage, grade, HR status, HER2 status, lymphovascular invasion, type of primary site surgery (mastectomy or lumpectomy), and the year of diagnosis, NAC increased the rate of ALND candidacy compared to AC (OR:2.03, 95%CI 1.9-2.1; p<0.001). Conclusion: Increasing the use of NAC in early-stage breast cancer is likely to increase the recommendation for axillary dissection, especially for HR+ tumors, which are less likely to respond. Therefore, the timing of chemotherapy utilization in early-stage breast cancers should be cautiously considered.

P5-08-02: Baseline dietary patterns among patients with early-stage breast cancer in the Healthy Living Program

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Background: Dietary patterns have been shown to impact breast cancer (BC) outcomes, including recurrence, survival, and quality of life. Clinical guidelines recommend a diet rich in fruits, vegetables, whole grains, and fiber with limited added sugar. The Healthy Living Program (HLP) at Memorial Sloan Kettering Cancer Center is a clinical initiative for patients with early-stage BC that provides individualized lifestyle optimization from the time of diagnosis. Here, we report baseline dietary patterns among the HLP cohort and associations with body mass index (BMI) and clinicopathologic variables.
Methods: Patients with stage 0-II BC enrolled in the HLP from September 2020 to August 2023 were included. Upon diagnosis, patients complete a survey that incorporates the National Cancer Institute Dietary Screener Questionnaire, which assesses consumption frequency of 26 food items in the prior month. Nutrient and food group intake estimates were calculated using Diet*Calc software. Adherence to guideline recommendations was assessed for each food group as follows: fruits/vegetables (3.5 cup equivalents [equiv] for females, 4.5 cup equiv for males), whole grains (3 ounce equiv), fiber (30 g), total added sugars (≤6 tsp for females, ≤9 tsp for males), and sugar-sweetened beverages (0 tsp equiv). BMI, race/ethnicity, tumor stage, and receptor status were extracted from electronic medical records. Univariable and multivariable regressions were performed to detect

associations between clinical variables and dietary patterns.
Results: A total of 1358 participants were evaluable (median age 59 years, interquartile range 49-68); 9 were male (<1%). 880 patients were White (65%), 191 were Asian (14%), 147 were Black/African American (11%), and 142 were Hispanic/Latinx (10%). BMI categorization was as follows: 372 (28%) had overweight BMI (25-29.9 kg/m2) and 389 (29%) were obese (BMI ≥30 kg/m2). 182 (13%) had carcinoma in situ, 983 (72%) had stage I BC, and 193 (14%) had stage II BC.
Median daily intakes were 2.56 cup equiv of fruits/vegetables, 0.61 oz equiv of whole grains, 15.41 g of fiber, 11.68 tsp equiv of added sugar, and 3.62 tsp equiv of sugar from sweetened beverages. 201 participants (15%) met the guidelines of fruits/vegetables intake. 0 met the guidelines for whole grains, fiber, total added sugar, and sugar from sweetened beverages intake.
Elevated BMI was associated with lower intake of fruits/vegetables ($p<0.001$), whole grains ($p<0.001$), and fiber ($p<0.001$) and higher intake of sugar from sweetened beverages ($p=0.01$). Compared to non-Hispanic Whites, Black/African American race and Hispanic/Latinx ethnicity were associated with lower intake of fruits/vegetables ($p=0.029$, $p<0.001$) and fiber ($p<0.001$ for both), and higher intake of sugar from sweetened beverages ($p<0.001$ for both). Hispanic/Latinx ethnicity was additionally associated with lower whole grain intake ($p<0.001$) and higher added sugar intake ($p=0.002$). Age ≥50 years was associated with lower added sugar ($p<0.001$) and sugar from sweetened beverages intake ($p<0.001$) compared to age <50. There were no significant associations between tumor stage or receptor status and fruits/vegetables, whole grains, or fiber intake. Triple negative BC was associated with lower added sugar intake compared to hormone-positive disease ($p=0.016$).
Conclusion: Few patients with early-stage BC met the recommended daily intake of fruits/vegetables, and none met the recommended daily intake of whole grains, fiber, and added sugar. Elevated BMI was associated with lower adherence to dietary guidelines. These findings highlight the value of dietary assessment at the time of BC diagnosis to identify patients for targeted dietary interventions and metabolic risk profile optimization.

P5-08-03: Significant overall survival (OS) benefit among patients with neoadjuvant chemotherapy use in T1c N0 triple negative and HER2 positive early breast cancer: a large National Cancer Database (NCDB) analysis.

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Purpose: In the late 1990s, an OS benefit with use of neoadjuvant chemotherapy (NAC) over adjuvant chemotherapy (AC) had not been identified in NSABP B18, and NAC was primarily used for surgical down-staging. However, an important correlation was noted between surgical residual disease and poorer survival rates. [1] After 2017, the CREATE-X and KATHERINE trials each showed an OS benefit in triple negative (TNBC) and HER2+ breast

cancers, respectively, when surgical residual disease after NAC was used to guide enhanced post-operative systemic therapy.[2,3] This has since made NAC the standard of care for T2 or N1 tumors.[4] However, T1c N0 tumors were largely underrepresented in these studies, leading to uncertainty about the true benefit and therefore variability in NAC use in this subset of patients. This study aims to review the overall trend of NAC use in T1c N0 TNBC and HER2+ tumors, with assessment of survival rate differences compared to AC use. We will also focus on how treatment facility and social determinants of health may be affecting these trends.

Methods: Using the NCDB between 2004 and 2021, we identified patients 18 or older with T1c N0 TNBC and HER2+ early breast cancer who underwent both surgery and chemotherapy. Categorical variables were assessed using Pearson's chi-square test, whereas continuous variables were analyzed using independent t-tests. Statistical significance was set at $p < 0.05$.

Results: 47,668 patients with T1c N0 HER2+/TNBC who received chemotherapy were included.

OS rates were higher in the NAC cohort compared to AC cohort for the entire 2004-2021 study period (91.8% vs. 87.7%, $p <0.001$). Median follow up times were 55 months for the NAC cohort and 79 months for the AC cohort. An OS benefit was maintained even when we analyzed by joint follow up time of ≥ 55 months (95.5% vs 92.5%, $p < 0.001$).

In the overall period of 2004-2021, 22.6% of the study population received NAC. There was an increasing trend in overall NAC utilization overtime, with the highest proportion being in the 2016-2021 period (69.5% compared to 39.4% in AC cohort) ($p < 0.001$). The rate of NAC use in T1c N0 patients increased from 8.3% in 2006 to 46.8% in 2021 ($p <0.001$). Patients who received NAC versus AC were overall younger (54.7 vs. 58.8 years), more likely to have private insurance (66.3% vs. 57.8%), more like to have median incomes in the top quartile (41.0% vs. 38.8%), were predominantly treated in academic facilities (56.9% vs. 50.7%),and were located in metropolitan areas (88.0% vs. 85.9%) ($p < 0.001$ for all).

Conclusion: Our study shows that there is an OS benefit with NAC over AC in T1c N0 patients with TNBC or HER+ early breast cancer. NAC utilization in this group has markedly increased overtime, particularly after 2016, marked by the impact of the CREATE-X and KATHERINE trials, both of which showed an overall OS benefit from the addition of enhanced post-operative systemic therapy for patients with surgical residual disease. This OS benefit can now be confirmed for even smaller T1c N0 tumors. Social determinants of health and treatment facility type can have an impact on the overall accessibility and use of NAC for T1c N0 tumors, highlighting the importance of bridging these gaps.

1. Fisher B, et al. J Clin Oncol. 1997;15(7):2483-2493. (NSABP Protocol B-18)
2. Masuda N, et al. N Engl J Med. 2017;376(22):2147-2159. (CREATE-X trial)
3. Loibl S, et al. 2023 SABCS. Abstract GS03-12. Presented Dec 8, 2023. (KATHERINE trial)
4. NCCN Clinical Practice Guidelines in Oncology: Breast Cancer. 2024. Retrieved from NCCN website.

P5-08-04: The Omission of Anthracycline Chemotherapy in Women with Early HER2-Negative Breast Cancer – A Systematic Review and Meta-Analysis

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Background: Anthracycline-taxane is the standard chemotherapy strategy for treating high-risk early breast cancer, despite the potential life-threatening adverse events caused by anthracyclines. In many cases, the combination docetaxel and cyclophosphamide (TC) is considered an alternative treatment option in select breast cancer populations. However, the efficacy of TC compared to anthracycline-taxane chemotherapy is unclear. This study aims to compare disease-free survival (DFS), overall survival (OS) and cardiotoxicity between adjuvant TC and anthracycline-taxane for patients with stage I-III, HER2-negative breast cancer. Methods: We conducted a systematic literature search on MEDLINE, Embase, Cochrane CENTRAL for trials published until March 11, 2024. Our search yielded 203 studies with 11,803 patients and 7 randomized-controlled trials (RCTs) were included, with a median follow up of 60 months. This research protocol was registered with PROSPERO. The Risk of bias (RoB) was assessed independently by two reviewers, using Cochrane RoB 2.0 tool for RCTs. Disagreements about study inclusion were resolved through discussion. Results: With a total of 11,803 participants, there were 725 DFS events in the anthracycline-taxane arm and 785 in the TC arm. TC results in little to no difference in DFS (HR 1.09, 95% CI 0.98 – 1.20; moderate-certainty of evidence). There were 411 and 400 death events in the TC and in anthracycline-taxane chemotherapy groups, respectively; OS (1.02, 95% CI 0.89 – 1.16; high-certainty of evidence); From the total of 9,732 patients who were treated and analyzed for cardiotoxicity, there were 13 events of cardiac-related side effects. Among those, 3 events were seen in the TC group and 10 in the anthracycline arm (RR 0.54, 95% CI 0.16 – 1.76; high-certainty of evidence). In all the study endpoints, DFS, OS and cardiotoxicity, the heterogeneity across all studies was likely not more than what is due to chance (I2 = 0%). In the subgroup analysis, there were 9,052 patients who had luminal ER positive breast cancer subtype, and 2,723 patients who had TNBC. There was no significant difference in DFS between TC and anthracycline-taxane in the subgroup analysis of the ER positive (HR 1.05, 95% CI 0.92 – 1.19; p = 0.49) and TNBC (HR 1.16, 95% CI 0.96 – 1.39; p = 0.12). The subgroup analysis based on lymph node (LN) status, in those who had LN negative there was no difference in DFS between anthracycline-taxane and TC chemotherapy (HR 1.06, 95% CI 0.87 – 1.28; p = 0.58). However, in patients with four or more lymph nodes, anthracycline-taxane was in fact, associated with better DFS, compared to TC (HR 1.48, 95% CI 104 – 2.12; p = 0.03). The OS analysis of LN positive, showed no benefit of anthracycline-taxane over TC in women with LN positive disease (HR 1.08, 95% CI 0.88 – 1.33; p = 0.47). Conclusions: There is high-certainty of evidence that there is no difference between TC and anthracycline-taxane chemotherapy in OS and cardiotoxicity. There is moderate-certainty of evidence that there is no difference between TC and anthracycline-taxane chemotherapy in DFS. In women with four or more positive lymph nodes, anthracycline-taxane was associated with a substantial reduction in relapse

events, compared to TC. Our study supports the current standard of practice, which is to use anthracycline-taxane regimens, and TC chemotherapy as a reasonable chemotherapy option in select cases.

P5-08-05: Impact of Inflammatory Breast Cancer on Survival Outcomes in Metastatic Breast Cancer Patients Treated with Topoisomerase 1 based Antibody-Drug Conjugates (ADCs)

Akshara Singareeka Raghavendra, Zhongya Wang, Roland Bassett Jr, Senthil Damodaran, Debu Tripathy

Background: Sacituzumab govitecan (SG) is an ADC targeting TROP2, coupled to SN-38, the active metabolite of irinotecan. SG is currently approved for metastatic triple negative breast cancer (BC) and hormone receptor (HR) positive metastatic BC after progression on prior therapies. Similarly, trastuzumab deruxtecan (T-DXd), an HER2 targeting ADC linked to topoisomerase I inhibitor deruxtecan is approved for HER2 low and HER2-positive BC. While both SG and T-DXd has significantly improved outcomes in metastatic BC patients, their activity in patients with inflammatory breast cancer (IBC) has not been well described. Here we aim to evaluate the impact of SG and T-DXd on survival outcomes in metastatic IBC compared to metastatic BC patients without inflammatory breast cancer (IBC).
Methods: We utilized Kaplan-Meier estimates to analyze progression-free survival (PFS) and overall survival (OS). Differences in survival curves between IBC and non-IBC patients were assessed using the log-rank test. Cox proportional hazards models evaluated associations between survival outcomes.
Results: The analysis included 834 breast cancer patients, of whom 76 (9%) had IBC. The median age at the start of treatment was 51 years (range: 20-85). The median PFS for the entire cohort was 6.4 months (95% CI: 6.0-6.9). The median OS for the entire cohort was 72.7 months (95% CI: 66.0-92.4). Half of the patients (415 patients) received SG as their first ADC treatment. IBC patients were more likely to be younger, with 25% aged 18-40 compared to 22% of non-IBC patients. More IBC patients had received greater than 1 prior line of therapy compared to non-IBC. Both groups had similar proportions receiving SG (49% for IBC patients vs. 50% for non-IBC patients) and T-DXd (51% vs. 50%). HR positivity was less frequent in IBC patients (30% vs. 41% for non-IBC patients), and a higher percentage of IBC cases were HER2-positive (32%) compared to non-IBC (25%). Disease progression or death occurred in 70% of IBC patients versus 69% of non-IBC patients. The median follow-up time was shorter for IBC patients (22.1 months) compared to non-IBC patients (28.4 months). The median PFS for the entire cohort was 6.4 months (95% CI: 6.0-6.9). There was no significant difference in PFS between IBC and non-IBC patients (p=0.85), with IBC patients having a median PFS of 6.3 months (95% CI: 4.7-7.7) compared to 6.5 months (95% CI: 6.0-7.3) for non-IBC patients. The hazard ratio for PFS was 1.03 (95% CI: 0.77-1.36). However, there was a significant difference in OS between IBC and non-IBC patients (p=0.006), with IBC patients having a median OS of 46.7 months

(95% CI: 34.1-Not estimable) compared to 74.6 months (95% CI: 67.8-95.2) for non-IBC patients. The hazard ratio for OS was 1.68 (95% CI: 1.16-2.43). 42% of IBC patients had died compared to 32% of non-IBC patients. There was no significant association between IBC and the clinical benefit rate (CBR), with a Fisher's exact test p-value of 0.37.
Conclusions: Despite similar initial treatments and best response rates to ADCs, IBC patients exhibited inferior survival compared to non-IBC patients, with a median OS of 46.7 months versus 74.6 months. The PFS was similar between the two groups, indicating that IBC status did not significantly affect the time to disease progression. Further research is warranted to understand the higher lethality of IBC and explore optimal and tailored treatment strategies to improve survival rates for IBC patients.

P5-08-06: Adjuvant capecitabine in combination with docetaxel and cyclophosphamide (TCX) versus anthracycline plus docetaxel & cyclophosphamide regimen (TAC) in women with high-risk, Her2-negative breast cancer: an open-label, randomized controlled trial

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Abstract: Background: The combination of anthracyclines and taxanes has been the standard regimen for the adjuvant treatment of early-stage, high-risk breast cancer. However, concerns exist regarding potential reduced efficacy in human epidermal growth factor receptor 2 (Her2)-negative patients due to co-amplification of topoisomerase IIα (TOPO IIα), the target of anthracyclines, and HER2 at the adjacent chromosomal loci (17q21.1 and 17q21.2, respectively). Some trials, eg. the West German Study Group PlanB trial, showed anthracycline-free chemotherapy (six cycles of docetaxel and cyclophosphamide [TC]) similar survival outcomes with standard anthracyclines plus taxanes regimens in Her2-negative high-risk early breast cancer patients. Meanwhile, some studies (such as FinXX, USO NO17629, ABCSG-24) suggest improved outcomes with capecitabine in HER2-negative patients. This rationale underpins our investigation comparing TAC (docetaxel, anthracyclines, and cyclophosphamide) to a novel regimen, TCX (docetaxel, cyclophosphamide followed by capecitabine), hypothesizing that replacing the anthracycline component with capecitabine (regimen X) might offer superior efficacy and potentially a more favorable toxicity profile in HER2-negative early-stage breast cancer.
Methods: This was an open-label, randomized, and controlled prospective trial. The study population enrolled patients with pT1 to pT3, node-positive, or node-negative high-risk Her2-negative early-stage breast cancer, between May 2011 and December 2013. Patients were assigned randomly as 2:1 to either TAC (docetaxel 75mg/m2, doxorubicin 50mg/m2 or epirubicin 75 mg/m2, plus cyclophosphamide 500 mg/ m2, cycled every 21 days for 6 cycles, with prophylactic administration of human granulocyte-colony stimulating factor [G-CSF]) or TCX (docetaxel 75 mg/m2, plus cyclophosphamide 500 mg/m2, followed by capecitabine 950 mg/m2, po, twice daily on days 1–14, cycled every 21 days for 6 cycles) regimens. Patients received radiotherapy or endocrine therapy after completion of

chemotherapy when clinically indicated. The primary endpoint was disease-free survival (DFS), defined as the time from surgery to locoregional or distant recurrence, second primary malignancy, or death. Secondary endpoints included distant disease-free survival (DDFS), overall survival (OS), disease-specific survival (DSS), and adverse event rate. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v3.0. This trial was registered in Clinicaltrials.gov (NCT01354522).

Results: A total of 204 patients were enrolled in this trial, with 136 patients in the TAC group and 68 patients in the TCX group (Table 1). The median age was 47 years for the TCX group and 48 years for the TAC group. The baseline characteristics were well-balanced between the two groups.

During a median follow-up time interval of 124 months, a total of 48 DFS events (35.29%) in the TAC group and 20 DFS events (29.41%) in the TCX group were observed (p=0.434, Table 2). There was no significant difference in 10-year DFS (TAC67.6±4.0% vs TCX 71.5±5.6%, p=0.477). There were significantly less distant metastases (TAC 29.41% vs. TCX 8.09%, p=0.040) and death event rates (TAC 26.47% vs. TCX 4.41%, p=0.003) in the TCX group. A tendency for statistical differences favoring the TCX group was observed in the 10-year DDFS (TAC 69.5±4.0% vs TCX 81.4±4.9%, p=0.054, Figure 1). The 10-year OS rate was significantly higher in the TCX group (91.0±3.5%) compared to the TAC group (77.2±3.6%, p=0.009). The 10-year disease-specific survival (DSS) rate was also significantly higher in the TCX group (92.5±3.2%) compared to the TAC group (78.5±3.5%, p=0.007).

Statistical differences were observed in the incidence of adverse events between the two groups ($p<0.05$, Table 3). The TAC group exhibited a higher frequency of infection (35.29% vs 14.71%), nausea (93.38% vs 25%), peripheral edema (16.18% vs 2.94%), and fatigue (93.38% vs 54.41%), while the TCX group exhibited a higher frequency of hand-foot syndrome (20.59% vs 86.76%) and diarrhea (17.65% vs 39.71%). The TAC group was prophylactically administrated G-CFS, and there was no significant difference in the incidence of neutropenia (TAC 69.82% vs TCX 60.29%, p =0.658) between the two groups.

Conclusions: TCX may be a promising option for high-risk HER2-negative breast cancer due to its potential for improved survival and tolerability compared to TAC. Further multi-center trials are needed to confirm the efficacy of TCX.

Figure 1. The Kaplan-Meier curves for 10-year survival analyses of breast cancer patients treated with TAC (n=136) and TCX (n=68) regimens. A) No significant difference in 10-year disease-free survival was observed between the TAC and TCX groups (p=0.477). B) The 10-year overall survival was significantly better in the TCX group compared to the TAC group (p=0.009). C) The TCX group showed a trend towards improved 10-year disease-free disease-specific survival compared to the TAC group (p=0.054). D) The 10-year disease-specific survival was significantly better in the TCX group compared to the TAC group (p=0.007). Abbreviations: TAC, docetaxel, anthracyclines, and cyclophosphamide regimen; TCX, docetaxel, cyclophosphamide followed by capecitabine regimen.

Table 1. The clinical and pathological characteristics of the TAC and TCX cohorts.

Table 2. Events in the TCX and TAC cohorts.

Table 3. Treatment-emergent adverse events during treatment.(Figures and Tables are to be uploaded)

P5-08-07: Survival Outcomes of Sequential Topoisomerase I Antibody-Drug Conjugate Therapies in Metastatic Breast Cancer Patients

Akshara Singareeka Raghavendra, Zhongya Wang, Roland Bassett Jr, Debu Tripathy, Senthil Damodaran

Background: Trastuzumab deruxtecan (T-DXd) and sacituzumab govitecan (SG) are ADCs with topoisomerase 1 payloads targeting HER2 and TROP2, respectively, that are approved for use in metastatic breast cancers (MBC). SG is approved for patients with triple negative and hormone receptor positive MBC, while T-DXd is approved for patients with HER2 low (IHC 1+ or IHC 2+, FISH negative) MBC. As such there will be a population of MBC patients that will meet eligibility for both SG and T-DXd. In this study we assessed clinical outcomes in patients treated with these ADCs, examining differences between sequential and non-sequential treatment approaches in a large cohort of MBC patients.
Methods: Progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method, and differences in survival curves between ADC treatment sequence (SG followed by T-DXd or T-DXd followed by SG) and between sequential or non-sequential ADC treatment were assessed using the log-rank test. Cox proportional hazards regression models were employed to evaluate the association between OS and PFS
Results: The analysis included a total of 125 breast cancer patients, with 67 (54%) receiving SG followed by T-DXd and 58 (46%) receiving T-DXd followed by SG. The median age at the start of ADC treatment was 52 years (range: 25–82 years). The median follow-up time for all patients was 13.9 months (range: 1.9–37.3 months). Among the study population, 35 (28%) had died, while 90 (72%) were still alive at their last follow-up. The majority of the patients (73%) received sequential ADC treatment. Patients in the SG -T-DXd group had more extensive prior treatment histories, with 55% having received three or more prior lines of treatment compared to 36% in the T-DXd - SG group. The T-DXd - SG group had a higher proportion of hormone receptor-positive patients (64%) compared to the SC - T-DXd group (18%). For PFS, no significant differences between the two ADC treatments sequence (T-DXd – SG vs. SG – T-DXd (HR: 1.30 (95% CI: 0.86 – 1.97), p = 0.52) or between sequential and non-sequential treatments (Hazard Ratio: 0.81 (95% CI: 0.53 – 1.24), p = 0.50) was observed. Median PFS was as follows: SG – T-DXd: 2.83 months (95% CI: 2.40 – 3.88); T-DXd – SG: 2.76 months (95% CI: 2.30 – 3.94); sequential: 2.66 months (95% CI: 2.30 – 3.94); non-sequential: 2.92 months (95% CI: 2.40 – 3.71). The median OS for all patients was 25.7 months. There was no significant difference in OS between the two ADC treatment sequence (T-DXd – SG vs. SG - T-DXd (HR: 1.34 (95% CI: 0.64 – 2.80), p=0.43) or between sequential and non-sequential treatments (HR: 1.22 (95% CI: 0.60 – 2.55), p=0.60). Median OS was as follows: SG – T-DXd: 25.7 months (95% CI: 20.3 – Not Estimable); T-DXd – SG: Not Estimable (95% CI: 16.5 – Not Estimable); sequential: 22.6 months (95% CI: 20.2 – Not Estimable); non-sequential: 25.7 months (95% CI: 21.3 – Not Estimable)

Conclusions: Our analysis indicates no clear survival differences between SG followed by T-DXd vs T-DXd followed by SG and whether these ADCs were used sequentially or non-sequentially. Further studies with rational biomarker analyses with larger sample sizes is warranted to draw more definitive conclusions.

P5-08-08: Predicting local recurrence in DCIS treated predominantly without radiation

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Problem statement: Approximately 14% of breast cancer patients are now diagnosed with ductal carcinoma in situ (DCIS). Predicting the prognosis of DCIS is imprecise, leading to substantial variability in treatment. Utilising a large patient cohort with conservative use of radiotherapy, we aim to identify markers of risk of relapse, and the nature of the relapses.
Methods: A cohort study including patients diagnosed at and/or managed by North Western BreastScreen and the Parkville Breast Service in Victoria diagnosed during 1994-2018. Demographic, treatment, pathology, and outcome data were extracted from medical records and via a linkage with the Victorian Cancer Registry and National Death Registry. Histopathological features of the index lesion were compared to subsequent ipsilateral breast cancer events (IBE).
Results: 1163 DCIS cases were identified: median age at diagnosis of 58 years and median follow-up was 11 years. Most patients were diagnosed through BreastScreen (n=1090, 94%). 565 (49%) were high-grade, 415 (36%) intermediate-grade and 183 (16%) low-grade, with the median size being 13mm. Most patients had wide excision (WE) (n= 1051, 90%) with 107 (9%) having mastectomy. 355/1051 (34%) were treated with radiotherapy after WE and 407/1163 (35%) with endocrine therapy. IBE occurred in 215 (18%) of patients: invasive cancer in 114 (10%) and DCIS in 101 (9%). There was a 1.6% incidence of breast cancer death at median of 11 years. No significant association between grade, size, margins or age and IBE was found. On multivariable analysis, radiotherapy was associated with a significantly lower rate of IBE (HR: 0.39, p: <0.0001). The level of radiotherapy benefit was the same for all grades of DCIS. On multivariable analysis, estrogen receptor (ER) positive status was associated with an increased invasive IBE rate (HR: 2.06, p: 0.0470). Conversely, progesterone receptor (PR) positive status was associated with a decreased invasive IBE rate (HR: 0.43, p: 0.003). Most invasive IBE following high-grade index DCIS were grade 2 or 3 (50/67, 75%) while those following low-grade DCIS were grade 1 or 2 (14/15, 93%). Most ER+ve DCIS recurred as ER+ invasive IBE (n=53/58, 91%) and ER-ve DCIS recurred as ER- invasive IBE (n=10/16, 63%). Two-thirds of PR+ve DCIS recurred as PR+ invasive IBE (n=29/43, 67%) and just over half of PR-ve DCIS recurred as PR-ve invasive IBE (n=17/31, 55%). HER2+ DCIS usually recurred as HER2+ invasive IBE (n=7/11, 64%) and most HER2- DCIS recurred as HER 2- invasive IBE (n=27/31, 87%). Of invasive IBEs with full phenotype data available (n=98), 56/98 (57.2%) were luminal A (ER+,PR+, HER2-), 17/98 (17.3%) were luminal B (ER+, PR-, HER2+:any), 12/98 (12.2%)

were HER 2+ (ER-, PR- and HER2+) and 7/98 (7.1%) were triple negative (ER-, PR- and HER2-). This matched the phenotype of the index DCIS in 23/53 cases (43.4%) where full information was available.
Conclusion: Invasive IBE is common after WE for DCIS with an association between the grade and phenotype of index DCIS and recurrence. Standard clinicopathological factors were not associated with IBE risk. Further advancement in DCIS patient stratification is required to ensure treatment is personalised and overtreatment is reduced.

P5-08-09: Validation of the Updated Breast Graded Prognostic Assessment (Breast GPA) for Stereotactic Radiosurgery (SRS) Treated Patients

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Background: Updated Breast Graded Prognostic Assessment (BGPA, Sperduto et al., 2020), a common prognostic index (PI) for overall survival (OS) among patients (pts) with breast cancer brain metastasis (BCBM), was developed from a dataset where only 1/3 received stereotactic radiosurgery (SRS) without whole brain radiotherapy (WBRT). However, in the last decade, SRS has largely replaced WBRT as preferred local therapy for BM, especially for fewer BM (</=4). There is a lack of external validation of BGPA in a SRS treated BM population. Objective of this study is to validate BGPA in SYBRA dataset (BCBM SRS database from University of Rochester) and explore additional variables that might improve PI in SRS population.

Methods: SYBRA includes pts with BCBM who have received their 1st or only SRS to brain between Jan 2010- June 2023. Baseline characteristics and proportion of pts in 4 GPA score categories (GPA cats) were compared between BGPA vs SYBRA with Chi square test. Following statistical approaches outlined by Royston and Altman (BMC Med Res Methodol, 2013) for external validation, the model fit, discrimination, and calibration of BGPA index was evaluated in SYBRA. Cox proportional hazards linear predictor (LP) from BGPA was calculated in SYBRA and its calibration slope estimated and evaluated. Original model predictors were re-estimated in SYBRA with LP offset to assess misspecification of fit (Global Chi Square test). Harrell's C-index was estimated and compared with the same index reported in the original paper. Kaplan-Meier curves and hazard ratios (HR) across GPA cats were estimated. Finally, two new predictors, timing of BM and lines or prior systemic therapy, were added to original model and tested for significance.

Results: Sample size in BGPA and SYBRA were 2473 vs 108; median OS (IQR) in months were 16 (7-34) vs 24 (8-45). % of pts in BGPA vs SYBRA for different variables: age in years <46 25 vs 14 (p=0.013); luminal A BC 31 vs 44 (p=0.076); >=10 BM 20 vs 8 (p=0.064); KPS=90 29 vs 35 (p=0.0085). There was insufficient evidence that distribution of pts in GPA

cats in SYBRA vs BGPA was different (p=0.22). Calibration slope of LP in SYBRA was 0.964 [Standard Error (SE)=0.211, 95% Confidence Interval (CI) 0.551-1.377] indicating strong evidence of validation (p<0.0001 for test of the null hypothesis that slope=0) & excellent calibration (p=0.87 for test of null hypothesis that calibration slope=1). Model misspecification assessment showed no overall evidence of misspecification of LP in SYBRA (p=0.54). Harrell's C-index was 0.642 (SE=0.035), similar to BGPA (c=0.648), indicating moderately good discrimination. SYBRA survival curves for GPA cats were well separated (p=0.0035, Log Rank) and similar to those reported in BGPA, except for highest GPA cat in SYBRA (n=7). Median OS for GPA cats 0.0-1.0, 1.5-2.0, 2.5-3.0 and 3.5-4.0 were 7.5, 22.0, 41.4 and 20.7, respectively. When comparing HR across GPA cats 1.5-2.0, 2.5-3.0 and 3.5-4.0 (vs. GPA 0.0-1.0) there was trend of incremental improvement in the risk of death [HR (95% CI) 0.639 (0.379 – 1.075), p=0.0913; 0.279 (0.140- 0.557), p=0.0003; 0.387 (0.145 – 1.036) p= 0.0587]. Lastly, in separate Cox models assessing the contribution of 2 novel variables with BGPA LP as an offset, recurred BM (vs de novo) was associated with a higher risk of death [HR=2.22 (95% CI: 1.37-3.60), p=0.0012]; prior lines of systemic therapy at BM diagnosis (>/=2 vs 0-1) was also associated with higher risk of death [HR=2.23 (95% CI: 1.41-3.52), p=0.0006].

Conclusion: This study suggests validity and excellent calibration of BGPA model in BCBM pts who have received SRS without WBRT. Adding BM timing (de novo vs recurred) or lines of prior systemic therapies for metastatic BC as a predictor may improve BGPA model in SRS population.

P5-08-11: Comprehensive characterization of the immune landscape in HER2-low and HER2-0 hormone receptor positive tumors

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Background: Targeting the spectrum of human epidermal growth factor receptor 2 (HER2) expression seen across breast cancer subtypes with the anti-HER2-drug conjugate (ADC) trastuzumab deruxtecan (T-DXd) has significantly impacted breast oncology, shifting the paradigm of anti-HER2 therapies. Despite the major clinical implications of targetable HER2-low tumors, the fundamental biological differences between HER2-low and HER2-0 breast tumors remain unclear. Here, using molecular profiling and multiplexed imaging techniques, we perform a comprehensive characterization of the immune landscape of HER2-low and HER2-0, hormone receptor positive (HR+) breast tumors.

Methods: We analyzed a cohort of treatment-naïve, HR+, and HER2-negative breast cancer patients obtained from Brigham and Women's Hospital (BWH) and Dana-Farber Cancer institute (DFCI). A total of 26 primary tumor resections were separated into HER2-low

(HER2 1+, 2+/FISH Neg., n=17) and HER2-0 groups (n=9). All patients had estrogen receptor (ER)-positive (91.2% positive cells, ranging from 50 to 100%). The average proportion of ER-positive cells was 90.35% in HER2-low and 91.5% in HER2-0. We performed bulk RNA sequencing (RNAseq), single-nucleus sequencing (snucSeq), and multiplex cyclic immunofluorescence (CyCIF) staining of FFPE sections. Significance was described according to p-value<0.05.

Results Gene set enrichment analysis (GSEA) of bulk RNAseq data revealed that HER2-0 tumors were significantly enriched in genes related to immune pathways such as "adaptive immune response" (padj<0.001) and "positive regulation of immune response" (padj<0.001) compared to HER2-low tumors. SnucSeq was used to investigate the contributions of individual cell types to the differential gene signatures. We observed that HER2-0 epithelial cells expressed gene sets derived from immune signatures such as "inflammatory response" (padj=0.01) and "interferon gamma response" (padj<0.001) while epithelial cells from HER2-low tumors expressed genes linked to cell cycle, metabolism, and estrogen signaling such as "MYC targets" (padj<0.001), "E2F targets" (padj<0.001), "oxidative phosphorylation" (padj<0.001), "glycolysis" (padj<0.001), and "estrogen early (padj<0.001) and late response" (padj<0.001). HER2-0 tumor displayed a greater, but non-significant, amount of immune cell infiltration compared to HER2-low tumors (23.4% vs 12.92% (p=0.12)). Accordingly, a non-significant increase in immune infiltration into HER2-0 tumors compared to HER2-low tumors was also observed using CyCIF on FFPE tissue sections (23.96% vs 17.57%, p=0.25). However, unsupervised analysis of individual lymphocyte and myeloid cell clusters within the snucSeq dataset revealed significant differences in immune population frequency between HER2-low and HER2-0 tumors. Amongst the differences we identified plasmacytoid dendritic cells (1.1% vs 0.5%, p=0.03) and DOCK4+ CD4+ T cells (3% vs 0.8%, p=0.01) to be significantly more abundant in HER2-0 tumors. Additionally, the gene set analysis of TAMs from HER2-0 vs HER2-low indicated an enrichment of immune pathways such as "positive regulation of immune response" (padj=0.001) and "lymphocyte activation" (padj=0.001) in the HER2-0 tumors.

Conclusions Our findings highlight subtle yet potentially relevant immunological differences between untreated HER2-low and HER2-0 HR+ tumors. Although there was a similar overall immune landscape, our data reveal notable discrepancies in biological pathways. HER2-0 tumors are marked by the presence of inflammatory and immune signatures, indicating an ongoing low-grade inflammatory response. Conversely, HER2-low tumors are characterized by elevated activity of cell cycle, metabolic, and estrogen signaling pathways suggesting a more active cellular proliferative program and luminal phenotype. The biological significance of these findings is currently being investigated.

Ethics Approval The ethics approval for this study was obtained from the Dana-Farber Cancer Institute, Boston, MA, under the ethics committee number 93-085.

P5-08-12: Association of Baseline Global Inflammation Score with Clinical Outcomes: Secondary Analysis from Alliance A011502

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Background: A011502 is a phase 3 randomized double-blind clinical trial that randomized high risk nonmetastatic breast cancer (BC) patients (pts) to aspirin 300 mg daily vs placebo. We previously reported on the association of lifestyle factors (linked with inflammation) with clinical outcomes and found that high perceived stress scale (PSS) scores and moderate/severe pain assessed with Brief Pain Inventory (BPI) were significantly associated with worse invasive disease free (iDFS) and overall survival (OS). We hypothesized that a global inflammation score encompassing lifestyle measures associated with inflammation (stress, poor sleep quality, depression, pain, being overweight/obese) could predict a subset of pts who are at the highest risk of worse outcomes.
Methods: 2257 BC pts enrolled on A011502 answered 4 validated questionnaires at baseline that, along with BMI, were used to calculate global inflammation score. These questionnaires included PSS, categorized as low: 0-13 (=0 (these patients were given a score = '0')) or moderate: 14-26 (=0.5) or high stress: 27-40 (=1); BPI, categorized as none: 0/mild: 1-3 (=0) or moderate/severe pain: 4-10 (=1); Pittsburgh Sleep Quality Index: PSQI, categorized as good: 0-5 (=0) or poor sleep quality: 6-10 (=1); Center for Epidemiologic Studies Depression Scale Revised: CESD-R, categorized as no depression (=0) or depression (=1); and BMI, categorized as normal weight = 0; overweight = 0.5; obese = 1. Thus, global inflammation score per pt was obtained by summing the individual scores and ranged from 0 to 5. Association between global inflammation score (both as a continuous and categorical variable as tertiles (T1: 0-1, T2: 1.5-2, T3: 2.5-5)) and iDFS and OS was performed with multivariable Cox models controlling for age, cancer stage, time since diagnosis, race, ethnicity, hormone receptor status and treatment arm.
Results: Median age was 53 (23-69) years, 83.2% White, 69.2% stage II, 89.1% hormone receptor positive; median time from diagnosis to answering questionnaires was 12.9 months. Median follow-up was 35 months. Median global inflammation score was 1.5. Pts with higher global inflammation score (continuous) had worse iDFS (events/total=195/2257), hazard ratio (HR) 1.14 (95% CI: 1.00, 1.30), p=0.04; and worse OS (events/total=89/2257), HR 1.24 (95% CI: 1.04, 1.49), p=0.02. Categorizing global inflammation score into tertiles revealed a non-linear relationship for iDFS: T1 reference, T2 HR 1.09 (95% CI: 0.77, 1.54), and T3 HR 1.44 (95% CI: 1.01, 2.05); and a non-linear relationship for OS: T1 reference, T2 HR 1.59 (95% CI: 0.92, 2.74), and T3 HR 2.04 (95% CI: 1.19, 3.51). Based on this, we dichotomized the global inflammation score as low (score in T1 or T2) and high (score in T3). Pts in high group had worse iDFS (events/total=68/643): HR 1.38 (95% CI: 1.02, 1.86), p=0.04; and worse OS (events/total=35/643): HR 1.59 (95% CI: 1.03, 2.45), p=0.04 compared to low group. There was no difference in iDFS for high or low inflammation group by treatment arm.
Conclusions: Pts with higher global inflammation score at baseline had worse iDFS and OS

in a randomized controlled trial of aspirin vs placebo in high-risk BC survivors. It is possible that some measures may be related to other non-cancer issues (e.g., chronic comorbidities, social determinants of health, endocrine therapy adherence) that were not captured in our study. Moreover, use of aspirin did not influence outcomes regardless of inflammation score. These results highlight the need for clinical trials to consider including measures affiliated with inflammation and BMI. Future studies are warranted to determine if measures that reduce inflammation would improve BC outcomes.
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P5-08-13: Real-world demographics, clinical characteristics, and treatment patterns among US patients with HER2-negative early breast cancer and germline BRCA mutations since 2021

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Background: Patients (pts) with germline BRCA1/2 pathogenic mutations (gBRCAm) and clinically high-risk HER2-negative (HER2−) early breast cancer (eBC) are eligible for adjuvant olaparib. In routine clinical practice, select pts with high-risk HER2− eBC may also receive CDK4/6 inhibitors or immuno-oncology therapies, but there is a lack of knowledge on preference and concurrent or sequential use of available treatments in this population. Here, we describe real-world gBRCA testing rates and treatment patterns prior to and post the U.S. FDA approval of adjuvant olaparib for HER2− eBC.
Methods: Data from US pts ≥18 years of age diagnosed with HER2− eBC (stage I–III) between January 1, 2021, and February 29, 2024, were captured from the nationwide Flatiron Health electronic health record-derived de-identified database. gBRCA testing rates were determined through abstraction from all successful BRCA tests in pt records. Demographics, clinicopathological characteristics, and treatment patterns were described across tumor subtypes (hormone receptor-positive [HR+]/HER2− or triple-negative breast cancer [TNBC]) and gBRCA status. High-risk eBC was defined, adapted from OlympiA criteria, as HR+/HER2− eBC with ≥4 positive lymph nodes (≥pN2), or TNBC with positive axillary node (≥pN1) or with negative axillary node (pN0) and invasive primary tumor size >2 cm (≥pT2).
Results: Of 2373 pts with eBC (HR+/HER2−, n=2040; TNBC, n=333; median age at diagnosis: 64 years), 1147 (48%) received a gBRCA test, with a lower testing rate in pts with HR+/HER2− eBC (n=905/2040, 44%) than in pts with TNBC (n=242/333, 73%). Grouping by year of eBC diagnosis showed consistent testing rates in 2021 vs 2023+ for pts with HR+/HER2− eBC (44 vs 46%) but a decline in pts with TNBC (76 vs 65%). A substantial proportion of pts eligible for gBRCA testing, including pts ≤50 years (n=117/417, 28%), with high-risk HR+/HER2− eBC (n=44/103, 43%) or with high-risk TNBC (n=20/87, 23%) had unknown/untested status.
Among tested pts, gBRCAm prevalence was lower in pts with HR+/HER2− eBC (n=66/905,

7%) than TNBC (n=57/242, 24%). Among pts with HR+/HER2− eBC and known gBRCA status tested for Ki67 and/or a 21-gene recurrence score (RS), a greater proportion of pts with gBRCAm than non-BRCAm had Ki67 ≥20% (86 vs 47%, respectively) and RS ≥26 (56 vs 11%, respectively).
Proportionally fewer pts with HR+/HER2− eBC (n=242/2040; 12%) than TNBC (n=151/333, 45%) received neoadjuvant therapy. Chemotherapy (CT; n=106/242, 44%) and endocrine therapy (n=81/242, 33%) were the most common neoadjuvant therapies for HR+/HER2− eBC. Pembrolizumab (n=92/151, 61%) and CT (n=54/151, 36%) were the most common neoadjuvant therapies for TNBC. A total of 60 pts (2.5%) received adjuvant olaparib, including 18/66 (27%) pts with gBRCAm HR+/HER2− eBC, 27/57 (47%) pts with gBRCAm TNBC, and 15/2250 (<1%) pts (7 HR+/HER2− eBC and 8 TNBC) who had non-gBRCAm/unknown gBRCAm status. Of the 25 pts with HR+/HER2− eBC who received olaparib, 20 (80%) were <pN2 (not high-risk). Of the 35 pts with TNBC who received olaparib, 16 (46%) were pN0 or <pT2 (not high-risk). Of pts with gBRCAm eBC who had received prior CT, 17/42 (40%) with HR+/HER2− eBC and 24/43 (56%) with TNBC received adjuvant olaparib.
Conclusions: Recently updated cancer guidelines endorse gBRCAm testing in all pts ≤65 years with a breast cancer diagnosis, pts >65 years who are candidates for PARP inhibitor therapy, and other high-risk individuals. This real-world analysis of pts diagnosed since 2021 showed that 55% of pts with HR+/HER2− eBC and 27% with TNBC did not receive a gBRCA test, and utilization of adjuvant olaparib was low, particularly among pts with HR+/HER2− eBC. Wider implementation of genetic testing is needed to ensure appropriate utilization of olaparib in eligible pts with eBC.

P5-08-14: Preliminary results of mini-invasive detection of residual disease in breast cancer patients in remission after primary chemotherapy: a further step toward omission of surgery?

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Background: Breast surgery may be overtreatment and can potentially be omitted when systemic neoadjuvant treatment (NAT) results in pathological complete response (pCR) in breast cancer (BC) patients. The accuracy of residual disease detection in patients with clinical/radiological response is increased by minimally invasive preoperative image-guided vacuum-assisted biopsy (VAB). Thus, patients with pCR – as indicated by negative VAB – might be spared surgery if pre-operative VAB findings closely correspond with definitive histology after breast surgery. We evaluated the ability of VAB to detect invasive residual disease in patients with cT1-T2-T3 cN0/N1 BC (either HER2-positive, triple-negative, or Luminal B HER2-positive) and complete clinical/radiological remission after NAT.

Methods: Patients were enrolled from April 2022 to April 2024 in a single-institute, ongoing, prospective pilot study (NCT 05951699). Patients had either complete clinical/radiological response, or residual breast disease (<1 cm) after NAT at imaging (ultrasound, mammography, contrast-enhanced mammography, and MRI). Breast VAB before surgery consisted of image-guided biopsy on the tumor bed, taking 5-10 cores using a 9G needle. The primary outcome was VAB accuracy, assessed by comparing the VAB findings with definitive histology on the surgical specimen. Cases with negative VAB and invasive residual disease at definitive histology were false negatives. Cases with negative VAB and ductal carcinoma in situ at histology were true negatives since no invasive residual disease was found. Cases with positive VAB (invasive or in situ) and negative histology after surgery were considered true positives (VAB had removed all residual disease).
Results: Thirty-nine patients gave their consent and had VAB after NAT. Median age was 53 years (IQR 47-61). Thirteen (33.3%) had HER2-positive disease, 23 (59%) had triple-negative disease, and 3 (7.7%) had Luminal B-HER2-positive disease. VAB failed to identify invasive residual disease in one case (triple-negative). Among true negatives there were 4 cases of ductal carcinoma in situ (3 HER2-positive and 1 triple negative). Furthermore, VAB identified both invasive and in situ residual disease in 3 cases with positive VAB but negative histology after surgery (1 ductal carcinoma in situ in an HER2-positive case, 1 ductal carcinoma in situ in a triple negative case, and 1 invasive disease in a triple negative case). Based on these considerations, VAB accuracy was 97.4% (38/39; 95% CI 92.5-100.0%); specificity was 100% (30/30), and sensitivity was 88.9% (8/9; 95% CI 68.4-100.0%)
Conclusion: These preliminary findings indicate that pre-operative VAB has good accuracy in predicting definitive histological findings in cT1-T2-T3 BC patients with HER2-positive, triple-negative, and Luminal B HER2-positive disease, who have a clinical/radiological response after primary chemotherapy. Although these findings require confirmation in larger studies, they suggest the use of VAB to select patients for prospective trials on the omission of surgery in patients who achieve a good clinical/radiological response after primary chemotherapy.

P5-08-15: Evaluation of efficacy and safety of sequential antibody drug conjugates (ADCs) in human epidermal growth factor 2 (HER2) negative metastatic breast cancer

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Background: Antibody drug conjugates (ADCs) are composed of a monoclonal antibody with a cytotoxic payload designed to deliver targeted therapy with minimal damage to non-cancerous cells.1 Recent trials of ADCs in the metastatic breast cancer (MBC) setting have demonstrated significantly improved clinical outcomes.2-5 Sacituzumab govitecan (SG) and trastuzumab deruxtecan (T-DXd) are FDA approved in patients with hormone receptor positive (HR+), human epidermal growth factor 2 (HER2) negative, HER2-low, and triple

negative MBC. Many of the developed ADCs share similar targets and cytotoxic payloads with similar mechanism of action; little is known regarding acquired resistance and impact on subsequent lines of therapy. There is limited data available assessing the efficacy, safety, and optimal sequencing of ADCs in this setting including patients (pts) with brain metastases (BrM) or leptomeningeal disease (LMD). Retrospective data suggests a reduction in response rates with subsequent ADC treatment in pts with MBC. This retrospective study aims to assess the efficacy, safety, and tolerability of sequential ADCs in MBC inclusive of both intracranial and extracranial disease, and to determine if the efficacy of subsequent cytotoxic chemotherapy may be impacted by ADC use.
Methods: This is a single-center, retrospective, cohort study in pts with HER2 negative or low MBC (immunohistochemistry (IHC) test of 0 (as negative), 1, or 2 and fluorescence in-situ (FISH) negative (as HER2 low) who received T-DXd and/or SG at the Duke Cancer Institute (DCI) from 04/2021 to 10/2023. Eligible pts were ≥ 18 years of age with a diagnosis of HR+, HER2 low, or triple-negative locally advanced or MBC. Adverse events (AEs) were graded using the Common Terminology Criteria for Adverse Events (CTCAE) v5.
Results: A total of 112 patients with HR+/HER2- or triple negative breast cancer (TNBC) who had received T-DXd and/or SG were included. A total of 59 (52.6%) pts had HR+ MBC, and 43 (38.3 %) TNBC. Approximately 22.3% and 2.7% of patients had brain and/or LMD metastases. Pts were divided into three cohorts: pts who received ADCs sequentially (cohort A, n=13), ADC then chemotherapy (CTX) thereafter (did not receive second ADC) (cohort B, n=46), or one line of CTX in between two ADCs (cohort C, n=13). The mPFS (mPFS) in the cohort A was 4.4 months in the SG immediately prior to T-DXd group and 3.0 months for T-DXd immediately prior to SG group. In cohort B, those who received CTX following T-DXd group, the mPFS was 3.1 months. Pts who received CTX following SG, the mPFS for chemotherapy was 2.5 months, whereas in pts who received both ADCs, PFS was 2.1 months with subsequent chemotherapy. In cohort C, the mPFS was 2.1 months for SG following T-DXd and CTX and 3.3 months for T-DXd following SG and CTX. The mPFS for ADC1 was longer than ADC2 regardless of sequence order. The mPFS for first ADC was 5.5 months for SG and 3.4 months for T-DxD. In both groups, those with BrM and/or LMD all demonstrated stable disease with extracranial response after the administration of an ADC. AEs were consistent with real-world data, and
Conclusion: The administration of a second ADC following prior ADC therapy results in a shorter PFS. The efficacy of chemotherapy is impacted with the use of multiple lines of ADC therapy prior to administration. Outcomes for pts with BrM and LMD who receive ADCs do not differ for those without recurrence to the brain. Sequencing of ADCs plays a role in efficacy of treatment and can likely confer a pattern of resistance. Additional prospective data is needed to further assess ideal sequencing.

P5-08-16: Best response to antibody-drug conjugates (ADCs), sacituzumab govitecan and trastuzumab deruxtecan, based on organ site of metastatic breast cancer (MBC) involvement

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Background: Sacituzumab govitecan (SG) is a TROP-2 directed ADC approved in hormone receptor positive (HR+)/HER2- and triple negative (TN) MBC. Trastuzumab deruxtecan (T-DXd) is a HER2 directed ADC approved in HER2+ and HER2 low MBC. TROP-2 and HER2 expression may vary by organ site, which may affect best response to SG and T-DXd in various metastatic sites. We studied best response to SG and T-DXd based on organ site of MBC.
Methods: A retrospective review of patients with MBC treated with SG or T-DXd (as the first ADC) at an academic institution between 6/2020-5/2024 was conducted. Sites of metastases before starting ADC were characterized through review of baseline imaging scans and duration of treatment was determined. Subsequent imaging scans for re-staging until ADC discontinuation were reviewed to determine the best response to ADC for individual organ sites of MBC, which was categorized using RECIST 1.1 definitions for complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Objective response rate (ORR) was defined as the percent of patients with CR and PR. The first organ sites of progression on the ADC were determined.
Results: Thirty-three patients were treated with SG. Median age at MBC diagnosis was 60 years (range 49-68 years). Of these, 61% had TN and 36% had HR+/HER2- MBC. Median number of prior therapies for MBC before SG was 2. Baseline organ site of MBC involvement included lung (82%), bone (79%), lymph node (70%), chest wall (36%), liver (36%), pleural effusion (30%), brain (18%), and soft tissue (12%). Median duration on SG was 4.3 months (range 1.6-9.9 months). Distribution of best response to SG varied by organ site (brain: CR 11%, PR 33%, SD 0%, PD 56%; lymph node: CR 4%, PR 12%, SD 54%, PD 29%; chest wall: CR 0%, PR 20%, SD 60%, PD 20%; lung: CR 4%, PR 28%, SD 52%, PD 16%; pleural effusion: CR 27%, PR 13%, SD 13%, PD 47%; soft tissue: CR 0%, PR 20%, SD 20%, PD 60%; liver: CR 0%, PR 29%, SD 21%, PD 50%; bone: CR 0%, PR 0%, SD 77%, PD 23%). The first organ sites of progression on SG were most commonly lung (33%), liver (21%), bone (18%), lymph node (15%), brain (12%), pleural effusion (12%), and ascites (12%). Forty-nine patients were treated with T-DXd. Median age at MBC diagnosis was 60 years (range 50-68 years). Of these, 65% had HR+/HER2-, 6% had TN, and 27% had HER2+ MBC. Median number of prior therapies for MBC prior to T-DXd was 3. Baseline organ site of MBC involvement included lung (82%), bone (76%), lymph node (59%), liver (51%), brain (31%), chest wall (22%), soft tissue (22%), pleural effusion (15%), and adrenal (2%). Median duration on T-DXd was 5.4 months (range 3.1-7.6 months). Distribution of best response to T-DXd varied by organ site (brain: CR 11%, PR 6%, SD 50%, PD 33%; lymph node: CR 10%, PR 38%, SD 41%, PD 10%; chest wall: CR 8%, PR 8%, SD 58%, PD 25%; lung: CR 2%, PR 5%, SD 85%, PD 7%; pleural effusion: CR 6%, PR 12%, SD 19%, PD 62%; soft tissue: CR 0%, PR 8%, SD 67%, PD 25%; liver: CR 4%, PR 38%, SD 35%, PD 23%; bone: CR

0%, PR 0%, SD 86%, PD 14%; adrenal: CR 0%, PR 0%, SD 100%, PD 0%). The first organ sites of disease progression on T-DXd were most commonly the bone (24%), brain (20%), liver (14%) lymph node (12%), and lung (12%).
Conclusions: Best response to SG and T-DXd varied by organ site. With SG, soft tissue, brain, and liver metastases and pleural effusions had high rates of PD as best response and low ORR was observed in bone, lymph node, chest wall, and soft tissue metastases. With T-DXd, pleural effusions and metastases of brain, soft tissue, and chest wall had high rates of PD as best response, and low ORR was observed in bone, lung, soft tissue, and chest wall metastases. Correlation of site response with tissue biomarker expression and cell-free DNA genomics is ongoing. Further study is merited to understand mechanisms of resistance to ADCs in various metastatic sites.

P5-08-17: Effect of vitamin D supplementation on the pathological complete response to neoadjuvant chemotherapy in women with breast cancer: a randomized clinical trial

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Background: Vitamin D supplementation may be a potential non-toxic and low-cost tool to improve the pathological complete response in breast cancer patients undergoing neoadjuvant chemotherapy.
Objective The study aimed to evaluate the effect of vitamin D supplementation on the pathological complete response (pCR) rate in women with breast cancer (BC) undergoing neoadjuvant chemotherapy (NCT).

Methods: A randomized clinical trial was conducted on 80 women aged ≥45years with a diagnosis of BC, who were eligible for NCT. Women were randomized to one of two groups that received either daily vitamin D supplementation with 2000IU of cholecalciferol (VD, n=40) or placebo (n=40) during 6 months. The primary outcome measure was pCR. Serum 25-hydroxyvitamin-D[25(OH)D] was measured at two time points, after BC diagnosis and at the end of NCT. Clinical, anatomopathological, immunohistochemical, and chemotherapy data were collected. Per-protocol analysis was performed using Student's t-test, chi-square test, ANOVA, and logistic regression (OR,odds ratio).
Results Seventy-five of the 80 randomized women completed chemotherapy and underwent surgery. Mean baseline 25(OH)D values indicated hypovitaminosis D in both groups (VD group:19.6±5.8ng/mL and placebo:21.0±7.9ng/mL,p=0.331). After 6 months of intervention, there was an increase in 25(OH)D values in the VD group compared to the placebo (28.0±8.7 vs 20.2±6.1ng/mL,p=0.032). The pCR rate was higher in women supplemented with vitamin D when compared to the placebo (43.2% vs 23.7%,p=0.036). In adjusted logistic regression, women with 25(OH)D values ≥20ng/mL were more likely to achieve pCR than women with vitamin D deficiency (OR3.65, 95%CI 1.085-12.78,p=0.036).

Conclusion: Women with breast cancer undergoing neoadjuvant chemotherapy who received supplementation with 2000IU of vitamin D were more likely to achieve pathological complete response than women in the placebo group.

Keywords Breast cancer; Vitamin D; Pathological complete response; Neoadjuvant chemotherapy
Trial registration ensaiosclinicos.gov.br, identifier RBR-10k4gqdg.

P5-08-18: Prognostic Value of FDG-PET/CT in Breast Cancer with Unfavorable Special Histology

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Background: Special type breast cancer represents 10% of all breast cancer cases, and it is recommended that the treatment approach be similar to that used for invasive ductal carcinoma. However, the prognostic and predictive factors specific to special type breast cancer have not yet been clearly established. Although the maximum standardized uptake value (SUVmax) on FDG-PET/CT has been identified as a prognostic factor for breast tumors, analyses specific to special type breast cancer remain unreported. This study investigates the prognostic significance of SUV max in special type breast cancer.
Methods: This retrospective cohort study, utilizing hospital database records, evaluated 150 patients with special type breast cancer who underwent curative surgery between February 2006 and December 2019 and had assessable pre-treatment SUVmax values. Since FAVORABLE HISTOLOGIES, as defined in the NCCN Guidelines, are associated with a favorable prognosis, these histologic types were excluded from this study. The cut-off value for SUVmax was determined using ROC curves for recurrence. The prognostic significance of SUVmax for Recurrence-free Survival (RFS) was evaluated using the log-rank test and Cox regression analysis.
Results: Median age was 58.9 years-old (IQR 50.1-69.0), and median follow up was 83.1 months (IQR 39.9-116.3). The most prevalent histological type was invasive lobular carcinoma (39 cases, 26.0%), followed by invasive micropapillary carcinoma (35 cases, 23.3%), microinvasive carcinoma (33 cases, 22.2%). Recurrences and deaths occurred in 15 (10.0%) cases. The cut-off value for SUVmax was determined at 5.4, with 24 cases (16%) ≥5.4 and 126 cases (84%) <5.4. SUVmax ≥5.4 was significantly associated with higher T stage, higher nuclear grade, and adjuvant chemotherapy in Fisher's exact test. The 5-year RFS was 54.4% for SUVmax ≥5.4 and 96.1% SUVmax <5.4 ($p<0.005$). In univariate analysis, T stage, nodal metastasis, SUVmax≥5.4, and adjuvant chemotherapy were identified as potentially associated with RFS. In multivariate analysis, nodal status and SUVmax was significantly factors for RFS (hazard ratio 9.3, 95% confidential interval 2.8-30.8, $p = 0.005$).
Conclusions: High SUVmax was significantly associated with breast cancer recurrence in

patients with special type breast cancer, indicating the potential of SUVmax for prognosis prediction in this patient group.

P5-08-19: A novel formula to improve the accuracy of predicting survival time after recurrent breast cancer

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Background: Recurrent breast cancer (BC) has a poor prognosis and is difficult to cure. The prognosis of each recurrent case also affects the social background and subsequent life plan of each patient. Recent studies have shown that advances in treatment have improved the prognosis after recurrence. To ascertain the survival time after recurrence (STR) is necessary for maintaining and improving QOL and selecting appropriate treatment. We investigated whether certain biomarkers would influence and improve the prognosis, and whether the STR can be predicted using the disease-free interval (DFI) and biomarkers.
Methods: A total of 1,254 cases with recurrent BC from January 2000 to December 2023 were enrolled in this study. The cases were divided into the 2000-2005 group (n=182), 2006-2011 group (n=331), 2012-2017 group (n=369), and 2018-2023 group (n=366). The sites of initial recurrence included bone (22.1%), lymph nodes (22.0%), and lung/pleura (18.7%). The clinicopathological factors examined were nodal status, tumor size, histological grade (HG), ER/PgR (cutoff points; 1%), HER2 (zero, low, and positive), the Ki-67 index value (cutoff points: 15% and 30%), and DFI (cutoff points: 24 months and 60 months). We applied a simple linear regression model to explain the relationship between STR and DFI. The overall survival after recurrence was calculated using the Kaplan-Meier method and analyzed using the log-rank procedure. Multivariate analysis for recurrent factors were performed using the Cox proportional hazard model.
Results:
1. Survival rates after recurrence significantly increased in cases after 2012. Improvement in prognosis was observed in most of the cases, but no improvement was observed in cases with a HER2-0 status and Ki-67 index value<15%.
2. In terms of subtypes and survival, there were significant improvements in survival in cases with Luminal A/B and Luminal/HER2 subtypes. However, the cases with HER2 enriched and triple negative subtypes had no significant improvement.
3. A multivariate analysis revealed that DFI, nodal status, ER, and the Ki-67 index values were significant factors for survival in all cases. However, the significant prognostic values varied among the year of recurrence. The Ki-67 index value was a significant factor in the 2000-2005 group. However, nodal status and HER2 status were significant after 2012.
4. The most significant correlation was found between DFI and STR in deceased patients with a Ki-67 index value>30% ($p<0.001$), followed by HER2-low, N≥4, DFI≤24 months, and ER<1%.
5. We developed a formula to predict the STR based on the following significant factors.

STR (mons)=α0 (39.6) +β0 (0.01) - DFI (mons)+ ∑Fi (αi +βi - DFI)
Without the 3rd term, this formula corresponds to a simple linear regression of STR by DFI, where α0 is the average level of STR and β0 is the sensitivity of STR to DFI. The 3rd term is a factor-dependent adjustment of α0 and β0 . The value Fi (i =1-5) can be either 0 or 1, depending on whether the factor meets the valid criteria or not. Therefore, the adjustment terms (αi+βi ·DFI) are effective only when the corresponding factors (Ki-67>30%, HER2-low, ER<1%, tumor size≤2 cm, and positive nodes: 1-3) are valid (i.e. Fi=1). The results suggest the factor1-3 (Ki-67>30% HER2-low and ER<1%) are significant to decline the level of STR, while factor 1 and 2 (Ki67>30% and HER2-low) significantly increase the sensitivity of STR to DFI.
Conclusion: Prognosis after recurrence clearly improved from 2011, but no significant improvement was observed in the cases with ER<1%, a HER2-0 status, and a Ki-67 index value<15%. In recurrent cases where the patients passed away, there was a strong correlation between DFI and prognosis after recurrence in cases with a Ki-67 index value>30% and a HER2-low status. As a result, we developed a predictive formula for determining the prognosis after recurrence. Further studies will be conducted in actual clinical settings to verify the accuracy of this new formula.

P5-08-20: Comparative study of the gut microbiome of newly diagnosed postmenopausal breast cancer patients and healthy controls, and pilot study on the acceptability of soy milk in the patient group

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Background: It has been reported that the composition of the gut microbiota in postmenopausal non-Hispanic Caucasians differs between breast cancer patients and healthy controls. The composition of the gut microbiota in Japanese breast cancer patients has not been thoroughly investigated, although Japanese dietary habits differ from those in the West, and the incidence of breast cancer is also different.
A case-control study in Japanese subjects reported a lower risk of breast cancer with a higher intake of soy isoflavones and a more frequent intake of Lactobacillus casei strain Shirota. In another nested case-control study, a higher blood concentration of aglycones, metabolites of isoflavones, was associated with a lower risk of developing breast cancer. Soy milk beverages fermented with Lactobacillus casei strain Shirota are expected to promote the metabolism and absorption of isoflavones into aglycones compared to regular soy milk beverages.
This study aims to compare the gut microbiota of postmenopausal, untreated Japanese early breast cancer patients and healthy subjects to determine whether dysbiosis occurs. Furthermore, we aim to investigate the acceptability of continuous consumption of fermented soy milk beverages and compare the gut microbiota composition between patient groups who drink or do not drink fermented soy milk beverages.

Methods: This study consisted of two parts: Study 1 and Study 2. Study 1 is a cross-sectional study. The subjects were 60 postmenopausal women with early-stage breast cancer and 60 healthy subjects. The key eligibility criteria for breast cancer patients were: 1) histologically diagnosed breast cancer, 2) untreated early-stage breast cancer without distant metastasis, 3) planned surgery for breast cancer, and 4) without history of other malignancies. Blood, stool, and urine samples were collected at enrollment. The primary endpoint was the composition of the gut microbiota in breast cancer patients and healthy subjects. Secondary endpoints were hormone-related and immunological biomarkers and a lifestyle questionnaire. Study 2 was a randomized controlled trial. Patients with early-stage breast cancer enrolled in Study 1 were included. The subjects were randomly assigned 1:1 to either an intervention group or a control group, and the intervention group was to drink the fermented soy milk beverage for approximately eight weeks during the perioperative period. The primary endpoints were the acceptability of fermented soy milk beverages and the comparison of gut microbiota composition between the intervention and control groups. Secondary endpoints were the composition of the gut microbiota before and after drinking fermented soy milk beverages, the composition of the gut microbiota before and after surgery, hormone-related and immunological biomarkers, the effect of lifestyle on the composition of the gut microbiota, and the effect of drinking compliance on the composition of the gut microbiota (UMIN000043339). The study began in February 2021, and enrollment was completed.

P5-08-21: Analysis of factors influencing the efficacy of NAC and prognosis between HER2-0 and HER2-low HR negative breast cancer

Jingjing Liu, Yi Zhang, Jin Zhang

Objectives: To explore the differences in clinicopathological characteristics, efficacy of neoadjuvant chemotherapy(NAC) and prognosis,and to analyze the influencing factors of the efficacy of neoadjuvant chemotherapy between HER2-0 and HER2-low HR-negative breast cancer patients, and to further clarify the influencing factors of the prognosis of HER2-0 and HER2-low HR-negative breast cancer patients. It provides a clinical basis for the precise treatment of HER2-0 and HER2-low HR-negative breast cancer patients.

Methods: Retrospective analysis of 319 breast cancer patients with HR-negative/HER2-negative completed neoadjuvant chemotherapy and surgery from August 2014 to August 2018 in Tianjin Medical University Cancer Institute and Hospital. The clinicopathological data of patients were collected, and the efficacy of patients' neoadjuvant chemotherapy was assessed by postoperative pathology. SPSS 26.0 statistical software was used for analysis and processing. Categorical variables described their component ratios, and comparisons between subgroups of different clinicopathological characteristics were performed using the chi-square test. The chi-square test was used for univariate analysis of the relevant factors affecting pathological Complete Response (pCR),followed by multivariate analysis of

the relevant factors affecting pCR using logistic regression model, Kaplan-Meier method was applied to calculate the survival rate and draw the survival curves, and the log-rank The log-rank test was used to compare the differences in survival between groups, and the independent risk factors affecting the Disease Free Survival(DFS) rate and Overall Survival (OS) rate were analyzed by using the COX regression model, and $p < 0.05$ was considered statistically significant.

Results: 1.319 HER2-0 and HER2-low HR-negative breast cancer patients among 207 patients (64.9%) with HER2-low,and the two groups had a statistically significant difference in terms of age at diagnosis(P=0.035),clinical N-stage(P=0.001), histological grading(P<0.001) and Ki67 (P=0.012).
2.319 HR-negative/HER2-negative breast cancer patients,92 patients(28.8%) achieved pathological complete response. pCR rate of HER2-low was 23.2% (48/207), and that of HER2-0 was 38.9% (44/112),with a statistically significant difference (P=0.004). HER2-0, HER2-1+, and HER2-2+/FISH(-) had decreasing pCR rates between them, which was statistically different(38.9% vs. 25% vs. 20%, P=0.012).
3. Univariate analysis of patients in the HER2-0 and HER2-low groups showed that: clinical T stage (P=0.004) and clinical N stage(P=0.046)were associated with pCR rate of HER2-0 HR-negative breast cancer; and clinical T stage(P=0.001) and pathological pattern (P=0.045) were associated with that of HER2-low HR-negative breast cancer; The results of multifactorial analysis of the two groups showed that clinical T-stage(OR=0.229, P=0.009; OR=0.110, P=0.001) and clinical N-stage (OR=0.359, P=0.010; OR=0.188, P=0.034) were the independent influences on pCR HER2-0 HR-negative breast cancer, and clinical T-stage (OR = 0.399, P = 0.022; OR = 0.178, P < 0.001) was an independent influencing factor for that in HER2-low HR-negative breast cancer.
4.The 3-year DFS rate of patients in the HER2-0 and HER2-low groups was 72.3% vs 78.3%, and the 5-year DFS rate was 67.0% vs 76.8%, and there was no statistically significant difference in recurrence/metastasis (P=0.397); and the OS rate of patients in the two groups was 79.5% vs 86.5%, and there was no statistical significance for the difference (P=0.366). HER2-0, HER2-1+ and HER2-2+/FISH(-) patients between the three groups had a 3-year DFS rate of 72.3% vs 78.8% vs 77.3%, and a 5-year DFS rate of 67.0% vs 78.3% vs 74.7%, and there was no statistically significant difference in recurrence/metastasis(P=0.625); and the OS rate of the two groups was 79.5% vs 85.6% vs 88.0%, with no statistically significant difference (P=0.308).
5.The univariate and multifactorial COX regression analyses showed that clinical T3-4 stage(HR=9.971; P=0.002) and post-NAC non-pCR (HR=3.420; P=0.018) were the independent risk factors affecting the DFS rate of patients with HER2-0 HR-negative breast cancers; post-NAC non-pCR (HR=4.693; P=0.038) was an independent risk factor affecting OS rate of them; clinical stage N1 (HR=3.333; P=0.001), N2-3 (HR=5.832; P<0.001), post-NAC non-pCR (HR=3.364; P=0.045), and lymphovascular invasion(HR=1.942; P=0.027) were independent risk factors affecting the DFS rate in HER2-low HR-negative breast cancer patients; clinical stage N2-3(HR=4.307; P=0.001), and lymphovascular invasion (HR=2.373; P=0.017) were independent risk factors affecting the OS rate in them.

6.There were statistical differences in HER2 evolution and histological grading ($p<0.001$)and Ki67 expression ($p=0.026$) after NAC, with significantly better DFS and OS in HER2-gain patients compared to HER2-stable patients, but the worst DFS and OS in HER2-loss patients compared to HER2-stable patients ($p=0.021$; $p= 0.031$).

Conclusions: 1. In HR-negative breast cancer, compared with HER2-0 patients, HER2-low patients were diagnosed at a greater age, had a later clinical N stage, higher Ki-67 expression, but a lower proportion of histological grade III.
2. HER2-low patients had a lower pCR rate than HER2-0 patients, and HER2-low patients with clinical T3-4 stage and clinical T2 stage had a lower pCR rate after neoadjuvant chemotherapy.
3. There was no difference in prognosis between HER2-0 and HER2-low patients in HR-negative breast cancer. Patients with HER2-0 HR-negative breast cancer in clinical stage T3-4 had a higher risk of recurrence/metastasis and those who were non-pCR after NAC had a higher risk of recurrence/metastasis and death. HER2-low HR-negative breast cancer patients with clinical stage N1, clinical stage N2-3, non-pCR after neoadjuvant chemotherapy, and lymphovascular invasion have a higher risk of recurrence/metastasis, and those who were with clinical stage N2-3 and lymphovascular invasion have a higher risk of death.
4. Patients with histological grade III are more likely to develop HER2-loss, and those with Ki-67 > 14% are more likely to develop HER2-gain. DSF and OS are prolonged in patients with HER2-gain compared with HER2-stable, which is more favourable than that in patients with HER2-loss.

P5-08-22: Effectiveness of Online Education in Improving Clinicians' Knowledge, Competence and Confidence in the Management of Metastatic Breast Cancer

Zhizhi Fiske, Stephen Dunn, Deborah Grainger, Eloise Ballard, Jamie Habib, Nicholas Turner, Giuseppe Curigliano

Background: There has been much progress in discovering novel therapies for the management of metastatic breast cancer (mBC). The objective of this study was to assess the effect of an online continuing medical education (CME) activity on clinicians' understanding of the key data for mBC in all subtypes presented at the European Society for Molecular Oncology (ESMO) Breast 2023 conference.

Methods: This CME activity consisted of a 30-minute video panel discussion between 3 expert faculty with synchronised slides. Educational effect was assessed using a repeated-pair design with pre-/post-assessment. 3 multiple choice questions assessed knowledge/competence, and 1 question rated on a Likert-type scale assessed confidence, with each individual serving as their own control. A McNemar's test assessed significance of

improvement in the percentage of correct responses to knowledge/competence questions from pre- to post-assessment. P values < .05 are statistically significant. The activity launched on 12th of June 2023, with data collected through 29th August 2023 being reported in the current study.

Results: The analysis set consisted of responses from oncologists (n=75) who answered all assessment questions during the study period. Analysis of pre- vs post-CME responses demonstrated a significant improvement in knowledge and competence of oncologists (P< .01). Overall correct responses increased from 39% pre- to 52% post-CME. Specific areas of improvement include:

- Knowledge of the latest clinical evidence evaluating novel and emerging therapeutic options for mBC (pre 26%, post 38%; P < .01)
- Competence related to personalizing treatment for patients with mBC (pre 65%, post 79%; P < .01)

Additionally, 32% of oncologists reported increased confidence in integrating new treatment strategies for patients with mBC in clinical practice, and that increase was, on average, 63%.

Conclusions: This analysis demonstrates the positive impact of an online CME activity on physicians' ability to interpret the key data in mBC presented at ESMO Breast 2023. As new evidence emerges, it will be important to educate clinicians on the latest developments to continue to close their knowledge gap.

P5-08-23: DIHYDROCERAMIDE DESATURASE 1 (DES1) AS A REGULATOR OF CASPASE 14 IS REQUIRED FOR ANCHORAGE-INDEPENDENT SURVIVAL AND METASTASIS OF HER2-POSITIVE AND BASAL BREAST CANCER

Deanna Peperno, Danielle Lambadis, Victoria Alvarado, Andrew Resnick, Christopher J. Clarke

The ability of cancer cells to survive in the absence of an extracellular matrix – termed anchorage-independent survival (AIS) – is central to the metastatic cascade and in recent years has emerged as a target biology of interest for the treatment of metastatic disease. We recently identified the SL enzyme dihydroceramide desaturase 1 (DES1) as a critical HER2-regulated node that was both necessary and sufficient for AIS in HER2+ BC. However, the underlying mechanisms connecting DES1 with AIS and the relevance of this for BC metastasis remains unclear. To begin to probe this, we conducted a microarray analysis of wild-type and DES1 knockout SKBR3 BC cells with results revealing that the loss of DES1 led to a significant reduction in the expression of Caspase 14—a poorly studied member of the caspase family. Notably, we found that targeting Caspase 14 led to significant loss of AIS and reduced growth in colony formation in both HER2+ (JIMT1) and basal BC cells (MDA-MB-231), while stable overexpression of Caspase 14 was able to overcome the effects of

DES1 loss on AIS. Extending these findings in vivo, our data found that DES1 KO led to striking decreases in both experimental and spontaneous metastasis despite no significant difference in primary tumor size for both basal and HER2+ BC cells. Additionally, IHC of spontaneous lung metastasis shows a substantial reduction in Caspase 14 staining in DES1 KO compared to vector controls. Finally, publicly available clinical data show that BC tumors with high DES1 and Caspase 14 expression have a significant reduction in relapse-free survival in patients who have undergone neoadjuvant chemotherapy (NAC). Taken together, these data establish DES1 as a key mediator of BC metastasis and define Caspase 14 as a novel effector of DES1 required for promoting AIS. Results further suggest that DES1 and Caspase 14 expression might have use as a biomarker and predictor of highly metastasis-prone BC tumors.

P5-08-24: Prognostic Value of the Geriatric Vulnerability Score in Older Patients with Metastatic Breast Cancer

Min Xiao, Lei Ji, Xi Chen, Song Ge, Qing Li, Qiao Li, Pin Zhang

Background: Comprehensive Geriatric Assessment (CGA) contains multiple dimensions and involves complex scales, thereby limiting its clinical application. This study aims to develop a clinically applicable geriatric assessment method (Geriatric Vulnerability Score, GVS) tailored to older patients with metastatic breast cancer (MBC) and to analyze its association with survival.

Methods: This study included MBC patients aged over 65 years. They underwent geriatric assessment(GA) including Charlson Comorbidity Index (CCI), Activities of Daily Living (ADL) ； Peripheral blood lymphocyte absolute count and albumin were recorded for assessment of immune system reserve and nutritional reserve. Data analysis included descriptive statistics, Cox multivariate regression, student’s t-tests, one-way ANOVA, Kaplan-Meier curves and Harrell's concordance statistic.

Results: A total of 233 patients were included with the median age of 69 years. The median CCI was 0 (0-4). 33 (14.2%) patients had an ADL score<90; 25 (10.7%) patients had a lymphopenia ≤1×109/L, and 17 (7.3%) patients had an albumemia ≤35g/L. 159 (66.8%) patients were treatment-naive in metastatic setting. Multivariate analysis showed that ADL score<90, lymphopenia ≤1×109/L, and albumemia ≤35 g/L were independent adverse factors for OS in patients (all P <0.05), whereas CCI was not an independent prognostic factor (P>0.05).When the significant GA items (ADL <90, lymphopenia ≤1×109/L, albumemia ≤35 g/L, each assigned a value of one) were scored to develop GVS, it showed that the higher GVS, the worse OS of patients (HR: 2.228, 95% CI: 1.640-3.027, P <0.001). Furthermore, this score was validated in the treatment-naive group (HR: 3.215, 95% CI: 1.975-5.232, P <0.001). Adding GVS to tumor characteristics improved the accuracy of prognostic prediction (C-index, all patients: from 0.670 to 0.709; treatment-naive group:

from 0.694 to 0.742).

Conclusion: GVS is an objective, simple, and feasible method that can serve as one of the dimensions for predicting the prognosis of old MBC. It is worth verifying its clinical application value in a larger sample.

P5-08-25: Inhibiting Breast Cancer Metastasis by Targeting Chemokine-Glycosaminoglycan Interactions

Mohammed Imad Malki, Ibrahim Elmakaty, Amr Ouda

Breast cancer exhibits a specific metastatic pattern potentially influenced by the interaction between the chemokine receptor CCR7 on breast cancer cells and its ligand CCL21, which is expressed at metastatic sites. Glycosaminoglycans (GAGs) on endothelial cell surfaces bind chemokines and are thought to be critical in presenting chemokines to responsive cancer cells. This study aimed to test the hypothesis that soluble GAG molecules, such as heparinoids, can disrupt the normal presentation of CCL21, potentially reducing metastasis of CCR7-expressing breast cancer cells. Human breast cancer samples were analyzed for the expression of CCR7, CCL21, and GAG, correlating this expression with tumor grade. The cytokines IFN-γ and TNF-α were found to influence the endothelial cell GAG's ability to present chemokines to passing cancer cells. Both GAG-expressing Chinese Hamster Ovary (CHO) cells and GAG-deficient mutant CHO cells were transfected with CCR7, and their intracellular calcium flux and chemotactic migration were assayed in response to CCL21 and heparinoid. It was discovered that GAG expression by the responding cells was not essential for chemokine receptor activation. However, heparinoids, including Low Molecular Weight Heparins, effectively blocked the binding of 125I-CCL21 to a monolayer of GAG-expressing CCR7 transfectants, inhibiting both CCR7 ligation and subsequent signaling. Further experiments with CCR7-expressing MDA-MB-231 breast cancer cells demonstrated that heparinoids significantly inhibited the chemotactic response within a CCL21 diffusion gradient. Additionally, heparin and both low and high doses of Tinzaparin treatment in SCID mice, at clinically relevant dosages, prevented both the number and area of metastases following intravenous injection of MDA-MB-231 cells. These findings suggest that heparinoids inhibit the interaction between CCL21 and CCR7 and may serve as effective agents to prevent breast cancer metastasis.

P5-08-26: Relapse after 10 years following treatment for early breast cancer. 18 years follow-up data from a Belgian single-center.

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Introduction: Early diagnosed breast cancer can recur even 20 years after initial treatment. Prognostic markers for recurrence beyond 10 years in patients who received contemporary locoregional and systemic treatment are lacking. We therefore investigated known risk factors for recurrence in a population with a 10-years disease-free interval.
Patients and methods: In this retrospective study, we included 3581 patients with early breast cancer treated at University hospital of Leuven between January 1st, 2000 until December 1stt, 2010 who remained disease-free for 10 years. Patients received local and systemic treatment according to institutional guidelines; 10% were triple negative, 84.3% were oestrogen receptor (ER) positive and 12.7% Human Epidermal growth factor Receptor 2 (HER-2) amplified but adjuvant trastuzumab was only available in the HERA study and routine for patients after 2007. We analysed patient and tumour characteristics for breast cancer recurrence in the second decade after diagnosis. Local and locoregional invasive disease-free interval was defined as the time till the first event of local or locoregional relapse. Metastasis-free interval was defined as the time till metastasis. Patients without event were censored at last follow-up. Death without event was considered as a competing risk. For the statistical analysis Cox proportional hazard models were used, with local and locoregional disease-free interval and distant metastasis-free interval as time-to-event outcomes. Furthermore, a multivariable model of independent predictors was constructed using backward selection. We focused on 2 outcome variables: local and locoregional and distant metastasis-free interval.
Results: We included 3581 patients (median age at diagnosis, 55years) who were disease-free after 10 years of follow-up. After a median follow-up of 8 years, 18 years since initial diagnosis, 334 (9.3%) had a recurrence (local, locoregional or metastasis) of which 147 (4.1%) were distant only and 38 (1%) combined local or locoregional and distant. A positive lymph node status at diagnosis was a significant risk factor for local and locoregional recurrence in the second decade after diagnosis in the univariable analysis hazard ratio (HR) = 1.190 (95% confidence interval: 1.003;1.412; p=0.0456). A multivariable analysis could not be built for local and locoregional recurrence as nodal invasion was the only significant risk factor. In a multivariable analysis for distant metastasis-free interval, tumour size HR=1.332 (1.084;1.636) p=0.0064, grade 2 (compared to grade 1 HR=0.456 (0.248;0.839) p=0.0116 and to grade 3 HR=1.958 (1.313;2.920) p=0.0010), lymph node invasion HR=1.824 (1.510;2.202) p<0.0001 and ER positivity HR=3.796 (1.619;8.899) p=0.0021 proved to be independent adverse prognostic in the second decade after diagnosis.
Conclusion: Our findings may help to differentiate between patients with high or low risk of recurrence beyond 10 years of diagnosis and as such, may help to provide a more personalized follow-up.

P5-08-27: Reliability of Immunohistochemical Biomarkers in Core Biopsy Versus Surgical Specimens for Breast Cancer: Implications for Treatment Planning

Hagigat Valiyeva, Nigar Mehdiyeva, Elcin Huseynov, Tahmina Kosayeva, Habil Muradov, Konul Ferhadzade, Rena Haciyeva, Emil Aghayev, Zarifa Caferzade

Background: Core biopsy is instrumental not only in diagnosing breast cancer but also in guiding individualized treatment plans through the immunohistochemical evaluation of biopsy samples. The advent of neoadjuvant chemotherapy (NAC) has revolutionized the treatment landscape of breast cancer, with subsequent adjuvant systemic therapies primarily based on the pre-treatment status of Estrogen Receptors (ER), Progesterone Receptors (PR), and Human Epidermal Growth Factor Receptor 2 (HER2) as determined by core needle biopsy. However, the reliability and concordance of these biomarkers post-NAC are not well understood, raising the question of whether their potential discordance necessitates changes in adjuvant treatment strategies. The literature reveals instances of multiple histological subtypes and varied immunohistochemical profiles within post-surgical pathological specimens, particularly in cases where the inherent heterogeneity of tumors and the presence of multiple foci in multifocal and multicentric tumors are not comprehensively sampled by biopsies or entirely identified. The initiation of NAC has underscored discrepancies between the immunohistochemical results of core biopsies and those obtained from final surgical specimens. Additionally, differences in techniques and reagents used during the processing of core biopsy material can significantly influence the results. These factors collectively raise the pertinent question of whether immunohistochemical re-evaluation of final surgical specimens is warranted. The primary objective of this study is to elucidate the differences between the immunohistochemical findings of core biopsy and subsequent surgical specimens in cases of breast cancer, specifically focusing on the concordance and discordance of ER, PR, and HER2 status.
Methods: Between 2018 and 2023 years, the Oncology Clinic at Azerbaijan Medical University treated 3383 patients with a diagnosis of breast cancer. Of these, 2815 patients (83.2%) underwent surgical intervention. A total of 1281 patients (37.8%) commenced treatment with NAC. The cohort included 92 patients (3.27%) with multifocal and multicentric tumors or additional foci identified during the pathological examination of the final surgical specimen. Among patients with multiple foci, 34 (1.21%) underwent direct surgical intervention, while 58 (2.06%) initiated treatment with NAC.
Results: Immunohistochemical re-evaluation was performed on 226 (8.03%) patients, revealing discordant results in 85 (3.02%) patients. In cases where discrepancies were observed between core biopsy and surgical specimens, 14 (0.49%) patients with multifocal lesions underwent upfront surgical intervention, while 22 (0.78%) patients underwent surgery following NAC. 18 (0.64%) underwent direct surgical intervention, while 31 (1.1%) received surgery after NAC.
Conclusion: The identification of discrepancies between the immunohistochemical results of core biopsy and final surgical specimens in breast cancer underscores the necessity for further extensive studies. These investigations should aim to determine whether routine or

selective re-evaluation of immunohistochemical markers in final surgical specimens should be incorporated into clinical protocols for optimal patient management.

P5-08-28: Randomized phase 2 window of opportunity trial comparing the effect of preoperative atirmociclib (PF-07220060) plus letrozole versus letrozole alone on Ki-67 tumor expression in postmenopausal women with HR+/HER2+ breast cancer

Shom Goel, Khalil Zaman, Seock-Ah Im, Ahmed Elkhanany, Chiun-Sheng Huang, Cristian Villanueva, Sara Lopez Tarruella, Chin-Hee Chung, Ann Alcasid, Yuan Liu, Cynthia Basu, Michail Ignatiadis

Background: In previous clinical trials, complete cell cycle arrest (CCCA, defined as Ki-67 ≤2.7%) in the neoadjuvant setting was correlated with relapse-free survival after definitive surgical resection. Cyclin-dependent kinase 4/6 inhibitors (CDK4/6is), in combination with endocrine therapy (ET), have consistently shown progression-free survival and/or overall survival benefits for patients with HR+/HER2− metastatic breast cancer (BC). High and/or continuous CDK4/6i dosing may result in dose-limiting cytopenia-related toxicity; neutropenia is believed to be mediated mainly by inhibition of CDK6 rather than CDK4. Atirmociclib (PF-07220060) is a novel, highly potent and selective inhibitor of CDK4. In preclinical studies, atirmociclib induced G1 cell cycle arrest in BC cell lines and significantly inhibited the growth of HR+/HER2− tumors, which are known to have a high degree of CDK4 dependency. Preliminary results from a phase 1/2a multi-part trial assessing atirmociclib in patients with HR+/HER2− metastatic BC have demonstrated encouraging efficacy, safety, and tolerability (Yap et al, JCO. 2023;41:3009; Giordano et al, JCO. 2024;42:3108). Based on these findings, this study aims to evaluate the potential impact of atirmociclib in combination with letrozole in the preoperative treatment of patients with HR+/HER2− early BC.

Methods: This international (16 countries, >80 proposed sites), phase 2, open-label, randomized clinical trial will evaluate the effects of atirmociclib plus letrozole versus letrozole alone on Ki-67 expression in tumors after 14 days of treatment in the preoperative setting. Postmenopausal women aged ≥18 years with newly diagnosed and treatment naïve HR+/HER2− BC will be enrolled. Additional inclusion criteria include primary tumor size ≥1.5 cm amenable to surgical resection, ECOG PS 0 or 1, and a baseline tumor Ki-67 score ≥10%. Exclusion criteria include any prior systemic therapy for the treatment of BC, inadequate bone marrow, renal, and liver function, any other active malignancy within 3 years prior to enrollment, certain severe medical conditions within 6 months of enrollment, known allergy to the study drug, inability to take oral medications, and certain psychiatric conditions. Approximately 118 patients will be randomly assigned 1:1 to receive atirmociclib (300 mg, orally, twice daily) plus letrozole (2.5 mg, orally, once daily) or letrozole alone (2.5 mg, orally, once daily), continuously for 14 days. After the 14-day treatment period, participants will come off study treatment and continue on standard-of-

care therapy (eg, ET, surgery, radiotherapy, chemotherapy) per physician's choice. Participants will have both a baseline biopsy and an on-treatment biopsy (Day 14). The primary endpoint will be CCCA (Ki-67 ≤2.7%) rates at Day 14 (based on centrally assessed post-treatment tumor biopsies). Secondary endpoints will be incidence of all adverse events (AEs), serious AEs, AEs leading to study drug discontinuation, circulating tumor DNA measurements at baseline and Day 14, Ctrough and peri-biopsy plasma concentrations of atirmociclib, and centrally-assessed Ki-67 immunohistochemistry. Assessment of changes in the tumor microenvironment using spatial omics, and evaluation of the association between tumor gene expression profiles and treatment outcomes will be exploratory objectives. Enrollment is to begin July 2024 (NCT06465368).

P5-08-29: Assessment of RNA Extraction Protocols from FFPE Breast Cancer Samples with the APIS Breast Cancer Subtyping Kit

Anna Gasior, Joanna Gorniak, Andreas Voss, Kimberly Howard, Mathew Harrison, Leanne Gough, Sara Rollinson, Zoe Pounce

Background: The APIS Breast Cancer Subtyping Kit detects the expression of mRNA target genes (ESR1, PGR, ERBB2, MKI67, CCNA2, PCNA, and KIF23) in formalin-fixed, paraffin-embedded (FFPE) pre-operative core needle biopsies or resected breast tumour tissue. RNA extraction from FFPE poses challenges due to degradation, cross-linking and base modifications introduced during the fixation process. The APIS' kit workflow includes RNA extraction using QIAGEN's RNeasy® DSP FFPE Kit. This study evaluates suitability of alternative kits (Promega ReliaPrep™ FFPE Total RNA Miniprep System, ThermoFisher PureLink™ FFPE RNA Isolation Kit and BioEcho EchoLUTION™ FFPE RNA Kit) for use in conjunction with the APIS kit, based on RNA yield and target call concordance.

Methods: 21 FFPE tissue sections were used in this study. All extraction procedures were carried out following the manufacturers' instructions. RNA was quantified and normalized to 2.5ng/µL for RT-qPCR analysis with the APIS kit. Relative RNA expression of all targets and reference genes was assessed using QuantStudio™ 5 Dx Real-Time PCR System. The agreement between the extraction kits was assessed by comparing calls (positive/negative) produced by testing RNA samples extracted using all four methods (QIAGEN kit, Promega kit, Thermofisher kit and Bioecho kit) with the APIS kit. The call was considered positive if ΔCt values (a measure of gene expression used by APIS kit) fell above the validated ΔCt cut-off values.

Results: All kits extracted RNA successfully. BioEcho and Thermofisher kits often yielded lower RNA concentrations, most likely due to higher elution volumes (30 µl vs 50 µl). Concordance rates for target calls ranged from 78-100%. To reduce miscalling, samples within 2x ΔCt intermediate precision either side of assay cut-offs were removed. Promega and BioEcho kits showed high concordance (100%). Lower agreement was seen for

Thermofisher kit (78% for ERBB2 and 91% for MKI67). 100% concordance was observed for ESR1 and PGR across all kits.

Conclusions: Sufficient RNA yield and high agreement confirmed that RNA derived using all described methods is of sufficient quality to accurately detect biomarker expression via the APIS kit. Promega and BioEcho kits demonstrated high target call concordance, making them viable alternatives to QIAGEN's kit. Lower concordance seen with Thermofisher kit is most likely a result of tumour heterogeneity. The extractions had to be performed using different FFPE sections, and therefore localized differences in marker expressions within tumors could have contributed to the variability observed, especially for ERBB2. Considering this inherent tumor heterogeneity, Thermofisher kit could also be considered for use. These results suggest that all assessed kits are suitable for use in APIS Breast Cancer Subtyping Kit workflow. It also confirms that APIS' kit is capable of detecting biomarker expression from highly fragmented, FFPE-derived RNA.

P5-08-30: The impact of tumor location on survival rates in invasive ductal carcinoma for patients who undergo breast-conserving therapy

Abdulah Jariri

Introduction: Invasive ductal carcinoma (IDC) is the most common type of breast malignancy, making about 75% of all breast cancer cases, usually the management for IDC contains breast-conserving therapy (BCT) which is a treatment strategy that removes the tumor while preserving as much as possible from breast tissue. There is still a debate how the tumor's location affects survival. This study aims to evaluate survival rates for IDC patients treated with BCT alone across different breast quadrants which improves patients outcomes and inrich our medical understanding.

Method: SEER Program was used to obtain the data, retrospectively. Patients diagnosed with infiltrating duct breast carcinoma treated with breast conserving therapy alone were included from (2000-2021). Patients were categorised into four groups based on the location of the tumor within the breast (upper outer quadrant , upper inner quadrant , lower inner quadrant, lower outer quadrant). All patients received BCT according to SEER breast cancer surgery codes (20-24). Relative survival rates were compared using Kaplan-Meier, log-rank analysis. IBM SPSS Statistics 27.0.1 was used to conduct the statistical analysis.

Results: A total of 36443 cases of IDC and treated with BCT alone were identified. Among the groups , 9.57%(n=3491) have a tumor located in lower inner quadrant, 21.55% (n=7854) in the upper inner quadrant, 57.47% (n=20947) in the upper outer quadrant, and 11.39% (n=4151) in the lower outer quadrant .Among all tumor locations, 5- years relative

survival was the highest for patients with tumors in the upper inner quadrant (95.3%), followed by upper outer quadrant (94.1%), then came Lower outer quadrant (92.5%),and lastly came lower inner quadrant (92.2%), with (p -value < .001).

Conclusion: Overall, patients who are diagnosed with invasive ductal carcinoma and treated with breast conserving therapy only showed better survival when tumors were located in the upper inner quadrant. In contrast patients with tumors in the (lower inner quadrant) showed the lowest survival rates among all quadrants . Our findings confirm that there is a significant difference in survival rates between different quadrants, it could be an entry for several researches and to spot the light on the importance of tumor location in IDC, and different management patterns.

P5-09-01: Some factors associated with re-excision in breast conserving surgery do not increase rates of re-excision after oncoplastic breast conserving surgery: a retrospective study

Kendall Vignaroli, Lana Mamoun, Amanda Daoud, Aldin Malkoc, Kevin Perez, Judi Ramiscal

Oncoplastic breast conserving surgery (OBCS) utilizes a multidisciplinaryapproach to provide tumor resection while minimizing deformities of the breast. The techniques used in OBCS are associated with similar rates of survival and decreased rates of re-excision, postoperative medical complications, and tumor recurrence when compared to breast-conserving surgery (BCS). Previous studies have revealed multiple factors associated with re-excision in patients undergoing BCS including tumor size, tumor pathology, multifocality, and patient age, however there is minimal literature discussing factors associated with re-excision in patients undergoing OBCS. The purpose of this retrospective study is to identify preoperative factorsassociated with re-excision in OBCS.
A retrospective review was performed on adult patients who underwent oncoplasticbreast conserving surgery between October 2021 and May 2024. Subjects were divided into those who required re-excision and those who did not. Amongst these groups, factors were evaluated including patient age, BMI, smoking status, presence of co-morbidities including hypertension and diabetes, presence of tumor multifocality or microcalcifications on mammogram, tumor size, HER2 positive status, triple negative (ER-/PR-/HER2-) status, and tumor pathologies including ductal carcinoma in situ (DCIS), invasive ductal carcinoma, and invasive lobular carcinoma. Univariate analyses was performed using Chi-Squared for categorical data and continuous data was analyzed using independent t-test or non-parametric Mann-Whitney U tests where appropriate. Of 48 patients total, all 48 patients (100%) were female. 31 patients (64.6%) underwentmastopexy and 17 patients (35.4%) underwent mammoplasty. 12 patients (25%) required re-excision for inadequate margins and 36 patients (75%) did not require re-excision. There was no significant difference in the need for margin re-excision based on patient age, BMI, smoking status, or the presence of hypertension or diabetes (p=0.738, p=0.171, p=1.000, p=1.000, p=0.522). There was no

significant difference in need for margin re-excision based on tumor size (25.2 +/-25.8mm in patients who required re-excision vs. 19.4 +/-10.7mm for patients who did not require re-excision, p=0.976). There was also no difference in need for margin re-excision based on the presence of invasive lobular carcinoma, triple negative status, presence of HER2,microcalcifications or tumor multifocality (p=0.614, p=0.404, p=0.131, p=0.394, p=0.614). 6 of the 12 patients (50%) with DCIS required margin re-excision, while 4 of the 32 patients (12.5%) with invasive ductal carcinoma required margin re-excision (p=0.021, p=0.005).
While invasive lobular carcinoma, younger patient age, tumor size, presence ofHER2, and tumor multifocality have been shown to increase the risk of re-excision inBCS, these factors did not significantly affect re-excision rates after OBCS in our study.However, similar to data published for BCS, our results show that DCIS has a significantly increased rate of re-excision in patients who undergo OBCS. Additionally, the presence of invasive ductal carcinoma seemed to have a significantly decreased need for re-excision after undergoing OBCS. While further studies with larger patient populations should be completed to confirm our results, our study has identified a pathology of DCIS as one factor that might be predictive of re-excision and a pathology of invasive ductal carcinoma that might be predictive of decreased re-excision in patients undergoing OBCS.

P5-09-02: Acellular Dermal Matrix (Braxon® and STRATTICE™) for immediate implant - based breast reconstruction - Our Initial experience

Asma Munir, Dean Kamyab, Nida Javed, Yousef Sharaiha, Sohail Khan, Anita Huws, Saira Khawaja

Background: Immediate implant reconstruction after a mastectomy is an option for breast cancer patients. The success associated with these procedures is related to providing extra implant coverage and anchoring to the pectoralis thereby accelerating healing. The use of acellular dermal matrix (ADM) is an alternative to titanium-coated polypropylene mesh (TCPM) for implant-based breast reconstruction (IBBR).

Our study is a prospective nonrandomized single-institution observational study of reconstructions using acellular dermal matrices.

Methods:Between October 2020 and April 2024, implant breast reconstructions after mastectomy using Braxon® or STRATTICE™ were performed in 24 patients (with 32 reconstructions). We analyzed short-term outcomes of such procedures and compared the outcomes to evaluate implant losses and surgical complications.
Complications were divided into major (need for additional surgery), minor (conservative treatment). The primary outcome was implant loss at 3 months. Univariate analyses were performed to determine the influence of the patient- and procedure-related factors on postoperative complications and implant loss.
Results:A total of 32 mastectomies with reconstructions were performed in 24 women.

Mean age was 50 years (range 29-73 years). The mean BMI was 26 (range 18.6 - 37.3). Twenty-four patients had therapeutic mastectomies with eight of them also having contra lateral risk reducing mastectomies.

There were 11 nipple sparing procedures and 21 skin sparing procedures.

The median follow-up period was 11 months. Implant loss rate was 3.1% (1 of 32 implants) at 3 months follow up. Immediate hematoma and subsequent delayed skin necrosis was responsible for this case.

One other patient had wound dehiscence and loss of implant at 3.5 months. Two other patients had revised reconstructions at later periods. One of these had the Braxon® replaced with a STRATTICE™ 13 months post reconstruction after developing Red Breast Syndrome. The other patient had a smaller implant placed 8 months post op for cosmetic purposes.

No risk factors were observed for immediate failure within 3 months. Univariate analysis revealed an increased risk for implant loss in patients with skin necrosis and wound dehiscence ($p<0.01$).

Conclusions:Acellular dermal matrices show acceptable complication rates and its use in immediate implant breast reconstruction is safe and effective. Going forward, we would like to compare the data between TCPM and ADMs.

P5-09-03: Localization combined with multidetector CT 3-dimensional image reconstruction guided precision breast conserving surgery versus conventional breast conserving surgery: cosmetic analysis of the single center, prospective, cohort study.

Yiqin Xia, Yangyang Cui, Yue Huang, Jinghui Peng, Meng Zhao, Hui Xie, Shui Wang

Introduction: Breast conserving surgery (BCS) with radiation is accepted as a standard local treatment for early-stage breast cancer. Advantages in survival and quality of life (QoL) have led to a higher demand for BCS. Success of BCS is characterized by negative margins and a good cosmetic outcome. The aims of this cohort study were to determine whether wire-guided localization (WGL) combined with multidetector CT (MDCT) guided 3-dimensional (3D) reconstruction could guide precision breast conserving surgery (PBCS), and to access the cosmetic outcome reported by patients.
Methods: 31 patients with unifocal breast cancer were enrolled for PBCS guided by WGL combined with MDCT guided 3D reconstruction from 2021 to 2022. Under local anesthesia, surrounded WGL was performed, followed by an immediate contrast enhanced MDCT scan. One day after the localization, PBCS guided by MDCT guided 3D reconstruction was performed. 61 women who underwent palpation guided breast conserving surgery (BCS)

were included as control. Two-sided Student t test, Fisher's exact test and chi-square test was applied.
Results: Breast Cancer Treatment Outcome Scale (BCTOS) cosmetic subscale was used to access patient reported cosmetic outcomes in our study. In the PBCS group, the results indicated no significant changes (P=0.168) between the baseline assessment and 1 month post operation. This is later followed by adverse changes (P=0.016) between 1 month post operation and 6 months post radiotherapy (RT). In the control group, the results indicated adverse changes (P 0.001) between the baseline assessment and 1 month post operation. This is later followed by deterioration (P=0.028) between 1 month post operation and 6 months post RT. Between the two groups, there was no significant difference noted for the score of the BCTOS at baseline, 1 month post surgery and 6 months post RT
Conclusion: For patients with unifocal breast cancer, WGL combined with MDCT guided 3D reconstruction could achieve better cosmetic outcomes.

P5-09-05: Median Five-Year Follow-Up Outcomes Based on Pathologic Grade in a Multi-Institution Trial Using Intra-Operative Electronic Brachytherapy for Treatment of Early-Stage Breast Cancer

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Background/Objectives: Grade (G) III breast cancer pathology received a separate AJCC Staging Manual 8th Edition classification due to increased evidence of higher tumor recurrence.1 The 2023 ASTRO Accelerated Partial Breast Irradiation Consensus Statement Guideline states that patients with GIII pathology may have a worse outcome, although this is an underrepresented subset in trials analyzed by this report.2 IORT is unique in that the final surgical pathology has not yet been determined at the time of IORT treatment. An IRB-approved single arm prospective multi-institution trial was designed to determine the efficacy and outcome of single fraction 20 Gy intra-operative radiation therapy (IORT) using disposable balloon electronic brachytherapy at the time of breast conserving surgery for early-stage breast cancer (women at least 40 years old, infiltrating ductal carcinoma [IDC] or ductal carcinoma in situ [DCIS], single lesion no larger than 3 cm, cN0). Differences in median 5-year follow-up ipsilateral breast tumor recurrence (IBTR) outcomes based on pathologic grade would be analyzed.
Methods: Between 2012-2018, 1199 enrolled patients were successfully treated per protocol with lumpectomy plus single 20 Gy fraction IORT using disposable balloon electronic brachytherapy. Data collection included demographics, Scarff-Bloom-Richardson graded pathology, IBTR (lumpectomy/index quadrant recurrence), and survival. The Exact Chi-square, 2-sided test was used for statistical analysis.
Results: 1199 patients completed IORT treatment per protocol. Post-biopsy, pre-IORT

classifications were IDC GI, GII, GIII (403, 437, 78 patients, respectively, 38 not recorded) and DCIS low, intermediate, and high grade (35, 108, 98 patients, 2 not recorded). The number of IDC patients in each group changed following surgical pathology (371, 439, 119 patients in the IDC GI, GII, GIII groups, 20 no residual tumor, 15 not recorded). DCIS patient numbers also changed (29, 92, 68 patients in the DCIS low, intermediate and high grade groups, 33 no residual tumor, 1 not recorded). Invasive lobular carcinoma was diagnosed in 12 patients.
There were 42 (3.50%) IBTR at median 5-year follow-up (IDC GI, GII, GIII, not recorded groups having 6, 14, 9, and 1 IBTR; intermediate and high grade DCIS groups having 5 and 7 IBTR). Of these, 49 patients with post-biopsy, pre-IORT IDC GI, GII pathology were re-classified as GIII on surgical pathology (3 IBTR) while 9 patients with low, intermediate grade DCIS were reclassified as high grade (2 IBTR). One post-biopsy, pre-IORT intermediate grade DCIS patient, reclassified as IDC GIII, remains recurrence free. At median 5-year follow-up, the 810 IDC GI, GII patients had a 2.5% IBTR, while the 121 low, intermediate grade DCIS patients had a 4.1% IBTR. 119 patients with IDC GIII and 68 patients with high grade DCIS were found to have a higher IBTR incidence (7.6% and 10.3%) which was statistically significant for DCIS ($p = 0.0014$), but not for IDC ($p = 0.0534$). There was 1 post-biopsy, pre-IORT IDC GIII breast-cancer related death.
Conclusions: At median 5-year follow-up, 1199 patients successfully treated with single 20 Gy fraction IORT per protocol using disposable ballloon electronic brachytherapy exhibited an overall 3.50% IBTR, with IDC GIII and high grade DCIS patients having a higher IBTR incidence, consistent with published reports.
References:

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P5-09-06: Acute Cardiovascular Events after Hypofractionated IMRT for Early Breast Cancer: Association with Coronary Calcifications in Radiotherapy Planning-CTs

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Radiotherapy (RT) for breast cancer (BC) has shown to increase the likelihood of acute coronary events (ACE) - infarction and angina - in older cohorts treated with obsolete techniques. Contemporary highly conformal RT, such as Intensity Modulated Radiotherapy (IMRT), and hypofractionation, which offers a theoretical radiobiological advantage by

reducing the equivalent biological dose to organs-at-risk, are mitigating the overall impact of RT on long-term cardiovascular (CV) toxicity and have shifted the focus towards the early diagnosis and management of patients' baseline cardiovascular risk factors (CVRF). Coronary arterial calcium, measured by a specific CT scan designed to quantify the calcium score, is a well-established early predictor of ACE. In recent years, it has been increasingly demonstrated that the incidental finding of coronary calcifications (CoC) on CT scans performed for RT treatment planning is significantly associated with an elevated risk of ACE.

The aim of this study is to determine the incidence of ACE or any other CV events in a modern and homogeneous cohort of patients receiving RT for early BC and to explore its association with the presence of CoC on the planning CT.

Materials and Methods

We included all BC patients treated with hypofractionated Forward Planned IMRT after breast-conserving surgery between 2009 and 2019. This monoinstitutional, descriptive, retrospective observational study is based on a manual review of the medical and RT planning records. To ensure cohort homogeneity, we excluded patients in indication for elective lymph node RT or those with previous mediastinal RT. Known CVRFs and those identified at diagnosis or during follow-up, were addressed with appropriate treatments. All RT-Planning CTs were performed in a supine position, without contrast, in free breathing, and were electrocardiogram non-triggered scans. The presence or absence of CoC in the planning CTs was registered.

Results:

882 patients (432 with left-sided BC) were included, with a median [Q1; Q3] age of 52 [46; 62] years. The presence of CoC was identified in 154 (17.5%) patients and was significantly associated with the patients' age at diagnosis and with other CVRFs, including diabetes, hypertension, dyslipidemia, overweight, and a personal history of CV events.

After a median follow-up of 8 years (ranging up to 14 years), the incidence of ACE was extremely low, at 0.7% (95% CI: 0.3%-1.5%). In women with CoC, the incidence of ACE was 3.9% (95% CI: 1.8%-8.2%), whereas none of the women without CoC developed an ACE and the risk was significantly higher for women with CoC compared to those without (RR 23.4 (95% CI: 3.4-233.9)).

In addition to ACE, 20 arrhythmias, 2 cases of severe valvular heart disease and 7 other non-ischaemic CV events were documented during the follow-up period, giving a total of 35 CV events (overall incidence 4.0% (95% CI: 2.9%-5.5%]). The occurrence of any of the latter in women with CoC was 9.7% (95% CI: 6.0%-15.4%), while in women without CoC it was 2.7% (95% CI: 1.8%-4.2%). The risk of experiencing any CV event was 3.5 times higher (95%: CI 1.9-6.8) for women with CoC compared to those without.

Conclusions

Although the incidence of ACE in this cohort is extremely low, both in women with and without CoC, a higher risk of ACE as well as of any CV event was detected in patients with CoC. These findings underscore the potential utility of detecting CoC on RT planning CT scans in conjunction with early management of classical CVRFs. This approach may provide

a valuable opportunity for identifying patients who could benefit from targeted primary prevention strategies for CV disease.

P5-09-07: Patterns of progression after first-line systemic therapy in metastatic breast cancer and the potential of ctDNA to guide metastasis-directed therapy: implications for breast oligometastases management

Jee Suk Chang, Min Hwan Kim, Byung Min Lee, Joohyunk Sohn, Gun Min Kim

Background: The role of metastasis-directed therapy (MDT) for oligometastases remains controversial in breast cancer. We analyzed the patterns of progression after first-line systemic therapy in metastatic breast cancer (MBC) in the contemporary era and investigated whether low-pass whole genome sequencing (WGS) ctDNA analysis can aid prediction of progression patterns.
Methods: We reviewed a previously established prospective ctDNA cohort (Kim et al., JNCI 2023) of 207 patients with MBC who received first-line systemic therapy from 2017 to 2020 in Yonsei Cancer Center, using. None of these patients received MDT. Imaging studies at baseline and during each progression were retrospectively reviewed to count the number of lesions and assess patterns of progression. Progression was categorized as either the progression of pre-existing lesions identified prior to treatment or the development of new lesions.
Results: An increase in disease burden (1-5 vs. 6-10 vs. >10 lesions) at baseline was significantly associated with decreased progression-free survival (PFS) and overall survival (OS). Patients with 1-5 lesions had a 5-year PFS and OS of 45.9% and 75.4%, respectively. With a median follow-up of 50 months, among MBC patients with 1-5 lesions at baseline, 49.2% showed no progression at the last follow-up, 30% had progression of 1-5 lesions, and 19% had progression of more than 5 lesions; among their progressions, 27% were progression of pre-existing lesions only, 43% were development of new lesions only, and 30% were mixed. These patterns varied by baseline number of lesions, mode of presentation (de novo vs. recurrent), and molecular subtype. A Sankey diagram was generated to identify the prevalence of progression of 1-5 lesions and PL progression through the fifth line of systemic therapy. A high ctDNA I-score was independently associated with a higher risk of widespread-metastasis (>10 lesions) progression (adjusted HR 3.25, 95% CI 1.54-6.85) in patients without widespread metastases at baseline.
Conclusions: Progression of pre-existing lesions was not the predominant pattern; poly-metastasis progression was not uncommon in oligometastatic breast cancer patients, highlighting the challenges in selecting patients for MDT based solely on the number of metastases at diagnosis. Incorporating ctDNA information may help identify patients at higher risk for widespread metastasis progression, who might not benefit from MDT.

P5-09-09: Prospective Evaluation of Shoulder Morbidity in Patients with Lymph Node-Positive Breast Cancer Receiving Regional Nodal Irradiation

Jose Bazan, Julie Stephens, Sachin R. Jhawar, Sasha Beyer, Karen Hock, Julia R. White

Purpose/Objective(s): Many women with axillary node-positive breast cancer benefit from regional nodal irradiation (RNI) in terms of improved cancer control outcomes. RNI unintentionally exposes the shoulder structures to radiation, which can lead to morbidity. We previously demonstrated that intensity modulated radiation therapy (IMRT) results in less radiation dose to the shoulder compared to 3D conformal radiation therapy (3DCRT). We set to determine if the dosimetric advantage of IMRT translates into a clinically meaningful reduction in shoulder morbidity.
Materials/Methods: This study is registered on clinicaltrials.gov (NCT03786354). We enrolled patients that were to receive RNI after mastectomy (Mx) or lumpectomy (Lump) with axillary staging (axillary lymph node dissection [ALND] or sentinel node biopsy [SNB]). All patients received 50 Gy in 25 fractions to the breast or chestwall and RNI. Patients were non-randomly assigned to either Arm IMRT or Arm B 3DCRT on the basis of a treatment planning algorithm that defaults to 3DCRT but transitions to IMRT based on dosimetric criteria. The primary endpoint was shoulder/arm morbidity at 1 year post-RNI in IMRT patients using the Disabilities of the Arm, Shoulder and Hand (DASH) questionnaire. Based on limited existing data at the time, we estimated that the 1-year DASH score would be 20 in patients that had undergone RNI with 3DCRT and that IMRT would reduce the 1-year DASH to ≤10. A sample size of 27 patients would have 80% power to detect this 10-point difference in DASH score. We enrolled 27 patients on Arm 3DCRT to establish the average DASH score in this cohort.
Results: Patients were enrolled from 1/2019-3/2022. After loss to follow-up, there were 29 patients evaluable for the primary endpoint in Arm IMRT and 27 patients in Arm 3DCRT with similar ages (median age=50 years vs. 51 years, p=0.83) but trend towards higher rates of Mx (72% vs. 51%, p=0.11) and ALND (90% vs.78%, p=0.23) in Arm IMRT vs. Arm 3DCRT. In arm IMRT, the mean baseline (post-surgery/pre-RNI) DASH was 12.8(SD 11.0) and increased to a mean of 15.4(SD 15.1) at 1-year. In arm 3DCRT, the mean baseline DASH was 14.0(SD 12.9) and increased to a mean of 14.8(SD 13.2) at 1-year. Decrease in DASH scores was more frequent in Arm IMRT vs. 3DCRT (51.7% vs. 37.0%, p=0.27). In the entire cohort, patients treated with Mx and patients treated with ALND tended to have worse 1-year DASH scores: 17.6 (SD 15.9) Mx vs. 11.0(SD 9.4) Lump, p=0.06; 16.4(SD 14.7) ALND vs. 8.4(SD 8.0) SNB, p=0.03.
Conclusion: This study did not meet its primary endpoint but the similar 1-year DASH scores in Arm IMRT and Arm 3DCRT may reflect IMRT compensating for the more extensive axillary and breast surgery in that arm. More than 50% of patients in Arm IMRT experienced a decrease in DASH score. These findings suggest that prospective investigation of IMRT for RNI in the setting of SNB remains worthy of study. Analysis of secondary endpoints including objective range of motion assessments and shoulder muscle dosimetry are forthcoming.

P5-09-10: Metastasis-directed radiotherapy and systemic treatment continuation for patients with extracranial oligoprogressive metastatic breast cancer (ESTER-RW): a single-center retrospective analysis

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Background: Following to an initial disease control to systemic treatment, 15-20% of patients (pts) with metastatic breast cancer (mBC) experience oligoprogression of disease (OPD) in ≤5 extracranial sites. These pts may benefit from radiotherapy (RT) to OPD sites, while maintaining the same systemic treatment, aiming at prolonging the progression-free survival (PFS) and postponing the time to systemic treatment failure (TTF). Data from large observational studies are missing.
Methods: Retrospective, single-center, observational study aiming at evaluating the post-RT-PFS (pRT-PFS) in consecutive pts with mBC and OPD, who received RT to all OPD sites (≤5 extracranial sites) at European Institute of Oncology (Milan), from Jan 2011 to Jun 2023, continuing the same systemic treatment. The primary endpoint was pRT-PFS, defined as the time from the first scan showing OPD to the subsequent PD. Secondary endpoints were: pRT-PFS in pts with luminal-like (HR+/HER2-) disease; post-RT TTF (pRT-TTF), defined as the time from the first scan showing OPD to treatment discontinuation; overall survival (OS), calculated from the OPD scan. A sample size of 130 pts allowed to estimate the lower boundary of the 95% confidence interval (95%CI) for the median pRT-PFS of 7 months (mo).
Results: 129 consecutive pts were included. Among all comers (N=129) and in the HR+/HER2- subgroup (N=99), respectively, median age was 60 (52-67) and 60 (52-68); 79% and 76% of pts had a ductal carcinoma, 77% and 100% a luminal-like disease, 15% and 0% a HER2+ disease, 9% and 0% a triple-negative disease.
The median line of treatment was 2 (interquartile range [IQR]: 1-2) and 1 (IQR: 1-2); 60% and 75% of pts were receiving an endocrine therapy (ET)-based treatment when OPD occurred, while 28% and 26% chemotherapy.
The median number of OPD sites was 1 (IQR: 1-1) and 1 (IQR: 1-1), and the median number of OPD lesions was 1 (IQR: 1-2) and 1 (IQR: 1-2); 9% and 6% of pts had visceral OPD, while 73% and 83% bone OPD.
Overall, 33% and 34% of pts received RT with cyberknife; 98% and 98% had in-field disease control rate as best response, with 47% and 47% of radiological complete responses; the median pre-RT PFS was 12.4 mo (95%CI 9.8-15.1) and 11.5 mo (95%CI 8.7-14.3).
After a median follow-up of 3.2 years (95%CI 2.7-3.8), 113 and 89 pRT-PFS events occurred: median pRT-PFS was 11.3 mo (95%CI 9.1-13.5) among all comers and 11.5 mo (95%CI 8.7-14.3) in the HR+/HER2- subgroup; median pRT-TTF was 13.6 mo (95%CI 11.7-15.5) and 14.2 mo (12.3-16.2); median OS was 6.4 years (4.7-8.1) and 6.7 years (4.8-8.1).
Among all comers, in pts with pre-RT PFS ≥12 or ≥18 mo, pRT-PFS was 13.5 mo (95%CI

10.2-16.7) and 14.9 (95%CI 12.2-17.7), respectively. Similarly, in pts from the HR+/HER2- subgroup with pre-RT PFS ≥12 or ≥18 mo, pRT-PFS was 13.0 mo (95%CI 8.1-18.0) and 14.8 (95%CI 12.1-17.4), respectively.
Conclusions: Pts with mBC, especially with HR+/HER2- subtype and who experience extracranial non-visceral OPD after at least 12 mo of treatment, benefit from RT to all OPD sites without changing systemic treatment.

P5-09-11: Cardiac assessment and safety of 5-fraction whole breast irradiation after breast-conserving surgery: an observational prospective cohort study (SAFE-FORWARD)

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Purpose. Whole breast irradiation (WBI) using 5 fractions after breast-conserving surgery (BCS) has shown non-inferior local control rates, an acceptable safety profile, and greater patient compliance. This study aims to assess the heart toxic effects using reliable cardiac assessments with standard and 3-dimensional (3D) echocardiography and left (LV) and right ventricular (RV) global longitudinal strain (GLS) in patients receiving 5-fraction postoperative radiation therapy (RT) for breast cancer (BC).
Patients and methods. SAFE-FORWARD is an observational prospective cohort study (NCT04842409). We included: (1) patients with invasive BC receiving ultra-hypofractionated WBI (26Gy in 5 fractions) after BCS, (2) without cardiovascular comorbidity, and (3) without previous thoracic irradiation. All enrolled patients were prospectively monitored for 12 months, receiving a complex cardiological assessment before RT start (baseline), and at 2, 6, and 12 months after the end of RT. Heart substructures of patients were delineated according to the cardiac contouring atlas. Both acute and early-late toxicity were evaluated according to CTCAE (v.5) scales. The primary endpoint was defined as the detection of any subclinical impairment in myocardial function and deformation (decrease ≥10%) measured with standard and 3D echocardiography LV and RV GLS.
Results. Overall, 50 women (median age 67 years; range 48-84) were enrolled in the study. All patients received ultra-hypofractionated WBI (26Gy in 5 fractions): left and right breast side patients were 27 and 21, respectively, and 2 patients had bilateral breast cancer. Patients were treated with tangential IMRT fields using 6 or 10 MV beams from an Elekta VHD linac. Adjuvant endocrine therapy was administered to 33 patients. The median PTV volume was 768 cc (range 220-1689) and the median heart volume was 576 cc (range 403-857). The average values of mean heart dose (Dmean) and maximum heart dose (dose covering 0.04cc of the Heart, D0.04) were 0.73Gy (SD 0.50) and 10.0Gy (SD 10.1), respectively. The left breast side group reported 0.94Gy (SD 0.49) and 15.0Gy (SD 9.03) for Dmean and D0.04, respectively, while right breast side patients had lower Dmean (0.44Gy,

SD 0.34) and D0.04 (3.14Gy, SD 7.12). Among patients who had completed the cardiological assessment at 12 months, GLS worsened by 4% or less, both for the left- and right-side treated breast, and remained in the normal range at all time points. The only exception was for RVGLS at 6 months for right-sided treatment where it reached a borderline value (-17.4±4.9 SE). 3D-LVEF remained stable during observation, both for the left- and right-side treated breast. At the end of RT, grade (G) 1 and 2 adverse events were observed in 31 and 3 patients, respectively. Eleven patients reported G1 toxicities at 12 months: 2 cases of radiation dermatitis, 2 of fatigue, 1 of telangiectasia, and 6 of fibrosis.
Conclusion. The 5-fraction WBI schedule was well tolerated, and the intensive 1-year cardiological monitoring showed no significant differences over time in cardiac functioning.

P5-09-12: Pre-Operative Radiation Boost Planning in a Phase II Clinical Trial Achieves Acceptable Tumor Volume Coverage

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Background: Radiation tumor bed boost after breast-conserving surgery (BCS) and whole breast irradiation (WBI) reduces local recurrence rates in women with ductal carcinoma in situ (DCIS) and early-stage breast cancer. This prospective trial investigated the feasibility of delivering the tumor bed boost pre-operatively instead of post-operatively. The primary endpoint, the incidence of wound complication rates, was previously reported as acceptable. Additionally, we have previously shown that pre-operative boost resulted in smaller treatment volumes compared to what would have been treated with a sequential lumpectomy boost based on the post-operative seroma cavity. The pre-operative boost approach may also allow for more accurate gross tumor delineation. We hypothesize that pre-operative imaging including mammography, MRI and CT simulation used in this trial would enable the accurate delineation of gross tumor volumes (GTV) that can adequately cover the entire tumor, as demonstrated by comparison with post-lumpectomy pathological tumor (pT) size. Methods: In this prospective phase II clinical trial (NCT04871516), enrolled patients all received mammography, breast MRI and CT simulation. Target volumes and treatment planning were designed based on the baseline mammography, MRI and CT simulation acquired for each patient, generally using the surgical clip placed at the time of biopsy as the center of the tumor. The GTV was expanded by 0.5 cm to define the clinical target volume (CTV) which was then expanded an additional 0.5 cm to define the planning target volume (PTV). Patients then underwent a pre-operative boost of 13.32 Gy in 4 daily fractions, followed by BCS 1-3 weeks later, and then WBI of 36.63 Gy in 11 daily fractions 3-8 weeks post-BCS. Clinical trial and patient records were reviewed to collect patient demographics, disease characteristics, and treatment details. The largest diameter of the GTV contoured from these baseline imaging studies was compared to the largest pathologic diameter of the tumor removed during BCS using a one-tailed paired sample t-test. Results: A total of 76 patients were included in this analysis. The median age was 64

(range: 40-80). Based on initial biopsy, 64 (84.2%) patients had invasive cancer while 12 (15.8%) had DCIS. Most patients, 70 (92.1%), have ER+ disease, 63 (85.1%) have PR+ disease, and 3 (3.9%) have HER2+ disease. The median clinical tumor size was 12 mm. The median GTV diameter was 22.35 mm (range: 11.1-58.1 mm) and the median pathologic tumor (pT) size was 10 mm (range: 0-34 mm). The GTV diameter was larger than the pT size in most cases (p = <0.001). For 8 (10.5%) patients, the largest diameter of the estimated GTV did not cover the pT diameter by a median of 4.35 mm (range: 0.6-6.8 mm). However, the largest diameter of the clinical target volumes (CTV) contoured for these patients were all greater than the pT size by a median of 9.1 mm (range: 2.7-19 mm). The PTV provided another 0.5 cm margin as well. Notably, for 5 (6.6%) patients, pre-operative boost resulted in no residual disease at time of BCS. Conclusions: For most patients, pre-operative boost GTV delineation resulted in a maximum diameter greater than pT size, suggesting that the GTV covered the entire tumor volume. In the minority of cases where the GTV diameter was not greater than the pT size, the tumor was adequately covered by the CTV with an added 0.5 cm PTV margin. These findings support the feasibility of delivering a pre-operative boost for patients with DCIS and early-stage breast cancer based on mammographic CT and MRI imaging.

P5-09-13: LumiSystem-guided lumpectomy enables informed customization of radiation therapy in select patients with breast cancer.

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Background: For most patients who undergo lumpectomy, adjuvant radiation therapy is standard of care (SOC) following surgery. There is an increasing emphasis on de-escalation or omission of radiotherapy, for which achieving the most complete tumor resection is particularly critical to minimize the risk of recurrence. When used as an adjunct to standard lumpectomy, the LumiSystem has been shown to remove residual cancer missed by the initial surgery and reduce the rate of positive margins. We evaluated whether eligibility for de-scalation or omission of radiotherapy changed based on the LumiSystem intervention. Methods: In this prospective trial, we assessed margin status with and without LumiSystem-guided surgery for patients with stage 0-3 breast cancer. After surgeons completed their standard lumpectomy procedure, patients were randomly assigned 10:1 to receive further LumiSystem-guided surgery for positive signal or not. For patients randomized to the LumiSystem intervention, eligibility for de-escalation or omission of radiotherapy, both after standard lumpectomy and after LumiSystem intervention, were retrospectively determined based on eligibility criteria from RTOG 9804, CALGB 9343, PRIME II, LUMINA, IDEA, BR007/DEBRA and the 2024 ASTRO Partial Breast Irradiation Guidelines. According to these criteria, the population of interest included only patients >49 yr. with various tumor characteristics intended to identify lower risk patients. Results: 357 patients received LumiSystem-guided surgery, and a retrospective chart

review was performed by two independent breast specialized radiation oncologists for the 166/357 (46.5%) patients that had additional LumiSystem-guided excisions taken after standard lumpectomy. 133/166 (80.1%) patients reviewed had negative margins after standard lumpectomy and 77/166 (46.4%) patients (age: median - 66 yr., range - 51-82 yr.) were eligible for omission of radiotherapy after standard lumpectomy based on the aforementioned eligibility criteria. 9/77 (11.7%) patients (age: median 51 yr., range 51-77 yr.) who were eligible for omission of radiotherapy following standard lumpectomy had residual tumor removed in subsequent LumiSystem-guided excisions. These residual tumor deposits included grade 3 histology and ranged from 1-11mm in size. 33/166 (19.9%) patients had a positive margin after standard lumpectomy, and 9/33 (27.3%) of these patients were converted to negative margins intraoperatively by LumiSystem-guided wider negative margins. 3/33 (9.1%) of these patients (age: median - 57 yr., range 54-73 yr.) with positive margins after standard lumpectomy had a change in eligibility for omission of radiotherapy based on LumiSystem-guided wider negative margins, and 2/33 (6.1%) patients also became eligible for smaller volumes of accelerated partial breast irradiation. Discussion: LumiSystem-guided surgery altered radiation therapy recommendations in a subset of patients. Among patients eligible for omission of adjuvant radiotherapy based on SOC negative margins, identification of fluorescence in the lumpectomy cavity resulted in removal of unsuspected residual tumor. These findings support the notion that those patients who historically have recurred without radiotherapy may have had residual disease left behind after standard lumpectomy. Using LumiSystem as an adjunct to standard lumpectomy may lead to the potential of the reducing targeted volume of radiation therapy and the option to omit radiation in additional patients. Long-term follow-up data is needed to determine if the risk of local recurrence is lower in patients undergoing LumiSystem-guided surgery, particularly in those who are candidates for radiation omission.

P5-09-15: Impact of Patient Navigation on Radiation Therapy Completion in Black Breast Cancer Patients: Early Phase I Trial Results From the Navigator-Assisted Hypofractionation (NAVAH) Program

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Purpose/Objective(s): Black breast cancer patients have substantially decreased access to optimal breast conserving cancer care (postlumpectomy radiation therapy) than White patients. Patient navigation (originally implemented in the 1990s to address cancer disparities and medical mistrust) has demonstrated improvement in breast cancer survival, yet has never been formally implemented into the receipt of radiation therapy (RT) for Black patients. The Navigator-Assisted Hypofractionation (NAVAH) program is the first to formally assess the impact of patient navigation on RT in Black breast cancer patients. Here, we present the initial results from an ongoing Phase I trial assessing the impact of NAVAH on RT completion.

Materials/Methods: NAVAH is a prospective single-arm Phase I pilot study. Patients of Black race age > 18 with pathologically confirmed breast cancer following operative resection are eligible. Patients referred for RT in multidisciplinary tumor board, seen by Radiation Oncology, and consented to receive RT were approached for trial participation. Participants were assigned a patient navigator to aid them throughout the course of radiation therapy and post-RT care, and were provided travel vouchers to offset the transportation cost of RT. The RT scheduled dose/fractionation, scheduled completion date, actual completion date, and verification of RT completion was recorded for each enrolled patient. Patients refusing trial participation were assessed to determine RT completion rate; Fisher's exact test was used for statistical analysis with significance assigned as $p<0.05$. The primary trial endpoint is RT completion rate following initiation of patient navigation; this trial is registered at clinicaltrials.gov, NCT05978232.

Results: Between March 27, 2024 and May 28, 2024, a total of 18 patients with scheduled CT simulation for RT planning were offered trial enrollment; 11 accepted and 7 declined. No patient had received navigation prior to being offered trial enrollment. Median fraction duration and total dose received (including boost) was 16 (range 5-20) and 40.05 Gy (range 26-52.56 Gy) among enrolled patients, and 16 (range 5-20) and 40.05 Gy (range 30-52.56 Gy) in those declining enrollment, with no significant difference in patient age or distance from RT facility between those who accepted versus declined trial enrollment. Of the 7 patients who declined trial enrollment, six (85.7%) completed RT (the seventh initially agreed to RT but subsequently failed to present for treatment); of these six, four (66.7%) completed RT without delay. Of the 11 patients who enrolled on trial, all (100%) completed RT, with eight (72.7%) completing RT without delay. The differences between groups were not statistically significant.

Conclusion: Early results of an ongoing Phase I clinical trial reveal that incorporation of patient navigation following initial Radiation Oncology consultation is associated with 100% radiation therapy treatment completion for postoperative early-stage breast cancer in Black patients, compared with lower rates among patients not receiving navigation, as well as numerically superior rates of RT completion without delay. Further work examining incorporation of patient navigation earlier in the treatment course of patients is ongoing.

P5-09-16: Real world experience with Carboplatin plus Nab-paclitaxel as neoadjuvant therapy in patients with early triple negative breast cancer.

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Background: Neoadjuvant therapy (NA) is the base treatment for the majority of patients with early triple negative breast cancer (TNBC). The achievement of pathological complete response (pCR) after neoadjuvant treatment translates into improvements in long term

outcomes. In the last decade, the arrival of new components or formulations have been assessed in clinical trials, such as carboplatin and nab-paclitaxel showing improvements in pCR rates or adverse events profiles. The aim of this study is to provide updated information on the efficacy of Carboplatin plus Nab-paclitaxel combined with other combinations (anthracyclines and immunotherapy) as neoadjuvant therapy in patients with TNBC in the real-world setting.

Methods: We analyzed a cohort of patients treated with neoadjuvant therapy diagnosed with stage I-II TNBC and ER/PR-low status in a single tertiary hospital in Spain from June 2015 to February 2023. TNBC was defined as ER and PR <1% and HER-2 negative. ER/PR-low was defined as an expression of estrogen or progesterone receptors ≤ 10% by immunohistochemistry (IHC). Both Carboplatin (AUC 2) and Nab-paclitaxel (125 mg/m2) were administered on days 1 and 8 every 21 days. Anthracyclines (Doxorubicin or Liposomal Doxorubicin) were administered every 2 or 3 weeks, at the physician's discretion. Pembrolizumab 200 mg was administered every 21 days concomitantly with chemotherapy. The response to NA was classified according to the residual cancer burden (RCB). pCR was defined as ypT0/is/ypN0. Cox's stratified proportional hazard model was used for event free survival analysis. Event free survival was define disease progression that prevented definitive surgery, local or distant recurrence or death from any cause.

Results: A total of 103 patients were included in the analysis. The median age at diagnosis was 53.1 years. The baseline caracteristhics of the patiens are in table 1. Regarding the systemic treatment, 91.2% of the patients were treated with anthracyclines sequentially and in 23% of these (n=30) a liposomal formulation was used. Dose-dense anthracyclines were used in the 23.7% of patients. Concomitant pembrolizumab was used in 27.1%(n=28) of the patients, 60.7% (n=17) of these were stage III at diagnosis. Only one patient did not undergo surgery due to intra-treatment systemic progression. The pCR rate (RCB-0) in the total population was 54.3%. Patients with residual disease after NA (n=47) were classified as RCB-I (17.4%), RCB-II (18.4%) and RCB-III (8.7%). The pCR rates according to different features is reported in Table1. The germline mutation carriers in HRD genes (n=14) obtained a pCR rate of 71,4%. In patients with concomitant use of pembrolizumab, the pCR rate was 60.7%. The pCR rate in TNBC and ER/PR-low groups were 54.2% and 55% respectively. The median follow-up time was 27.7 months. Tha hazard ratio for event of progression between pCR vs residual disease groups was 0.21, 95%CI: 0.06 to 0.76; (p=0.018), with 3 events in the RCB-0 group (n=53) and 12 events in the residual disease group (n=47).

Conclusion: In the real world setting, the use of carboplatin-nabpaclitaxel plus other combinations, such as anthracyclines and immunotherapy obtain a higher rate of pathological complete response translating to improvements in long term outcomes.

P5-09-17: Patterns and Predictors of Recurrence After Neoadjuvant Chemo-Immunotherapy in Early-Stage Triple-Negative Breast Cancer

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Background: Adding pembrolizumab to neoadjuvant chemotherapy (NAC+P) for stage II-III triple-negative breast cancer (TNBC) improves pathologic complete response (pCR) rates and event-free survival compared to neoadjuvant chemotherapy (NAC) alone. Recurrence patterns and factors associated with disease-free survival (DFS) in a real-world cohort are not known. We compared recurrence rates and assessed factors associated with DFS in patients receiving NAC+P versus NAC for early-stage TNBC.
Methods: This retrospective analysis compared two consecutive cohorts of patients with stage II-III TNBC: 344 treated with NAC+P from 6/1/21-4/30/24 based on the KEYNOTE-522 regimen and 513 treated with NAC from 7/1/09-3/15/19. Demographic, clinicopathologic characteristics, and recurrence data were collected. Tumor infiltrating lymphocytes (TILs) were scored on pre-treatment core needle biopsy for NAC+P only. Primary outcomes were local recurrence (LR), locoregional recurrence (LRR), and distant recurrence (DR). Secondary outcomes were DFS and factors associated with DFS. Kaplan-Meier time-to-event analyses assessed for DFS. Univariate and multivariable Cox regression models assessed for factors associated with DFS.
Results: In the NAC+P group, most patients presented with cT2 (70%), cN0 (53%) disease at a median age of 52 years (IQR 41, 62). In the NAC group, most presented with cT2 (48%) or cT3/4 (28%), cN1 (52%) disease at a median age of 51 years (IQR 41, 60). Rates of breast, nodal, and overall pCR (58% vs 36%, p<0.001) were higher with NAC+P. At 1-year, crude LR rates were not different between NAC+P and NAC: 2.3% vs 4.3%, p=0.13. However, 1-year LRR and DR rates were lower with NAC+P: LRR (0.6% vs 4%, p=0.01) and DR (8% vs 12%, p=0.03). Thirty-one NAC+P patients had DR. The most common sites of first progression were liver (29%), lung (29%), or brain (26%). Among 121 NAC patients with DR, the most common sites of first progression were multiorgan (50%), lung (19%), or liver (12%). Of 61 patients with multiorgan DR, 36 (59%) had brain metastases.
At a median follow-up of 1.2 years (IQR 0.8, 1.6), the 1-year disease recurrence rate for NAC+P was 10% (95% CI: 6%-13%). With a median follow-up of 6 years (IQR 3.3, 7.7), the 5-year disease recurrence rate for NAC was 24% (95% CI: 20%-28%). Factors associated with worse DFS for NAC+P included extreme breast density (HR 3.78, p=0.004) and cN1 disease (HR 2.68, p=0.04). On univariate analysis only, cN2/3 disease and comprehensive RT were associated with worse DFS, while lower cT stage, immune-related adverse events, breast pCR, nodal pCR, and overall pCR were associated with improved DFS. Using a 10% cutoff, stromal TILs on pre-treatment core needle biopsy were not associated with DFS. Factors associated with improved DFS for NAC were cT2 vs cT3/T4 (HR 0.56, p=0.01), ACT-based regimen (HR 0.61, p=0.02), and overall pCR (HR 0.32, p<0.001), while lymphovascular invasion on biopsy (HR 2.1, p<0.001) was associated with worse DFS. Univariate associations for worse DFS included cN1 and cN2/3 disease.

Conclusions: Compared to NAC, NAC+P resulted in improved crude 1-year rates of LRR and DR, along with numerical improvements in DFS with shorter follow-up. Factors associated with DFS (including pCR) were not similar across cohorts and isolated brain metastases as a site of first progression were more common in patients treated with NAC+P who developed DR.

P5-09-18: Real-World Analysis of Patients with Triple-Negative Breast Cancer and Germline BRCA Mutation Undergoing Neoadjuvant Treatment Following the Keynote 522 Protocol

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Background: Triple-negative breast cancer is the most immunogenic and the most prevalent among patients with germline BRCA1/2 (gBRCA) mutations. Regardless of the mutation, the current treatment for initial localized triple-negative breast cancer is based on chemotherapy combined with pembrolizumab, following the KEYNOTE 522 protocol.
Methods: We collected data from patients diagnosed with stage II or III triple-negative breast cancer who were treated according to the KEYNOTE 522 protocol at A. C. Camargo Cancer Center, Brazil, from January 2022 to December 2023. We identified patients with gBRCA mutation and analyzed their clinical, pathological, and treatment characteristics and outcomes, comparing them with the general population.
Results: We analyzed 118 patients, of which 19 (16%) had a gBRCA mutation, 90% gBRCA1 and 10% gBRCA2. The average age of patients was 40 years versus 43 years in the cohort without BRCA1/2 mutations. Additionally, we observed differences in menopausal status (pre-menopausal 84% vs. 55%), histological grade 3 (HG3) (84% vs. 78%), HER2-low rate (62% vs. 15%), KI67 >20% (100% vs. 94%) between patients with and without gBRCA mutation. Average BMI (28 kg/m^2 vs. 26 kg/m^2) and obesity frequency (21% vs. 26%) were similar in the two subgroups. According to the stage, there was no difference in T stage, N stage, or grouped clinical staging. Regarding the treatment regimen, 13 (70%) received neoadjuvant ACdd, compared to the cohort of non-gBRCA mutated patients 65 (53%). Pathological complete response (RCB-0) was 58% vs. 62%. There was a higher incidence of reported adverse events in patients with gBRCA1/2 mutation (68% vs. 52%), but hospitalization rates were the same.
Conclusion: The cohort of patients with gBRCA consists of slightly younger patients with a higher percentage of pre-menopausal status and with high BMI. They have a higher likelihood of high-grade HG3 tumors, KI67 > 20%, and HER2-low tumors larger tumors T3/T4, and stage III. Interestingly, the likelihood of achieving pathological complete response (RCB-0) was lower in patients with gBRCA, and they had a higher rate of adverse effects.

P5-09-19: Survival outcomes of carboplatin plus taxanes neoadjuvant chemotherapy in triple negative breast cancer and cell-free DNA whole methylome based biomarker analysis: A prospective multi-center cohort study

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Background: The survival outcomes of carboplatin plus taxane neoadjuvant chemotherapy(NAC) in triple negative breast cancer(TNBC) was lack of a large-sample study. The predictive biomarker of efficacy based on cell-free DNA (cfDNA) whole-methylome sequencing (WMS) has not been reported yet.

Methods: The prospective multi-center cohort study was conducted in four hospitals of China between 2016 and 2023. Stage II-III TNBC patients were enrolled to receive NAC of carboplatin (AUC 5) every 3 weeks or carboplatin (AUC 4) every 2 weeks plus taxane(standard dose) for 4-6 cycles. Plasma samples were prospectively collected at baseline(T1) and end of NAC (T2). Chromosomal aneuploidy of featured fragments (CAFF), fragment size index (FSI) and methylation density score (MD) of cfDNA were detected with WMS. The primary endpoint were relapse-free survival (RFS) and exploratory biomarker analysis.

Result: A total of 267 patients were included in the study. The median age was 49 years, 156 patients (58.4%) were stage III disease. cT3-T4 were 28.1% (75/267), cN3 were 17.2% (46/267). 40.3% patients (106/263) achieved pathologic complete response (pCR). The 3-year RFS and overall survival (OS) were 77.9%, 87.6%, respectively. Patients who achieved pCR had a significant better RFS(95.5%) and OS(97.7%) than those non-pCR (68.5%, 81.2%, all $P < 0.001$). Survival of patients with minimal residual disease (ypT1mi/1a/1b N0) are comparable to those pCR, 3-year RFS and OS were 80.2% vs 92.3%(Log-rank P=0.060), 90.5% vs 97.4% (Log-rank P=0.247), respectively, after excluding cN3 patients. Patients with residual node number >2 had a poor 3-year RFS and OS compared to number≤2(All Log-rank $P < 0.0001$). A total of 66 patients with 120 plasma samples (64 samples at T1, 56 samples at T2) were included in WMS analysis. Patients with CAFF, FSI or MD positive at T1 had a higher tumor burden (stage III or cN2-3, all P values <0.05). The proportion of patients with FSI negative at T2 was significantly higher in pCR group compared to non-pCR(86.2% vs 59.3%,P=0.034). Similar tendency were observed in patients with CAFF negative. A linear SVM model was developed to predict pCR with an AUC of 0.90 in the training datase and an AUC of 0.86 in the testing dataset. Moreover, patients with MD positive at T1 was significantly associated with poor RFS compared to MD negative (HR = 7.36, Log-rank P=0.028).
Conclusion: Our large-sample study further confirmed that carboplatin plus taxanes as NAC in TNBC was a preferred regimen with the comparable survival outcome, especially for

patients who cannot tolerate anthracyclines or immunotherapy. Biomarkers based on cfDNA WMS may provide predictive and prognostic information, warranting further investigation.

P5-09-20: The impact of HER2 expression in early-stage triple negative breast cancer

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Background: Whether the expression of human epidermal growth factor receptor 2 (HER2) has an impact on the clinical-pathological characteristics and on outcomes in early-stage, triple negative breast cancer is unknown.
Methods: A single center, retrospective cohort study comprising all patients diagnosed with early-stage triple negative breast cancer between 2012-2022. Data on clinicopathological characteristics and outcomes, including disease free survival (DFS) and overall survival (OS), were extracted. Data on systemic therapy, pathological response in patients who were treated with neoadjuvant chemotherapy and type of surgery were also extracted. Differences between patients with HER2-low (defined as HER2 immunohistochemical staining of +1 or +2, FISH negative) to HER2-0 (defined as no staining for HER2) were evaluated.
Results: After exclusion 41 patients whose intensity of HER2 expression was not reported explicitly, 93 patients were included in the final analysis: 56 patients with HER2-0 disease and 37 patients with HER2-low disease. Median age was 50, 41% were BRCA carriers, 56% presented with clinical T1, 48% had clinical node positive disease, 85% had grade 3 disease, 38% had presence of lymphovascular invasion and 91% had ductal carcinoma. Age, BRCA status, and clinico-pathological characteristics were comparable between the groups. Neoadjuvant chemotherapy was given to 57%, adjuvant chemotherapy to 33% and 10% did not receive chemotherapy. Complete pathological response was achieved by 64% of the patients who were treated with neoadjuvant chemotherapy. 60% underwent lumpectomy, 40% underwent mastectomy and 28% completed axillary lymph node dissection. HER2 expression was not associated with systemic therapy, the response to neoadjuvant chemotherapy and type of surgery. There was also no difference in clinical outcomes, with 3-year OS rate of 86.1% and 89.1% for HER2-low and HER2-0, respectively ($p = 0.933$) and 3-year DFS rate of 82.8% and 79.5% for HER2-low and HER2-0, respectively ($p = 0.620$).
Conclusions: In this early-stage, triple negative cohort there were no differences between HER2-low to HER2-0 with respect to clinic-pathological characteristics, treatment approach and outcomes. More research is warranted to further investigate the impact of HER2 expression and to identify whether treatment approach should be tailored based on HER2 expression in early-stage triple negative disease.

P5-09-21: Hidden Challenge: Atypical Ductal Hyperplasia in Vulvar Ectopic Breast Tissue

Yolcar Chamorro, Niloofar Nassseri-Nik, Ana C. Sandoval-Leon

Introduction: Breast tissue develops during embryogenesis along the milk line, extending from the axilla to the perineum. Although rare, ectopic breast tissue (EBT) can persist anywhere along this line, most commonly the axilla. Similar to breast tissue, EBT is influenced by hormonal changes. Benign tumors, particularly fibroadenomas, are the most frequent pathology associated with EBT. Breast cancer (BC) in EBT is uncommon (0.3%-0.6% incidence), mostly affecting axillary EBT. Diagnosis is challenging due to varied differential diagnoses, including skin pathologies. Atypical ductal hyperplasia (ADH) is noncancerous but is classified as a high-risk lesion due to its association with a fivefold increase in BC risk. To our knowledge, ADH of ectopic breast tissue in the vulva has not been previously described in the literature.

Case presentation: A 63-year-old postmenopausal woman had a lump in her labia for several years. She had past medical history of endometrial polyps and family history of breast cancer in her mother and in her sister. Upon physical exam, she had a 5mm cystic lesion in the right labia majora. She was evaluated by her gynecologist for endometrial polyps, and she asked to remove the vulvar cystic lesion during the procedure. Pathology of the cystic vulvar lesion revealed EBT with foci of ADH. The mass was immunoreactive for GATA3, estrogen receptors, and progesterone receptors. Myoepithelial cells were identified via p63/SMM staining. The diagnosis of ADH was supported by the pattern of staining for CK5 and estrogen receptors. A stain for PAX8 and ERG was negative. The mass incompletely excised and additional surgery was recommended. Upon re-excision, no residual ADH or EBT were seen.
Due to these findings, she was referred to the Breast Cancer Prevention Clinic. The decision was to have updated breast imaging with a mammogram that showed that the breasts were almost entirely fat and no lesions were seen. We also recommended a breast MRI that was also negative. Due to her family history, she had genetic testing that was negative. Her lifetime risk of developing BC was calculated using the Tyrer-Cuzick model and it was less than 20%. For this reason, she was not recommended to have additional breast MRIs. Chemoprevention was discussed with the patient and was not recommended.

Discussion: Ectopic mammary tissue found along the embryonic milk lines is rare. It occurs in 0.22%-6% of women most commonly found in the axilla with less frequent occurrences in the vulva. Variability in presentation depends on the degree of breast tissue development along the milk line. Diagnosis requires histological examination, as these tissues may be mistaken for lipomas or malignant lesions. In our case, immunoreactivity for GATA3, estrogen, and progesterone receptors confirmed breast tissue origin, while negative PAX8 and ERG stains ruled out Müllerian and vascular neoplasms, respectively. The etiology of ADH within ectopic tissue remains unclear. There are currently no established guidelines on

how to screen, treat, or follow-up patients with atypical lesions in ectopic breast tissue. Our treatment approach was excision of the ADH and EBT, which aligns with the standard of care for ADH in the breast. Since patients with ectopic breast tissue were not included in the chemoprevention trials, we do not recommend chemoprevention in these patients.

Conclusion: This case underscores the importance of considering ectopic mammary tissue in vulvar masses and highlights the need for further research to establish management guidelines. Additionally, this report illustrates the value of histological and clinical correlation in indeterminate tumors that are along the mammary line.

P5-09-23: Programmed death-ligand 1 expression in a Mexican cohort of triple-negative breast cancer

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Programmed death-ligand 1 (PD-L1) expression predicts response to immunotherapy in a range of malignancies, including triple-negative breast cancer (TNBC). However, observational studies show a significant variability in the rates of PD-L1 positivity in TNBC. Currently, PD-L1 expression rates remains unknown in the Mexican population.
A retrospective multicenter study of women with newly diagnosed TNBC between 2006 and 2021 was carried out. PD-L1 was assessed at a central laboratory using the IHC 22C3 assay. Expression was characterized according to the combined positive score (CPS), with positivity defined as >1%. Descriptive statistics were employed to calculate means, percentages, and standard deviations.
Among 298 cases with available tissue for analysis, 285 (96%) samples had sufficient cellularity for adequate CPS evaluation. In the entire cohort, the PD-L1 positivity rate was 29.1%, while 13.3% had a CPS >10%. The prevalence of PD-L1 positivity differed significantly according to histological grade, percentage of tumor-infiltrating lymphocytes, and the tissue type analyzed.
The majority of CPS evaluations were performed on tissue from biopsies of the primary tumor, which can underestimate PD-L1 positivity by 20-30% when compared to matched surgical specimens. Furthermore, biopsies from metastatic sites were not analyzed for PD-L1 expression, which can impact overall CPS positivity rates. Our data underscore the importance of considering the source of the tissue sample when interpreting PD-L1, as different types and sites of biopsy may yield different expressions.

P5-09-24: Neoadjuvant (NA) immunotherapy (IO) combined with chemotherapy in triple negative breast cancer (TNBC): A UK real world experience from three centres.

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Background: The KEYNOTE (KN)-522 study, a practice-changing phase III trial, demonstrated improved outcomes with the addition of pembrolizumab (P) to Paclitaxel (T) & Carboplatin (C) then to Epirubacin (E) & Cyclophosphamide (Cy) in high-risk early-stage TNBC. While highly effective with unprecedented pathologic complete response (pCR) rates and clinically meaningful improvements in event-free survival, it comes with significant high-grade treatment-related toxicity.

Methods: This is a retrospective, multicentre, longitudinal cohort study. Data on 92 patients (pts) who received the treatment from December 2022 to November 2023 were analysed focusing on efficacy and toxicities. In this analysis, pCR is defined as no invasive residual in the breast or nodes (i.e ypT0/is ypN0). Statistical analysis was done using SPSS program.

Results: The cohort median age was 52 years (26-80) with majority (74%, n=68) of Caucasian ethnicity. Invasive ductal carcinoma (IDC) was the predominant histology (97%, n=89). Grade (G) 2 and G3 documented in 35% (n=32) and 65% (n=60) respectively. HER-2 low (1+ or 2+/in situ hybridization (ISH) negative phenotype) was noted in 39% (n=36) of pts.

All pts received first block of the treatment (P/T/C), with only 89% (n=82) completed four cycles. While 78% (n=72) who started second block (P/E/Cy), only 57% (n=52) completed the whole NA treatment. Reasons for non-completion varied, including intolerance (n=22, 24%), excellent response (n=8, 9%), local progression (n=2, 2.2%), patient choice (n=4, 4.3%) and other causes (n=4, 4.3%).

pCR was achieved in 53% (n=49) of the pts. No residual in the axillary nodes (ypN0) were observed in 76.1% (n=70).

Among the adverse events observed, neutropenia was the most common, affecting 89.1% (n=82 pts), with 43.3% (n=40 pts) experienceing severe grade (G) 3/4. Anaemia was also prevalent, occuring in 85.9% (n= 79 pts) but only 10.9% (n=10 pts) were severe (G 3/4). Other notable adverse events include fatigue (75%, n=69 pts), transaminitis (45.6%, n=42 pts) and low platelets (34.8%, n=32 pts) with severe G 3/4 represent 1.1% (n=1 pt), 6.5% (n=6 pts) and 2.2% (n= 2 pts) respectively. Less frequent adverse events were adrenal insufficiency (8.7%, n=8 pts), hypothyroidism (20.7%, n=19 pts), and rash (19.6%, n=18 pts), with minimal severe cases reported.

About 32% (n=29) of pts were admitted to the hospital during the treatment (average 2

times) with median length of stay of 3 days (1-45). Infection (including neutropenic sepsis) was the most documented diagnosis in 16 (4) occasions.

Conclusion: In comparison to KN-522, a lower pCR rate was observed in our cohort of pts with a higher haematological toxicities. Small sample size and retrospective analysis are the limiting factors.

P5-09-25: Immunotherapy in Neoadjuvant Treatment of triple-negative breast cancer: First Insights and Treatment Monitoring with MRI - Single Institution Retrospective Analysis

Bibiana Vertakova Krakovska, Lucia Vanovcanova

Background: Neoadjuvant immunochemotherapy represents an essential tool for the treatment of triple-negative breast cancer (TNBC). This approach has resulted in increased rates of pathologic complete response (pCR) compared to chemotherapy alone, however, with numerous serious side effects. Factors influencing the response to this regimen remain poorly characterized. There is a lack of accurate imaging tools to monitor early response to immune checkpoint inhibitor (ICI) therapy to help tailor the following therapeutic strategies for patients without response and avoid the toxicity associated with ineffective treatment.
Methods: In Slovakia, full access to pembrolizumab for the therapy of TNBC started on April 1, 2024. From September 2023 to June 2024, 14 patients (median age 47) with TNBC received neoadjuvant chemotherapy (NACT) and immunotherapy (pembrolizumab) according to the Keynote-522 study regimen. Eight patients (57.1%) received NACT with pembrolizumab initially, while 6 (42.9%) received it sequentially due to local reimbursement rules. Biopsies revealed 13 invasive ductal carcinomas and one invasive lobular carcinoma, all with high Ki-67 expression (median 55%). Tumor-infiltrating lymphocyte (TIL) levels were examined in 10 patients (2 with <30%, 4 with 30-40%, and 4 with >40%). MRI scans were performed before the first cycle of NACT alone or with pembrolizumab. Interim MRIs were conducted after 1., 2, or 3. cycle of pembrolizumab. The following parameters were recorded and assessed: tumor response (according to iRECIST), lymph node (LN) response (axillary ipsilateral/contralateral, internal mammary, intramammary), among other changes.
Results: Tumor responses were as follows: radiologic complete response (rCR) as well as radiologic partial response (rPR) in 6 patients (42.86%), radiologic non-response (rNR) and pseudoprogression in one patient (7.14%). LN responses showed an enlargement in 9 patients (64.29%): axillary ipsilateral in 4, bilateral in 2, contralateral in 1, and internal mammary in 2 patients. One patient exhibited axillary and intramammary LN enlargement, while another with inflammatory carcinoma also showed progression of skin oedema.
Conclusions: Breast MRI is a powerful tool to monitor tumour response to ICI, although it brings several associated challenges. Though relatively rare in breast cancer compared to melanoma or lung cancer, primary tumour pseudoprogression can occur as a response to

immunotherapy. As evident from our results, ICI's significant impact on LN can confuse the imaging via MRI (e.g., axillary and intramammary lymph nodes). The enlargement of axillary LN imitates the progression of the disease and results in inappropriate restaging, while activated intramammary LN may be incorrectly classified as a satellite lesion. In patients with inflammatory carcinoma, the lymphocyte infiltration of the skin is displayed in MRI as progressive thickening and oedema. Due to the short history of this unique treatment and limited experiences with immunotherapy response presentation in breast MRI, this method may be slightly less reliable. Our results show that MRI has a solid potential to remain the best method for treatment monitoring. However, further research is needed to refine imaging techniques and improve the interpretation of MRI findings.
Keywords: Triple-negative breast cancer, immunotherapy, neoadjuvant treatment, MRI, pathologic complete response, pseudoprogression, lymph nodes.

P5-09-26: KEYNOTE-522 Regimen Outcomes: Real World Experience from a Single Institution with a Predominantly African American Population

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Background and Objectives: High-risk early triple-negative breast cancer is frequently associated with early recurrence and high mortality. [1] In 2020, the Keynote 522 trial changed the way high-risk early triple-negative breast cancer (TNBC) is managed. It showed that the addition of pembrolizumab to neoadjuvant chemotherapy improved the pathological complete response rate (pCR) compared to placebo with neoadjuvant chemotherapy. It has been reported in the literature that TNBC disproportionately affects the Black population in the United States. [2] In the Keynote 522 trial, approximately 4.8% of patients in the pembrolizumab with chemotherapy arm were African Americans (AA). In our institution, most of the patient population are AA or Blacks. Limited data have suggested a pCR rate of around 50% in the AA population. Thus, we conducted a retrospective study in our institution to evaluate the pCR rate in AA female patients who received the Keynote 522 regimen as part of their early breast cancer treatment.

Methods: We conducted a chart review of adult patients (> 18 years) who were diagnosed with TNBC between January 2020 to May 2024 who received the Keynote 522 regimen. We recorded demographic data including age, race, ethnicity, and clinical parameters such as the stage of breast cancer, hormone receptor status, HER2 receptor status, menopausal status, performance status, pCR rates, and complications leading to discontinuation of treatment. Computations were performed using SAS statistical software.

Results: We identified 24 patients with TNBC who received the Keynote 522 regimen during the specified period. Of the 24 patients, 75% were AA and 25% were Caucasian. In our study, 41.6% (two-sided p-value 0.6653) of patients had pCR overall. Specifically, 38.8% of African American patients and 50% of Caucasian patients had pCR. Regarding age, 14% of patients younger than 50 years and 52% of patients older than 50 years had pCR, with a two-sided p-value of 0.1718. All patients had a performance status of 0 or 1. The pCR rate

was 41.67% (two-sided p-value: 1.000) regardless of performance status. All patients had either stage II or III, except one who had stage IV. The pCR rate in stage II patients was 44.44%, and in stage III patients, it was 40%, with a two-sided p-value of 1.000. Only three patients had to discontinue the Keynote 522 regimen due to side effects (hepatitis, dermatitis, sclerodactyly, and hepatitis). No pCR was achieved in the three patients who discontinued Keytruda.

Conclusion: In this real-world analysis at a single institution with a predominantly AA population, total pCR rates were lower (41.6%) compared to the original study (64.8%)3. pCR rates were lower in the African American patient population compared to Caucasians. The results are not statistically significant, which is likely due to the small sample size. The original study included only 4.8% African American patients, and a subset analysis was not performed to evaluate pCR rates in this subgroup. Thus, we conclude that larger studies including more AA patients might be beneficial to evaluate the efficacy of the Keynote 522 regimen in this subset of the population.

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P5-09-27: Neoadjuvant versus adjuvant systemic therapy in Stage I triple negative breast cancer - An institutional review

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Background: Most patients with early-stage triple negative breast cancer (TNBC) receive neoadjuvant treatment (NT). With the data from the KEYNOTE-522 most Stage II and III TNBC patients receive NT. However, whether Stage I patients require NT or adjuvant treatment (AT) is debatable. We performed a retrospective analyses of Stage I TNBC who received NT or AT at our institution.

Methods: We reviewed patients >18 years age who were diagnosed with clinical Stage I based on either mammogram or ultrasound (US) histologically proven invasive TNBC from January 1, 2018 until December 31, 2022. All patients must have received at least one cycle of systemic treatment, whether in the NT or AT setting. All patients must have undergone definitive surgical treatment. We analyzed patients till December 31, 2024. We used Kaplan-Meier estimates for overall survival (OS) and progression-free survival (PFS) and Cox-Proportional Hazards Model to assess treatment effect on OS and PFS.

Results: We analyzed 72 patients (52 AT and 20 NT arms) and the patients in the NT arm were much younger than the AT arm (51.4 years vs 65.6 years respectively). Invasive ductal

cancer was the most common histology (94% AT vs 80% in NT arm), while the rest were metaplastic (6% AT vs 20% NT) In the AT arm 9 patients were upstaged after surgery and 50% achieved a pathological complete response (pCR). Comparing AT vs NT, there was no statistically significant improvement in PFS (72.5% vs 73.4% p=0.79) or OS (74% vs 74.5%, p=0.43 However, we found that tumor size is a significant predictor for the risk of recurrence with HR 1.13 (p-value=0.003, 95% CI 1.05-1.22) times higher risk of recurrence when tumor size is more than 10mm, with patients receiving AT doing better.

Conclusion: In Stage I TNBC there is no survival difference between NT or AT treatments. We did notice an improvement in PFS in patients with T1c tumors receiving AT. Prospective trials and trials incorporating immune checkpoint inhibitors can help us answer these questions.

P5-09-28: A phase I, prospective exploratory clinical study of neoadjuvant pyrotinib plus trastuzumab, dalpiciclib, and letrozole for patients with HR-positive, HER2-positive early or locally advanced breast cancer

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Background: Triple-positive breast cancer (TPBC) is a breast cancer characterized by positive expression of ER, PR and HER2. The first choice of neoadjuvant treatment is anti-HER2 targeted therapy combined with chemotherapy, and the pCR rate is lower than that of HR-/HER2+ breast cancer. Therefore, it is still necessary to explore new treatment models for TPBC patients.This study aims to explore the efficacy and safety of pyrotinib plus trastuzumab, dalpiciclib, and letrozole as neoadjuvant therapy in patients with TPBC.
Methods: This is a single-arm, open-label study that enrolled breast cancer patients with early or locally advanced TPBC and an ECOG performance status of 0 to 1. After enrollment, patients first received 2 cycles of pyrotinib (320 mg po qd, d1-28) plus trastuzumab (8 mg/kg in the first cycle, 6 mg/kg in the second to eighth cycles, intravenous drip, q3w) , dalpiciclib (150 mg po qd, d1-21, q4w) and letrozole (2.5 mg po qd, q4w) as neoadjuvant therapy, Tumour response was assessed by investigators according to RECIST 1.1 using MRI, If patients achieved a confirmed partial response (PR) after 2 cycles, they would continue to receive an additional 4 cycles until surgery; patients who did not achieve PR were treated with pyrotinib, trastuzumab and chemotherapy selected by the doctor for 4 cycles until surgery.The primary endpoint was the total pathological complete response rate (tpCR: ypT0/is, ypN0), and the secondary endpoints were objective response rate (ORR) and safety. This study is registered with ClinicalTrials.gov (NCT05800756).
Results: From August 2022 to June 2024, 30 patients were assessed for eligibility, Among the 20 patients completed neoadjuvant therapy and surgery, 17 patients (85.00%) were immunohistochemical HER2 3+. Among the 20 patients who completed 6 cycles of

neoadjuvant therapy and underwent surgery, the overall response rate (ORR) was 100% (20/20). Postoperative MP grading evaluation: tpCR and bpCR were both 20% (4/20), the complete remission rate of positive lymph nodes was 13/19 (68.42%); RCB (0-I) patients were 7/20 (35.00%). The breast conservation rate was 30.00% (6/20), and the sentinel lymph node biopsy rate for downstaging was 35.71% (5/14). Compared with before neoadjuvant therapy, the Ki67 positive rate was reduced after neoadjuvant therapy (41.67±18.48% vs 19.67±21.89%, p=0.003). The most common adverse events were diarrhea, oral mucositis, and decreased neutrophil counts, with diarrhea occurring in 95.00% (19/20), 10 patients (50%) with grade ≥3, and no grade 4 or higher adverse events. Conclusion: Pyrotinib plus trastuzumab, dalpiciclib, and letrozole as neoadjuvant therapy in patients with early or locally advanced TPBC has a high ORR rate, which is conducive to downstaging surgery and breast conservation, and a high complete remission rate of positive lymph nodes, which is conducive to downstaging sentinel lymph node biopsy to preserve axillary function.In conclusion, the combination of pyrotinib plus trastuzumab, dalpiciclib, and letrozole has demonstrated good efficacy and safety, which may be an alternative option instead of neoadjuvant chemotherapy for patients with early or locally advanced TPBC.

P5-09-29: Trastuzumab Combined with Pyrotinib and Capecitabine as Postoperative Adjuvant Therapy in Non-Pathological Complete Response HER2-Positive Early Breast Cancer Following Neoadjuvant Therapy: A Multicenter Phase II Study

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Background: HER2-positive early breast cancer patients achieving a pathological complete response (pCR) during neoadjuvant therapy demonstrate a more favorable prognosis. The KATHERINE study indicated that for HER2-positive early breast cancer patients who did not achieve pCR (residual tumor lesions present) after neoadjuvant therapy, the administration of TDM-1 during postoperative adjuvant therapy significantly reduces the risk of disease recurrence compared to trastuzumab. This study aims to evaluate the efficacy and safety of trastuzumab in combination with pyrotinib and capecitabine in HER2-positive early breast cancer patients who did not achieve pCR following neoadjuvant therapy.

Methods: This single-arm, open-label, multicenter study enrolled patients with HER2-positive early breast cancer who did not achieve pCR (defined as residual invasive tumor >1 cm or lymph node metastasis) following neoadjuvant therapy. Eligible patients received trastuzumab (initial loading dose of 8 mg/kg followed by 6 mg/kg every three weeks for one year, encompassing both neoadjuvant and adjuvant settings), pyrotinib (400 mg/day

for one year post-surgery), and capecitabine (1000 mg/m^2 twice daily for six 3-week cycles, with continuation at the investigator's discretion beyond six cycles). Primary prophylaxis with loperamide was administered to mitigate pyrotinib-induced diarrhea. The primary endpoint was 3-year invasive disease-free survival (iDFS), with secondary endpoints including distant disease-free survival (DDFS), overall survival, and safety. This study is registered with ClinicalTrials.gov (identifier NCT05292742).

Results: From July 2021 to November 2023, a total of 102 patients were enrolled, all of whom did not achieve pCR following neoadjuvant therapy. As of May 31, 2024, with a median follow-up of 11.96 months, 8 iDFS events (all local recurrences or bone metastases, without distant metastases) and 4 DDFS events were reported. Regarding safety, 22 patients (21.6%) experienced grade ≥3 adverse events, with the most common being diarrhea (13.7%), white blood cell count decreased (2.0%), and hand-foot syndrome (1.0%). Adverse events led to the interruption of any agent in 66 patients (64.7%), discontinuation of any agent in 8 patients (7.8%), and dose reduction of any agent in 37 patients (36.3%).

Conclusions: For patients with HER2-positive early breast cancer who did not achieve pCR (residual invasive tumor >1 cm or lymph node metastasis) following neoadjuvant therapy, the combination of pyrotinib, trastuzumab, and capecitabine as postoperative adjuvant therapy appears to improve prognosis. The overall safety profile of the regimen was acceptable, with no new safety signals identified. Primary prophylaxis with loperamide effectively reduced the incidence of grade ≥3 diarrhea during adjuvant therapy with pyrotinib.

P5-10-01: Demographic, Clinical Characteristics, and Overall Survival of Patients with Metastatic Breast Cancer and Brain Metastases: A Single Center Retrospective Cohort Study

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Background: Approximately 30% of patients with metastatic breast cancer (MBC) develop brain metastases (BM). It is critical to better understand the risk factors, natural history, and treatment outcomes, including patients in a modern cohort.
Methods: In this single center retrospective cohort study, we identified patients with MBC and BM diagnosed between 1997-2024. Review of medical records and institutional cancer registry were completed to identify key demographic and clinical characteristics, treatment history, and real-world overall survival (rwOS).
Results: We identified 484 patients with MBC and BM. Most patients were female (n=481, 99.4%) with a median age of 47.4 years (range 25-92 years) at the time of MBC diagnosis. Most patients had ductal histology (n=391, 81.0%). Patients had the following breast cancer

subtypes: HR+/HER2- (n=187, 38.6%), HER2+ (n=182, 37.6%), and triple negative breast cancer (TNBC; n=115, 23.8%). Median time from diagnosis of MBC to first BM was 8.1 months (range 0-177.1 months) and varied by MBC subtype, with longer median time for patients with HR+/HER2- (16.2 months) than for patients with HER2+ (8.1 months) or TNBC (2.1 months) ($p<0.01$). Patients had the following number of BMs at initial BM diagnosis: 1 BM (n=129, 26.7%), 2-5 BMs (n=183, 37.8%), 5-10 BMs (n=42, 8.7%), and >10 BMs (n=84, 17.4%). 18.6% of patients (n=90) developed leptomeningeal disease (LMD) in addition to BMs; median time from first BM to LMD was 6.6 months (range 0-61.7 months). After BM diagnosis, most patients received central nervous system-directed locoregional therapy (n=474, 99.1%), including BM surgical resection (n=119, 23.7%), stereotactic radiation therapy (n=401, 82.7%), and/or whole brain radiation therapy (n=218, 46.0%). Patients received a median of four lines of systemic therapy for MBC (range 1-13; HR+/HER2- median 5, HER2+ and TNBC median 3).
Among all patients, median rwOS from the diagnosis of first BM to death was 21.7 months (range 0.2-224.9 months) with the longest median rwOS in patients with HER2+ disease (median 30.9 months) vs. patients with HR+/HER2- (median 19.6 months) or TNBC (13.3 months) ($p<0.01$). By date of BM diagnosis 1997-2014 vs. 2015-2024 (divided by ~50% of patients in each time period), patients with HER2+ and TNBC lived longer in the more modern cohort compared to prior years (HER2+: 41.2 vs. 26.5 months, $p<0.001$; TNBC 16.2 vs. 9.0 months, $p=0.03$); there was no statistically significant difference for patients with HR+/HER2- disease (18.2 vs. 20.4 months, $p=0.37$). Patients with both BM and LMD had a shorter median rwOS from time of first BM compared to patients with BM only (18.5 vs. 21.6 months, $p=0.048$). Median rwOS was similar in patients with ductal vs. lobular histology (21.9 months vs. 21.5 months, $p=0.98$). On multivariable analysis, HER2+ disease (HR 0.63, 95% CI 0.48-0.84, $p=0.002$), stereotactic radiation therapy (HR 0.55, 95% CI 0.40-0.75, $p<0.001$), and/or BM surgical resection (HR 0.51, 95% CI 0.38-0.70, $p<0.001$) were associated with longer survival and having 6-10 brain metastases at baseline (HR 1.94, 1.22-3.09, $p=0.01$) was associated with shorter survival.
Conclusion: In a cohort of nearly 500 patients with MBC and BM spanning >25 years, median rwOS from the diagnosis of first BM to death was 21.7 months. Patients with HER2+ disease and those who underwent BM resection and/or SRS had an improved prognosis, and patients with a greater number of BMs had a worse prognosis. Patients with HER2+ and TNBC lived longer from the time of first BM to death in a more modern cohort compared to prior years. In contrast, there was no statistically significant difference in rwOS in patients with HR+/HER2- BMs over time, suggesting that this is an area of ongoing unmet clinical need.

P5-10-03: Impact of gBRCA1/2 Status on Survival Outcomes in Metastatic Breast Cancer Patients Treated with Topoisomerase 1 based Antibody-Drug Conjugates (ADCs)

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Background: ADCs combine the tumor specificity of monoclonal antibodies with the cytotoxic effects of chemotherapeutic drugs, allowing for targeted delivery of potent anticancer agents while minimizing systemic toxicities. Sacituzumab govitecan (SG) and trastuzumab deruxtecan (T-DXd) are antibody drug conjugates with topoisomerase 1 payloads targeting TROP2 and HER2, respectively. Defects in homologous recombination repair through gBRCA1/2 alterations have been postulated as a potential biomarker of response to DNA damaging agents such as topoisomerase I inhibitors. Consequently, we sought to assess the impact of gBRCA1/2 status and clinical outcomes in metastatic breast cancer patients treated with SG or T-DXd.
Methods: We estimated the distribution of progression-free survival (PFS) and overall survival (OS) using the Kaplan-Meier method. Differences in survival curves between gBRCA-positive and BRCA-negative patients were assessed using the log-rank test. Cox proportional hazards regression models were employed to evaluate the association between each survival outcome and BRCA status, and Fisher exact test was used to assess the association with the clinical benefit rate (CBR).
Results: The analysis included a total of 322 breast cancer patients treated with SG or T-DXd with a median follow-up time 30.1 months (Range: 0.5 – 299 months). Of these, 49 (15%) were gBRCA positive (gBRCA1 n=27 and gBRCA2 n=22). gBRCA1/2-positive patients were more likely to be younger, with 43% aged 18-40 compared to 22% in BRCA-negative patients. The median age at the start of either ADC was 49 years (range: 20-83). gBRCA-positive patients had received more prior lines of treatment, with 49% having three or more prior treatments compared to 40% of BRCA-negative patients. Out of the gBRCA1/2 positive patients, 39(80%) received PARP inhibitors, with 15(31%) of them receiving it as first-line treatment.53% of gBRCA-positive patients received SG, while 47% received T-DXd, similar to the BRCA-negative group (56% and 44%, respectively). 45% of BRCA-positive patients had hormone receptor (HR) positive cancers compared to 37% of BRCA-negative patients, and 88% of gBRCA-positive patients had HER2 negative cancers compared to 77% of BRCA-negative patients. Among the gBRCA-positive cohort, 51% had died by the last follow-up compared to 34% of BRCA-negative patients. Additionally, 80% of gBRCA-positive patients experienced disease progression or death, compared to 72% of BRCA-negative patients. The median PFS was 3.9 months (95% CI: 2.9-6.4 months) in gBRCA1/2 positive compared to 5.6 months (95% CI: 5.1-6.7 months) (hazard ratio 1.48 (1.05 – 2.10), p = 0.025) in BRCA1/2 negative patients. Median OS was 64.5 months (95% CI: 39.8 – Not estimable) in gBRCA positive patients compared to 71.4 months (range 63.5 – 99.6 months) in BRCA1/2 negative patients (HR 1.39 (0.89 – 2.16), p = 0.15). No statistically significant difference in CBR was noted between gBRCA positive and negative patients (p = 0.23).
Conclusions: There is very limited data describing the survival outcomes of BRCA-tested patients receiving topoisomerase based ADCs. In our study, significant difference in PFS, favoring BRCA1/2 negative patients was noted compared to gBRCA1/2 positive patients treated with SG or T-DXd; while no differences in OS and CBR was observed between the two groups. Further study regarding the impact of DNA repair pathway defects and clinical outcomes with DNA damaging agents are warranted.

P5-10-04: Trimodal Therapy is Associated with Higher Overall Survival than Chemotherapy only in Patients with Metastatic Inflammatory Breast Cancer

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Background: Metastatic inflammatory breast cancer (MIBC) is a rare and aggressive breast cancer with poor overall survival. Current standard of care treatment of MIBC consists of systemic therapy only with locoregional therapy to the primary site (surgery and radiation) utilized inconsistently and generally reserved for palliation. The purpose of this study is to examine overall survival (OS) among MIBC patients receiving chemotherapy only versus trimodal therapy in the National Cancer Database (NCDB).
Patients and Methods: All patients diagnosed with MIBC from 2004 to 2021 were identified in the NCDB. The cohort was divided into a chemotherapy only group versus trimodal therapy. Trimodal therapy included chemotherapy, modified radical mastectomy, and radiation therapy. Radiation therapy included radiation to the breast, chest wall, and/or regional lymph nodes. We fitted Overlap Propensity Score Weighting (OPSW) Cox proportional hazard models to account for confounding in the relationship between OS and trimodal therapy status. Variables included in the propensity score model included age, Charlson-Deyo score, molecular subtype, and metastatic site.
Results: A total of 4258 patients with MIBC were included in this study, of whom 1029 (24.17%) underwent trimodal therapy and 3229 (75.83%) received chemotherapy-only. Patients in the trimodal therapy group were younger (median age 54 vs. 59 years old; $p<0.0001$) compared to the chemotherapy-only group. Additionally, a higher percentage of patients in the trimodal therapy group had a Charlson Deyo Score of 0 (87.66% vs 82.25%, p-value<0.0001), held private insurance (58.16% vs 41.64%, p-value<0.0001), had a median income of $74,063 or higher (31.20% vs 27.50%, p-value=0.0109) and had less than 2 metastatic sites (90.43% vs 67.19%). There were no statistically significant differences in cancer subtypes between the treatment groups (p=0.158). On multivariable analysis, patients who underwent trimodal therapy were at 48% lower hazard of mortality compared to those on chemotherapy only (aHR: 0.52, 95% CI: 0.46-0.58, p<0.0001). Moreover, the median OS for those who received trimodal therapy was 49.5 months compared to 21.7 months (p<0.0001) for those who received chemotherapy only.

Conclusions: In this cohort of patients with MIBC in the NCDB, trimodal therapy improves overall survival compared to chemotherapy only. Future prospective studies with patient-reported outcomes are needed to confirm these findings.

P5-10-05: Differences in drug efficacy between primary and metastatic sites and their prognosis for de novo stage IV breast cancer: an exploratory analysis of a phase III trial, JCOG1017

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Background: Breast cancer is a highly heterogeneous disease, and there is a biological diversity. Biological factors such as estrogen receptor, progesterone receptor, and HER2 receptor may vary between primary and metastatic sites in breast cancer. This variation could influence treatment responses in stage IV breast cancer.
This exploratory analysis aims to differentiate drug efficacy and long-term prognosis between primary and metastatic sites in patients with de novo stage IV breast cancer.

Methods: In JCOG1017, patients diagnosed with de novo stage IV breast cancer received primary systemic therapy (PST) according to subtypes after first registration. In this analysis, the efficacy of PST was evaluated prior to second registration (about 3 months post-initiation of systemic therapy), using both JCOG1017 criteria (progressive disease [PD] defined as a 10% or greater increase in long diameter) and additionally RECIST v1.1 criteria. In addition, the efficacy at about 6 months of PST in only patients with non-PD response at the second registration and assigned systemic therapy alone group was evaluated.
Patients were categorized based on their treatment responses at primary versus metastatic sites into four groups: discordant group I (primary non-PD and metastatic PD), concordant group I (both primary and metastatic PD), discordant group II (primary PD and metastatic non-PD), and concordant group II (both primary and metastatic non-PD). We assessed clinicopathologic characteristics, including subtype of primary tumor and menopausal status, and overall survival (OS).

Results: Of 570 patients who enrolled in JCOG1017, 315 patients were included in this analysis. In the evaluation at second registration using JCOG1017 criteria, the proportions of each group were 21.3% (67/315) for discordant group I, 8.3% (26/315) for concordant group I, 0.3% (1/315) for discordant group II, and 56.2% (177/315) for concordant group II. There were 14.0% (44/315) of patients with not evaluable. The overall discordance proportion of treatment effect between primary and metastatic sites was 25.1%. Subtype-specific discordance proportions were notably higher in luminal (28.9%) and triple-negative (25.0%) subtypes compared to HER2-enriched (11.1%). Menopausal status did not significantly impact discordance proportions (pre: 25.3% (24/95): post: 25.0% (44/176)). However, among the triple-negative subtype, the discordance proportion is much higher in the premenopausal population (45.5%:5/11) than in the postmenopausal population

(7.7%:1/13).
Median survival times were 3.8 years for discordant group I, 4.6 years for concordant group I, 1.7 years for discordant group II, and 5.7 years for concordant group II. No differences in prognosis were observed between discordant and concordant groups I. However, differences in prognosis between discordant and concordant groups II tended to be observed. The Kaplan-Meier curve for OS is similar based on both evaluation by JCOG1017 criteria and RECIST v1.1.

Conclusions: We found a difference in the treatment efficacy of PST between primary and metastatic sites in the early phase of treatment for stage IV breast cancer, and most cases were primary non-PD and metastatic PD. In the early phase of PST for de novo stage IV breast cancer, not only primary tumors but also metastatic sites need to be examined.

P5-10-06: Retrospective, population-based cohort study on associations between tumor size, receptor status, & regional nodal positivity rates for early-stage breast cancer: implications for decision between upfront surgery vs neoadjuvant systemic therapy

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Purpose: For clinically node-negative T1-T2 breast cancers, the ASCO/Ontario Health guidelines discourage staging axillary ultrasound (AxUS). At the same time, neoadjuvant systemic therapy (NST) is recommended for HER2-positive(+) and triple negative (TN) breast cancers over 2 cm, with controversy whether clinical T1cN0 HER2+ and TN breast cancers should receive NST or upfront surgery. We evaluated the rates of regional nodal positivity across T1a, T1b, T1c and T2 tumor sizes for hormone receptor (HR)+HER2-, HR-HER2+, HR+HER2+, and TN breast cancers.
Methods: We performed a population-based, retrospective cohort study using administrative health databases at ICES Ontario. Data from all patients diagnosed with invasive breast cancer between 2000 – 2019 in Ontario, Canada were extracted from the Ontario Cancer Registry. Demographic, tumor, and treatment data were collected from the linked databases. The following variables were analyzed: T stage, number of positive regional lymph nodes, and estrogen, progesterone, and HER2 receptor statuses. The proportion of patients with positive lymph nodes was calculated for each receptor subtype. Chi-square tests (significance of $P < 0.05$) with post-hoc Marascuilo correction were conducted. Analyses were conducted using SAS® and Python software.
Results: There were 52,888 invasive breast cancers, including 39,265 HR+HER2- (74%), 5,511 HR+HER2+ (10%), 2,523 HR-HER2+ (5%), and 5,589 HR-/HER2- (11%). Increasing tumor size was associated with increasing rates of regional nodal positivity across all receptor subtypes ($P < .001$ for all). Among TN tumors, T1a lesions had a nodal positivity rate of 19% (95% CI 0.12 – 0.24), T1b 19% (95% CI 0.15 – 0.23), T1c 28% (95% CI 0.26 – 0.30), and T2 42% (95% CI 0.40 – 0.43). Among HR-HER2+ tumors, T1a lesions had a nodal

positivity rate of 18% (95% CI 0.13 – 0.24), T1b 30% (95% CI 0.24 – 0.37), T1c 42% (95% CI 0.37 – 0.46), and T2 54% (95% CI 0.51 – 0.57). Among HR+HER2+ tumors, T1a lesions had nodal positivity rate of 23% (95% CI 0.19 – 0.29), T1b 20% (95% CI 0.17 – 0.25), T1c 32% (95% CI 0.29 – 0.34), and T2 56% (95% CI 0.54 – 0.58). Among HR+HER2- tumors, T1a had a rate of 16% (95% CI 0.15 – 0.18), T1b 16% (95% CI 0.15 – 0.17); T1c 30% (95% CI 0.29 – 0.30), and T2 54% (95% CI 0.54 – 0.56).
For T1a lesions, nodal positivity rates were similar across all receptor subtypes. For T1b and T2 lesions, pairwise comparisons were significantly different between all receptor subtypes, except between HR+HER2+ and TN, and HR+HER2- and HR-HER2+, respectively. For T1c, HR-HER2+ had the highest rates of nodal positivity, followed by HR+HER2+, HR+HER2-, and TN ($P < .001$).
Conclusion: While current guidelines discourage AxUS for cT1-T2N0 breast cancers, at the population-level, pathologic regional nodal positivity rates are not insignificant for T1-T2 tumors, especially TN and HER2+ tumors. Taking these patients with pathologically positive lymph nodes to upfront surgery results in undertreatment. Furthermore, for clinical T1c tumors, regional nodal positivity rates reached 42%, 32%, and 28% for HR-HER2+, HR+HER2+, and TNBC, respectively. These data need to be shared with patients with cT1c tumors when deciding between a NST or upfront surgery approach. Finally, we advocate that staging AxUS should be considered for all HER2+ and TN T1-T2 tumors, irrespective of tumor size.

P5-10-07: Longitudinal survey on supportive care needs and coping strategies in women with recurrent/metastatic breast cancer

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Background: Patients with advanced cancers face a wide range of physical and psychosocial needs, often dissatisfied by available means of support. As the treatment of metastatic breast cancer (mBC) has evolved and life expectancy increases, it is crucial to identify patient needs throughout the disease trajectory, particularly contrasting the overwhelming experience of diagnosis with the chronicity of ongoing treatment.
Methods: From April 2021 to May 2024, adult women with mBC enrolled in the EMBRACE: Ending Metastatic Breast Cancer for Everyone cohort study and treated at the Dana Farber Cancer Institute were invited to complete a survey that assessed quality of life (5-point Emoji Scale, PROMIS Global Health), coping strategies (Brief COPE and SHINE), anxiety (PRO-CTCAE), depression (PHQ-9), and supportive care needs (SCNS-SF34). Following baseline assessment, the survey was repeated after 6 and 12 months. Patients reporting needs related to pain, professional counseling, depressive symptoms or sexual relationships, triggered a question offering to share this information with their clinical team. We summarize baseline survey data with descriptive statistics.
Results: Of 81 respondents to baseline surveys, mean age was 58.2 ±8.2 years, 96% were of non-Spanish/non-Hispanic ethnicity and 90% were of Caucasian race. Most patients were

diagnosed with recurrent mBC (69.1%) and responded to the baseline survey within 1 year of their mBC diagnosis (44.4%). At the time of baseline survey, 29.6% were receiving treatment with an antibody-drug conjugate, 24.7% with a cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitor combined with endocrine therapy, 14.8% with chemotherapy and 14.8% with anti-HER2 therapy; 22.2% were receiving their treatment through a clinical trial protocol. Using the 5-point emoji scale, most (74.1%) patients reported feeling happy (4-5) on the day they completed the survey. At least occasional anxiety (PRO-CTCAE) was reported by 65.8%, and at least mild depressive symptoms (PHQ9) by 50.7%. Prominent coping strategies included acceptance (mean 6.4 ±1.4), self-distraction (5.8 ±1.6), use of emotional support (5.7 ±1.7) and active coping (5.3 ±1.8) and only rarely substance use (2.3 ±0.8. Supportive care needs were expressed in multiple domains; most frequently related to psychological needs (40.8%-73.6% reporting at least some need on an individual domain-related item), and with the highest need reported for "Uncertainty about the future" (8.3% no need – not applicable, 18.1% no need-satisfied, 31.9% some need – low, 25% some need – moderate, 16.7% some need - high). Needs were also reported regarding physical and daily living (30.1-53.5%) and less frequently in relation to sexuality (33.3-19.7%,) health system and information (11.3-35.2%), and patient care and support (11.0-24.7%). Prompts offering to notify the clinical team of high needs were triggered by 55 participants, of which only 20% agreed to share this information.
Conclusion: While needs related to information communication and patient cancer are generally met, many patients with mBC report symptoms of anxiety and depression and subsequent psychological needs. Future analyses will aim to identify distinct needs through the disease trajectory.

P5-10-08: Real-world clinical outcomes of patients with HER2- BRCAm early breast cancer treated with adjuvant capecitabine

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Background: In the CREATE-X trial, addition of adjuvant capecitabine after standard of care neoadjuvant chemotherapy improved clinical benefit in patients (pts) with early-stage triple-negative breast cancer (TNBC). The effect of adjuvant capecitabine in pts with BRCA1/BRCA2 mutation (BRCAm) has not been specifically examined, although some studies showed decreased benefit in pts with basal-like tumors, which are enriched in BRCA1m breast cancers. This study explored the effect of adjuvant capecitabine in a real-world cohort of pts with HER2-negative (HER2−) early breast cancer (eBC), stratified by BRCAm status and tumor subtype (hormone receptor positive [HR+]/HER2− or TNBC).
Methods: This retrospective study included de-identified data from US pts aged ≥18 years in the Tempus real-world database who received adjuvant capecitabine for HER2− eBC between 1 Jan 2016 and 11 Nov 2022 and had known BRCA test results (BRCAm or non-BRCAm by germline, somatic, and/or tumor tissue testing). Pts who received neoadjuvant capecitabine ≤90 days from diagnosis, or adjuvant CDK4/6 inhibitor, PARP inhibitor, or

immunotherapy were excluded. Demographics, clinical characteristics, and treatment patterns were described. Invasive disease-free survival (IDFS) and distant disease-free survival (DDFS) were estimated using a Kaplan-Meier approach after the first breast surgery with curative intent.
Results: In total, 269 pts receiving adjuvant capecitabine with known BRCAm status were followed for a median of 23 months. Most pts had TNBC (n=192; 71.4%); 71 (26.4%) had HR+/HER2− eBC, and 6 had unknown subtype. BRCAm was reported in 22/269 (8.2%) pts (TNBC n=15/192, 7.8%; HR+/HER2− n=7/71, 9.9%).
Median age at eBC diagnosis was 53 (range 20–77) years, and was lower among pts with BRCAm (44 [29–70] years) versus non-BRCAm (54 [20–77] years). Most pts (n=245, 91.1%) had received ≥1 neoadjuvant treatment (n=232, 86.2% received only chemotherapy) for a median of 133 (interquartile range [IQR] 105–148) days. The median duration of adjuvant therapy was 168 (IQR 126–210) days and most pts (n=219, 81.4%) had received adjuvant capecitabine monotherapy.
Median IDFS (95% confidence interval [CI]) was 14.1 (10.7–not estimable [NE]) months in pts with BRCAm and 16.9 (15.1–20.8) months in pts with non-BRCAm. At 6, 12, and 18 months respectively, IDFS (95% CI) rates were 95% (72–99%), 55% (32–74%), and 40% (19–60%) for pts with BRCAm, and 96% (93–98%), 69% (62–74%), and 48% (40–54%) for pts with non-BRCAm. Median IDFS (95% CI) was 21.2 (15.8–29.8) and 15.6 (12.8–17.8) months for pts with HR+/HER2− eBC and TNBC, respectively. At 6, 12, and 18 months respectively, IDFS (95% CI) rates were 98% (90–100%), 77% (64–86%), and 59% (45–71%) for pts with HR+/HER2− eBC, and 95% (91–97%), 64% (56–71%), and 41% (33–49%) for pts with TNBC.
Median DDFS (95% CI) was 14.6 (10.7–NE) and 19.1 (16.4–22.6) months in pts with BRCAm and non-BRCAm, respectively. At 6, 12, and 18 months respectively, DDFS (95% CI) rates were 100% (NE), 60% (36–78%), and 40% (19–60%) for pts with BRCAm, and 98% (95–99%), 73% (66–78%), and 52% (44–59%) for pts with non-BRCAm. Median DDFS (95% CI) was 22.4 (16.2–29.8) and 16.9 (14.9–20.8) months in pts with HR+/HER2− eBC and TNBC, respectively. At 6, 12, and 18 months respectively, DDFS (95% CI) rates were 100% (NE), 79% (65–87%), and 61% (47–73%) for pts with HR+ eBC, and 97% (93–99%), 69% (62–76%), and 46% (38–54%) for pts with TNBC.
Conclusions: In this real-world study of pts with known BRCAm status who received adjuvant capecitabine for HER2− eBC, there was a trend for shorter IDFS and DDFS in pts with BRCAm versus non-BRCAm. However, these results are descriptive, unadjusted, and limited by the small number of pts with BRCAm. Further investigation in a larger cohort of pts is warranted, considering that targeted therapeutic options exist for pts with BRCAm.

P5-10-09: A comparative analysis of model and age independence of a plasma-based real-time qPCR clinical assay for the non-invasive detection of stage I breast cancer.

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Background: In the US, approximately 13% of women will be diagnosed with breast cancer in their lifetimes, of which 8.8% will die within 5 years. This is in stark contrast to only 0.4% of patients diagnosed with early-stage breast cancer who will die within 5 years, highlighting the value of robust early-stage diagnostics for breast cancer. To date, diagnostics have faced limitations in reliability, validity, and clinical flexibility due to multiple confounding variables. When considering potential biases in diagnostic research, patient demographics pose as a key limitation as potential confounding variables. This consideration is particularly essential when considering age, as the normal expression patterns for many biomarkers naturally evolve with age. It is also common for diagnostic assays to rely entirely on specific statistical modeling in order to demonstrate their significance and clinical value. Their brittle nature is illuminated when the clinical and statistical significance deteriorates upon the utilization of other statistical models. Demonstrating independence between biomarkers and patient demographics, as well as significance with a variety of statistical methods, is essential in producing a robust diagnostic assay.

Methods: In plasma obtained from women with stage I breast cancer and healthy controls (n=87), data was stratified across women ages 35-60 and >60 and assessed for statistical significance of gene expression by diagnosis for 8 mRNA targets using the Wilcoxon test with Bonferroni correction. A Pearson analysis was then performed to determine correlation between age and gene expression of the 8 targets. Additionally, a model comparison between XGBoost Classification and Logistic Regression was performed on the results of the 8 mRNA target assay (genTRU) to assess model dependency. The XGBoost model performance was assessed using the Sonrai Discovery platform (xgboost==1.4.1.1:CRAN) (XGBoost v1) and Optuna + KFold method (XGBoost v2), while Logistic Regression was assessed with the Optuna + KFold method (Logistic Regression v1).

Results: Strong statistical significance ($p<0.001$) of gene expression was observed by diagnosis for all targets with very weak correlation observed between each target and patient age (R 0.00-0.06). XGBoost v1 demonstrated >99% sensitivity, 89% specificity. XGBoost v2 demonstrated 80% sensitivity, 95% specificity. Logistic Regression v1 demonstrated 51% sensitivity, 96% specificity.

Conclusion: Our analyses confirmed the efficacy and validity of our plasma-based real-time qPCR clinical assay in detecting early-stage breast cancer, independent of statistical method, and gene expression of the targets is not strongly correlated with age. Further, results from the model comparison highlight the dynamic range of sensitivity and specificity of the genTRU clinical assay, enabling clinical flexibility in maximizing PPV or NPV, depending on the clinical application.

P5-10-10: PELICAN-IPC 2015-016/Oncodistinct-003: a randomized phase II study of pembrolizumab in combination with neoadjuvant chemotherapy in HER2-negative inflammatory breast cancer

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Background: Inflammatory breast cancer (IBC) is a rare and highly aggressive clinical entity requiring multimodal treatment, including neoadjuvant chemotherapy, mastectomy, and radiation therapy. With the advent of anti-HER2 agents, significant progress has been made in HER2-positive subtypes but the outcome remains unsatisfactory in HER2-negative IBC. Pembrolizumab in combination with neoadjuvant chemotherapy improves pathological complete response (pCR) and survival in stage II/III triple-negative breast cancer (TNBC) and has become standard of care in this setting. Pembrolizumab also improves pCR in high-grade, HER2-negative, and ER-positive stage II/III breast cancer. Yet, little is known about the specific impact of chemo-immunotherapy in IBC.

Methods: We conducted a prospective, national, multicenter, randomized, phase II study evaluating the efficacy of pembrolizumab in HER2-negative IBC (NCT03515798). Patients aged ≥18 years with stage III disease were randomized (1:2) to receive neoadjuvant chemotherapy with epirubicin/cyclophosphamide (EC) +/-5FU x 4 followed by weekly paclitaxel × 12 (arm A) or the same regimen with pembrolizumab administered 3-weekly (arm B). Patients were then subjected to complete mastectomy and axillary lymph node dissection, prior radiation therapy. Randomization was stratified according to hormone receptor (HR) status (HR+, i.e. ER or PR >10%; TNBC, i.e. ER and PR <10%). Primary endpoint was central assessment of pCR rate, as defined as no residual invasive disease in breast and axilla (ypT0/is, ypN0), in arm B. Secondary endpoints included central pCR rate in arm A, local pCR rates and safety. Exploratory objectives included pCR rates according to HR status and PD-L1 expression, as evaluated by combined positive score (CPS).

Results: From 29/08/2018 to 22/06/2022, 53 stage III, HER2-negative, IBC patients meeting eligibility criteria were randomized in arm A (n=20, including 9 HR+ and 11 TNBC) and arm B (n=33, including16 HR+ and 17 TNBC). All patients were female, median age was 52 (33-71) and 54 (26-73) in arm A and B, respectively. Histology was of no special type in 70% of patients in both arms. PD-L1 expression on baseline tissue was available in 43 patients: CPS was >10 in 17 (39%) patients, with a non-significant numerical difference between arms (50% and 33% in arm A and B, respectively), and was negatively associated with HR expression. Four patients came off the study before surgery: 3 in arm B (1 consent withdrawal, 2 progressive disease) and 1 in arm A (investigator decision). The central pCR rate in arm B (pembrolizumab) was 24%, 90%CI [12-41] (21%, 90%CI [6-47] in HR+ and

27%, 90%CI [10-51] in TNBC). In arm A (control), the central pCR rate was 35%, 90%CI [17-58] (29%, 90%CI [5-66] in HR+ and 40%, 90%CI [15-70] in TNBC). The pCR rates per local assessment were consistent with central evaluation. There was no significant association between PD-L1 expression and pCR in both arms. Grade >3 treatment-related adverse events (AE) were reported in 65% and 69% of patients (including Serious AE in 20% and 39%) in arms A and B, respectively. Pembrolizumab-related grade >3 AEs were noted in 30% of patients (including SAE in 12%). Immune-related AE included skin rash (n=1, grade 2), myocarditis (n=2, grade 1 and grade 2), adrenal insufficiency (n=2, grade 2 and grade 3), hepatitis (n=2, grade 2 and grade 3), thyroiditis (n=2, grade 2), and diabetes (n=1, grade 3).

Conclusions: In HER2-negative IBC, pembrolizumab combined to neoadjuvant EC-paclitaxel chemotherapy induced a moderate pCR rate. Translational studies are ongoing to identify the determinants of therapeutic resistance. Innovative strategies including alternative cytotoxic backbones and novel immune-modulating agents are warranted in this aggressive disease.

P5-10-11: Immediate breast reconstruction using smooth round implants: a retrospective analysis

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Background: Breast reconstruction with implants is the most common method of immediate reconstruction after mastectomy in patients with breast cancer. Currently, implants with different characteristics are used for this. The choice of implant is most often determined by the thickness of the subcutaneous fat, anatomy, physiology, the patient's wishes and the surgeon's experience with various manufacturers. This directly affects most of the studies conducted, which as a result do not reveal a significant difference between the implants. This study we compare the results of immediate breast reconstruction using smooth implants with textured and polyurethane surface.
Methods: This retrospective review was performed using a database from two medical centers. The study included patients who underwent skin-sparing/nipple-sparing mastectomy with immediate breast reconstruction in the period from February 2020 to March 2024. Complications (early and late), rehabilitation period, patient satisfaction were analyzed. Early complications included hematoma, seroma, infection, skin redness, extrusion. Late complications included: capsular contracture, rippling, rotation and malposition. In all cases, lymph node dissection was performed through a separate incision, and the lymph node dissection and mastectomy zones were drained separately. The authors of the study do not use additional coverage of endoprostheses using special meshes or ADM.
Results: A total of 681 breasts in 426 patients underwent one-stage reconstruction with smooth (n=401), textured (n=176), and polyurethane (n=104) implants. Early

complications were much less common with smooth implants: seroma lasting 2 weeks or more 0.5% with smooth round implants, 4% and 3.5% with textured and polyurethane implants, respectively; skin hyperemia occurred only with the use of polyurethane implants (5.2%). The incidence of capsular contracture, regardless of adjuvant radiation therapy, was significantly lower with the use of smooth round implants and polyurethane implants than with the use of textured implants. There was no statistically significant difference in complication rates (hematoma, infectious complications, extrusion of the endoprosthesis) between patients with different implants. Skin rippling occurred only with the use of textured implants (8.9%), which in most cases required either lipofilling procedures or repeat surgery. Implant rotation occurred 3 times more often with textured implants (2.7%) compared to smooth implants (0.8%). However, malposition of smooth implants occurred significantly more often (7%) in the long term, compared to textured (1.7%) and polyurethane (0.8%) implants. In all cases of polyurethane reconstruction, vertical malposition was observed, with equal frequency of vertical and horizontal malposition with the placement of textured and smooth implants. Animation with the same frequency for textured and smooth implants placed under the muscle. Patients with smooth round implants were hospitalized for an average of 5±1 days, with textured implants 10±1 and with polyurethane implants 9±1 days.
Conclusion: Smooth round implants have demonstrated a reduced risk of complications, capsular contracture, and accelerated rehabilitation period compared to other implants in immediate breast reconstruction. However, patients more often sought care for displacement of smooth implants, which required more repeat surgical interventions for implant replacement and pocket correction, which is likely due to the features of placement (requires a tighter muscle pocket with complete muscle coverage) and the formation of a thin capsule. The advantages of smooth round implants make them the method of choice in immediate breast reconstruction with a small thickness of subcutaneous fat.

P5-10-12: Bria-IMT CD8+ Tumor Infiltrating Lymphocytes Turn “Cold” Tumor “Hot” in Metastatic Breast Cancer

Ephraim Parent, Carmen Calfa, Saranya Chumsri, Blaise Bayer, Tamar Aghajanian, Giuseppe Del Priore, William Williams, Russ Kuker

Introduction Transforming "cold" tumors into "hot" ones is critical for the success of immuno-oncology therapies, but there has been little evidence that “cold” tumors can be turned “hot”. SV-BR-1-GM, an allogenic human cancer cell line (Bria-IMT) with antigen-presenting capabilities, is designed to counteract the immunosuppressive tumor microenvironment. Zr-89 crefmirlimab berdoxam is a radio-labeled truncated mini-antibody specific to human CD8α developed for CD8 ImmunoPET imaging. We conducted CD8 imaging before and after Bria-IMT treatment to evaluate baseline and subsequent intra-lesional changes in CD8+ T cell tumor infiltration. We now present additional data from the nested feasibility trial of immunoPET in late state MBC. Methods Nested feasibility trial of subjects from two CD8 immunoPET capable tertiary care sites participating in

NCT03328026 a randomized phase 2 of Bria-IMT in combination with a check point inhibitor (CPI). Standard Uptake Value (SUV) was evaluated pre-dose and following therapy. Results 6 patients, median age 60.5 (44-66), enrolled in the ongoing Bria-IMT trial in advanced heavily pretreated metastatic breast cancer. Median number of prior therapies was 7 (4-8). Prior lines included antibody-drug conjugates (ADCs) and CPIs. All patients had progressed on prior treatment. Four of these patients were randomized to start the CPI after 2 cycles of Bria-IMT to "train" the host immune system before adding the CPI; 2 patients were randomized to begin CPI concurrent in C1 with Bria-IMT. Four patients matched at least 2 SV-BR-1-GM HLA loci, which in previous publications is associated with greater clinical benefit. Sites of disease included in situ breast mass, multiple lymph nodes, visceral and osseous metastases. On follow-up ImmunoPET, each patient demonstrated an increase in SUV in at least one metastatic lesion (range –57.4 - 442.9%) including lung, soft tissue, liver, lymph node, dural based and bony metastases. There was no consistent change recognized in any of these groups except as noted below. Evaluable subjects with HLA matches (3/4) did not experience any increases in CD8+ ImmunoPET SUV at inguinal lymphatic sites while those without HLA matches (n=2) showed increase in CD8 SUV at these sites. In addition, bilateral axillary lymph nodes presented with a median SUV of 6.4 (1.4 - 15.1) after treatment as compared to a median SUV of 7.4 (2.1-18.9) at baseline. 3 out of the 6 patients showed a decrease in neutrophil/lymphocyte ratio NLR at cycle 2 when compared to baseline values. The longest survivor on trial (>1yr, after 6 prior lines, ER/PR +, HER2+) CD8 PET also had the largest percentage (442.9%) increase of any patient in SUV (right frontal dural based). This lesion completely resolved around cycle 8. Additional correlation with tumor genomics will be reported. Conclusion We report additional data supporting future hypotheses and that "cold" tumors can become "hot" when treated with Bria-IMT in combination with an anti-PD-1 CPI, as demonstrated by metastatic site-specific CD8+ PET results. Subjects in a now nearly completed randomized phase 2 demonstrated responses of metastatic lesions on CD8 ImmunoPET. The nonspecific nodal localization of CD8 ImmunoPET may indicate a systemic activation of CD8 positive lymphoid cells in response to peripheral non-lesional SV-BR-1-GM injections. These results suggest a potential value of CD8 ImmunoPET in identifying lesions that are progressing on treatment versus pseudo progression. It also provides support that the Bria-IMT combination immune-based therapy can result in an increase of CD8+ tumor infiltrating lymphocytes in breast cancer metastatic sites as well as in lymphoid organs. This advance may aid in triaging patients, adjudicating pseudo-progression and predicting clinical benefit of immune based therapies.

P5-10-13: Estimation of biological aging and non-cancer mortality rate in breast cancer survivors due to chemotherapy using a computational model of senescence

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Purpose: The steady reduction in deaths from breast cancer has heightened the importance of breast cancer survivorship, as emphasized in the new phase of the Cancer Moonshot initiative. One key concern is the adverse effects of breast cancer therapies causing the early onset of other age-related diseases and deaths from non-cancer causes. Chemotherapy has been shown to increase the level of senescent cells in breast cancer survivors, measured using the expression level of the p16 protein. Senescent cells secrete phenotypes, including inflammatory cytokines and proteases, which are main factors behind several age-related diseases. In fact, mathematical modeling of stochastic accumulation and removal of senescence has been shown to model the mortality rate and the incidence rate of several age-related diseases in humans. Therefore, we need a computational approach to model the impact of breast cancer diagnosis and treatment on the enhanced accumulation of senescence and the subsequent increase in the non-cancer mortality rate.
Method: We used a stochastic model of the accumulation and removal of senescent cells (SnC) with age to simulate the SnC trajectories for 6 scenarios, women without cancer, women with breast cancer but without senescence-enhancing therapies, and women with breast cancer and with 4 groups of chemotherapy regimens (for which the data on the change in p16 expression were available). The 4 groups of chemotherapy regimens were, (1) doxorubicin, cyclophosphamide, and paclitaxel, (2) docetaxel and cyclophosphamide +/- anti-HER2 therapy, (3) docetaxel and carboplatin + anti-HER2 therapy, and (4) doxorubicin and cyclophosphamide + paclitaxel and carboplatin. We used the probability distribution of SnC for each age in the non-cancer group as a biomarker for the true biological age. The clinical data on the change in p16 expression level due to breast cancer and subsequent chemotherapy was used to modify the parameters of the stochastic model to generate the SnC probability distributions for the other 5 scenarios. Biological age for the breast cancer and chemotherapy groups were estimated by using the difference in the SnC probability distributions in each of the 5 breast cancer groups vs. the non-cancer group. Non-cancer mortality rate for the 5 breast cancer groups was determined using the computationally-estimated biological age rather than the chronological age.
Results: We obtained quantitative maps from chronological age to biological age for breast cancer survivors depending on two variables, the age at diagnosis of breast cancer and the chemotherapy regimen. The divergence of biological age from the chronological age is higher for women diagnosed with breast cancer at a younger age. This divergence can be as high as 30+ years for women diagnosed with breast cancer before the age of 50, leading to a significantly higher non-cancer mortality rate. Therefore, chemotherapy-enhanced senescence can be a critical factor for deciding the specific regimen for younger women with breast cancer.
Conclusions: We present a novel computational approach to generate chronological age to biological maps and chronological age to non-cancer mortality rate maps for breast cancer survivors as a function of the age at diagnosis and the chemotherapy regimen. These maps can be useful for clinical decisions on the impact of different chemotherapy regimens on subpopulations of breast cancer survivors. The chronological age to non-cancer mortality rate maps also provides new inputs for epidemiological simulations of breast cancer survivorship, to discern the impact of different therapies on breast cancer survivorship.

Furthermore, we will be able to incorporate tumor marker subtype and race/ethnicity as variables for generating these senescence-based maps as more data become available.

P5-10-14: Early Invasive Lobular or Ductal Carcinoma with Differential Clinical Outcomes Across Molecular Subtypes among Young Women Who Received Neoadjuvant Chemotherapy

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Background: Young women in the US are experiencing rising rates of breast cancer, necessitating effective treatment strategies. Neoadjuvant chemotherapy (NACT) is widely used in clinical practice, yet research on post-NACT outcomes among young women is limited, especially for those with less common histology, e.g., invasive lobular carcinoma (ILC) or invasive ductal and lobular carcinoma (IDLC). In this study, we examined differential pathologic complete response (pCR) and overall survival (OS) between invasive ductal carcinoma (IDC), ILC, or IDLC among female patients (pts) aged ≤40 years with stage I-III disease who received NACT, stratified by molecular subtype.
Methods: This hospital-based, retrospective study analyzed data from the 2010-2020 National Cancer Database. We assessed 4 molecular subtypes: HR+/HER2–, HR+/HER2+, HR–/HER2+, and TNBC. pCR, defined as ypT0/Tis ypN0, was modeled using logistic regression, and adjusted odds ratios (aOR) were calculated. OS was event or censored at the time of death from any cause or last known contact, with 5-/10-year OS rates estimated using the Kaplan-Meier method and compared using log-rank tests. We performed Cox regression to generate adjusted hazard ratios (aHR). All models were stratified by molecular subtype, adjusting for clinical T/N stage, tumor grade, PR status (HR+ only), race/ethnicity, year of diagnosis, and comorbidity score.
Results: Of 26,480 young women (median follow-up 52.9 [IQR 32.9, 79.2] months), 95.8% had IDC, 2.1% ILC, and 2.1% IDLC. In the HR+/HER2– cohort, 14.9% of IDC pts achieved pCR compared to 3.3% of ILC and 4.1% of IDLC pts (p<.001). After covariate adjustment, pts with ILC (aOR 0.45, 95% CI: 0.23-0.86) or IDLC (aOR 0.49, 95% CI: 0.27-0.89) had lower odds of pCR than IDC pts. In the HR+/HER2+ cohort, a higher pCR rate was observed among IDC pts (33.8%) than ILC (24.3%) or IDLC (27.5%) pts (p=.033). However, the odds of pCR were not significantly different between IDC and ILC (aOR 0.90, 95% CI: 0.56-1.43) or IDLC (aOR 0.77, 95% CI: 0.52-1.13). In the HR–/HER2+ cohort, pCR rates were similar across histologic types (IDLC: 70.6%; ILC: 58.8%; IDC: 49.9%; p=.180). On multivariable regression, pts with ILC (aOR 1.68, 95% CI: 0.60-4.70) or IDLC (aOR 1.96, 95% CI: 0.67-5.73) had similar odds of pCR as those with IDC. In the TNBC cohort, IDC pts achieved a higher rate of pCR than ILC or IDLC pts (36.0%, 21.3% vs. 18.0%; p=.003). Compared to IDC pts, IDLC pts had lower odds of pCR (aOR 0.40, 95% CI: 0.18-0.9) while ILC pts had similar odds of pCR (aOR 0.70, 95% CI: 0.34-1.42). IDC was associated with significantly longer 5-/10-year OS rates (compared to ILC or IDLC) among pts with HR+/HER2– (p=.012) or TNBC (p<.001). In the adjusted models, pts with HR+/HER2– ILC (aHR 1.55, 95% CI: 1.15-2.10) or

IDLC (aHR 1.52, 95% CI: 1.15-2.03) had a greater mortality risk than IDC pts. HR–/HER2+ ILC pts also had a higher risk of death than IDC pts (aHR 3.51, 95% CI: 1.10-11.15). In the TNBC cohort, ILC was associated with an increased mortality risk compared to IDC (aHR 2.28, 95% CI: 1.41-3.69).
Conclusions: In this US national registry of young women with early-stage breast cancer who received NACT, pts with ILC or IDLC consistently exhibited lower pCR rates and poorer OS than those with IDC across molecular subtypes. These disparities underscore the need for tailored treatment interventions that account for histologic type, particularly for ILC, among young women. Our findings not only highlight the importance of informed NACT counseling, but also underscore the necessity for oncology programs to optimize therapeutic approaches and continue multidisciplinary efforts to reduce disparities specifically for young women with ILC.

P5-10-15: Effectiveness and tolerance of Trastuzumab Deruxtecan (T-DXd) in HER2-Positive and HER2-Low Metastatic Breast Cancer (MBC): a large real-life French Cohort Study

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Background: Trastuzumab deruxtecan (T-DXd) has demonstrated promising efficacy in MBC patients enrolled in clinical trials but limited data is available on the real-world outcomes.

Methods: This retrospective multicenter study enrolled patients with HER2-pos and HER2-low MBC receiving T-DXd from April 2019 to November 2023 across 10 French centers. All data were obtained from patient medical records. Overall survival was estimated using the Kaplan-Meier method.

Results:
Throughout the study period, 238 patients were included, 51% with HER2-pos MBC and 49% with HER2-low MBC. Among them, 76% were HR+ and 24% were HR-. Median age was 59 years (IQR 50-69) and median prior regimens in the metastatic setting (PRMS) were 2 and 4 for HER2-pos and HER2-low, respectively. A total of 56 (24%) had a PS >1 before T-DXd introduction. The best overall response was evaluable in 223 patients.
Complete/partial response (CR/PR), stable disease (SD), and progressive disease (PD) rates were 61%, 20%, 19% in the HER2-pos MBC population, and 42%, 30%, 28% in the HER2-low MBC population. The median time to treatment discontinuation (TTD) with T-DXd was 9.1 months, 12.8 months in HER2-pos MBC and 6.9 months in HER2-low MBC. Overall survival (OS) was 20.9 months, 24.7 months in HER2-pos MBC and 16.9 months in HER2-low MBC. PS >1 was associated with lower CR/PR in HER2-pos MBC (50% vs 64%, p=0.04) but not in HER2-low MBC (43% vs 42 %, p=0.3). In terms of tolerance, dose reduction

occurred in 43 patients (18%), and 22 patients (9.2%) developed interstitial lung disease.

Conclusion:This large cohort study confirmed the favorable real-life activity of T-DXd in HER2-pos and HER2-low MBC, although objective response rates appeared shorter than those observed in the DESTINY-Breast trials. Dose reductions and incidence of drug-related interstitial lung disease were consistent with the findings from clinical trials.

P5-10-16: HER2-Low Breast Cancer: The Impact of Online Education on Oncologists' Knowledge and Confidence in Understanding the Latest Advances and Implications for Practice

Zhizhi Fiske, Stephen Dunn, Deborah Grainger, Jamie Habib, Giuseppe Viale, Nadia Harbeck

Background: HER2-low has been receiving wide-spread interest and is now a therapeutically relevant biomarker in breast cancer. The objective of this study was to assess the effect of an online continuing medical education (CME) activity on clinicians' knowledge in the heterogeneity of HER2 expression in breast cancer, knowledge in the key data from the latest clinical trials investigating novel and emerging therapies in HER2-low breast cancer, and confidence in their ability to individualize treatment plans for patients with HER2-low breast cancer.

Methods: This CME activity consisted of a 15-minute video discussion between 2 expert faculty with synchronised slides. Educational effect was assessed using a repeated-pair design with pre-/post-assessment. 3 multiple choice questions assessed knowledge, and 1 question rated on a Likert-type scale assessed confidence, with each individual serving as their own control. A McNemar's test assessed significance of improvement in the percentage of correct responses to knowledge questions from pre- to post-assessment. P values < .05 are statistically significant. The activity launched on 5th of December, 2023, with data collected through 26th April, 2024 being reported in the current study.

Results: The analysis set consisted of responses from oncologists (n=73) who answered all assessment questions during the study period. Analysis of pre- vs post-intervention responses demonstrated a significant improvement in overall knowledge of oncologists (P< .001). Overall correct responses increased from 27% pre- to 49% post-CME, representing an 81% relative increase, with 12 times more leaners answering all questions correctly after education. Specific areas of improvement include:

- Knowledge of the heterogeneity of HER2 expression in breast cancer (pre 14%, post 56%; P < .001)
- Knowledge of the key data from the latest clinical trials investigating novel and emerging therapies in HER2-low breast cancer (pre 34%, post 46%; P < .01)

After education, 42% of oncologists had a measurable increase in confidence in individualizing treatment plans for patients with HER2-low breast cancer, and that increase

was, on average, 72%.

Conclusions: This analysis demonstrates the positive educational impact of an online CME activity on oncologists' knowledge regarding HER2-low breast cancer, particularly in areas related to the heterogeneity of HER2 expression. However, there is still an educational need in a large percentage of oncologists on this topic.

P5-10-17: Impact of Pathologic Response and Individual Prognosis After Neoadjuvant Treatment (NAT) in Patients (pts) with Early HER2+ and Triple-Negative Breast Cancer (TNBC)

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Background: Pathologic complete response (pCR) is prognostic in pts with early breast cancer (eBC). This study aims to assess individual prognostic factors for recurrence-free survival (RFS) and overall survival (OS) in pts with pCR vs residual disease (RD).

Methods: This retrospective study included pts diagnosed at our institution with TNBC or HER2+ eBC who underwent surgery post-NAT and had available data for pCR/RD, RFS, OS. Clinical tumor size (cT), nodal status (cN), and subtype were evaluated as prognostic factors using Cox models and Kaplan-Meier method, in all pts and in pts with pCR (ypT0/is, ypN0) and RD. RFS and OS were defined as time from surgery to first recurrence or death from any cause, whichever occurred first, and as time from surgery to death from any cause, respectively ($p<.05$ significant).

Results: 863 pts were identified with a median age of 50.2 yrs (21.0-85.4) who underwent surgery between 2016-2021. Median follow-up was 3.5 yrs (2.6-6.0). 387 pts (44.8%) had TNBC, 295 (34.2%) ER+HER2+ eBC, 181 (21.0%) ER-HER2+; 80 (9.3%) were stage I, 602 (69.8%) stage II, 181 (21.0%) stage III. Regarding NAT, 370 pts (42.9%) received chemotherapy-only, 473 (54.8%) received HER2-targeted regimens, 14 (1.6%) received immunotherapy-based regimens. Overall, 374 (43.3%) pts had a pCR, 489 (56.7%) RD. In TNBC, 125 pts (32.3%) had a pCR, 262 (67.7%) RD; in ER+HER2+ eBC, 119 pts (40.3%) had a pCR, 176 (59.7%) RD; in ER-HER2+ eBC, 130 pts (71.8%) had a pCR, 51 (28.2%) RD. 108 pts (12.5%) had disease recurrence, of whom 74 (68.5%) had TNBC, 22 (20.4%) ER+HER2+, 12 (11.1%) ER-HER2+ BC. Most common recurrence sites were brain (39, 36.1%), bone (37, 34.3%) and lung (33, 30.6%).
Median survival measures were not reached. 3-yr RFS was 87% for the entire cohort (95% CI, 85-89), 80% (95% CI, 76-84) in TNBC and 93% (95% CI, 91-96) in HER2+ eBC. 3-yr RFS was 98% (95% CI, 97-100) in pts with pCR vs 79% (95% CI, 75-82) in pts with RD. In TNBC,

3-yr RFS was 97% (95% CI, 95-100) in pts with pCR vs 72% (95 CI, 66-77) in pts with RD; in HER2+ eBC, 3-yr RFS was 99% (95% CI, 97-100) in pts with pCR vs 87% (95% CI, 82-92) in pts with RD.
Overall, higher cT (cT3-4 vs cT1-2, hazard ratio [HR]: 2.72; 95% CI, 1.88-3.94, p<.001), positive cN (cN+ vs cN0, HR: 2.43; 95% CI, 1.65-3.59, p<.001), TNBC subtype (TNBC vs ER+HER2+, HR: 2.57; 95% CI, 1.63-4.04, p<.001; ER-HER2+ vs ER+HER2+, HR: 0.94; 95% CI, 0.49-1.80, p=.8) and RD (pCR vs RD, HR: 0.11; 95% CI, 0.06-0.22, p<.001) were associated with poorer 3-yr RFS.
In pts with pCR, 3-yr RFS was numerically higher in cT1-2 cases (99%) vs cT3-4 (95%) and in cN0 cases (99%) vs cN+ (97%), but differences were not significant (cT3-4 vs cT1-2, HR: 2.97; 95% CI, 0.84-10.52, p=.09; cN+ vs cN0, HR: 2.85; 95% CI, 0.74-11.01, p=.1); stratification by subtype revealed no associations.
In pts with RD, higher cT (cT3-4 vs cT1-2, HR: 2.26; 95% CI, 1.54-3.33, p<.001), positive cN (cN+ vs cN0, HR: 2.31; 95% CI, 1.54-3.47, p<.001) and TNBC subtype (ER-HER2+ vs ER+HER2+, HR: 1.66; 95% CI, 0.79-3.49, p=.2; TNBC vs ER+HER2+, HR: 2.42; 95% CI, 1.51-3.86, p<.001) remained associated with poorer 3-yr RFS.
In a multivariate model for RFS, no associations were found in pts with pCR. In pts with RD, higher cT (cT3-4 vs cT1-2, HR: 1.86; 95% CI, 1.24-2.80, p=.003), positive cN (cN+ vs cN0, HR: 2.13; 95% CI, 1.39-3.28, p=.001) and TNBC subtype (TNBC vs ER+HER2+, HR: 2.60, 95% CI, 1.63-4.17, p<.001) remained associated poorer 3-yr RFS. These results were consistent using ER<10% to define ER negativity. 3-yr OS will be presented at the meeting.

Conclusions: The study confirms that pts experiencing pCR have better outcomes than those with RD. Overall, 3-yr RFS and 3-yr OS varied by cT, cN, and subtype at presentation. In pts with RD, cT, cN, and subtype remained independent prognostic factors. In pts with pCR, few events occurred, and no associations were found.

P5-10-18: What to measure in Real-World Evidence (RWE) Studies: A Patient Informed Conceptual Disease Model in Early-Stage Breast Cancer

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Background: The treatment of early-stage breast cancer (eBC) is rapidly evolving, with new advancements in early diagnosis and targeted therapies. Given the heterogeneity of breast cancer, it is crucial to understand specific needs of eBC patients to ensure they are well supported in their treatment journey. Conceptual disease models (CDM) provide a means to organize and visualize concepts of importance for a patient population. A CDM can support real-world studies to understand the multidimensional aspects of eBC and aid in the identification of appropriate patient-reported outcome measures (PROMs).
Objectives: To develop a comprehensive CDM of the patient experience and understand the concepts that are of greatest importance to patients with eBC.
Methods: A targeted literature review (TLR) was conducted to inform a preliminary CDM. A

Patient Steering Committee (PSC) comprised of three US patient experts with history of breast cancer contextualised the findings, provided input on the TLR results and further advised on concepts of importance to eBC patients.
Results: The preliminary CDM represented eBC signs and symptoms, side-effects, and proximal and distal impacts identified from the TLR, which comprised of 88 empirical studies and grey literature. Although some patients may be asymptomatic, fatigue and pain/discomfort were reported as common symptoms. eBC has a major impact on multiple aspects of life like cognitive functioning (ability to concentrate), physical functioning/daily activities, roles, emotional/psychological impact, social impacts, relationships, work/school, and financial concerns. Anxiety was identified as a pervasive impact across the treatment journey (testing, diagnosis, pre-, during and post-treatment, survivorship, and recurrence) and may shape how patients cope with eBC. A need for access to quality care and support with physical, practical, social, cognitive and economic impacts in the post-treatment period was also highlighted, along with support for multiple languages and cultures.
The PSC emphasized prominent themes in the CDM like importance of more timely quality of life (QoL) discussions between patients and healthcare providers during early diagnosis. The PSC discussed several areas affecting QoL: 1) Energy levels (fatigue/tiredness) which can directly impact work, family, and social relationships; 2) Body image and concerns related to sexual functioning and affecting self-confidence; and 3) Survivorship which is often related to fear of recurrence/disease progression, guilt, grief, and mourning.
Other critical unmet needs raised by the PSC were a necessity for more information on treatment options/surgery, inconsistency in doctor-patient communication and need for improvements in shared decision making. Financial toxicity was also seen as a critical issue for some patients undergoing treatment for eBC.
Discussion: This is the first comprehensive CDM in eBC developed based on information from the literature and patient expert insights. Improved understanding of the eBC patient experience necessitates identification of the wide-range of different concepts related to treatment and how they impact patients' daily lives. The CDM will be further refined following in-depth qualitative interviews with eBC patients, followed by discussion with patient experts to validate key concepts that should be measured in RWE settings. The CDM will be used to inform the selection of PROMs in future studies aimed at understanding the patient perception and experience of eBC treatments with the ultimate aim to better support patient treatment and care needs. Although these findings are focused on the RWE setting, the application of the CDM demonstrates what matters to patients and should also be considered in patient focused drug development of new treatments.

P5-10-19: Impact of Online Case-Based Education in Improving Clinicians' Knowledge and Confidence in Managing HER2-Low Metastatic Breast Cancer

Zhizhi Fiske, Stephen Dunn, Deborah Grainger, Jamie Habib, Judy King

Background: HER2-low breast cancer has generated great interest recently due to the introduction of novel antibody-drug conjugate (ADC) therapy into the clinical practice and the remarkable outcomes it has achieved. The objective of this study was to assess the effect of an online case-based continuing medical education (CME) activity on clinicians' knowledge of the available evidence supporting the use of novel ADC therapy for patients with HER2-low metastatic breast cancer and their confidence in individualizing treatment plans for those patients.

Methods: This 18-minute segmented multi-media online CME activity consisted of videos portraying realistic physician–patient interaction followed by a test question and expert commentary. Educational effect was assessed using a repeated-pair design with pre-/post-assessment. 3 multiple choice questions assessed knowledge, and 1 question rated on a Likert-type scale assessed confidence, with each individual serving as their own control. A McNemar's test assessed significance of improvement in the percentage of correct responses to knowledge questions from pre- to post-assessment. P values < .05 are statistically significant. The activity launched on 28th of August, 2023, with data collected through 29th December, 2023 being reported in the current study.

Results: 63 oncologists and 36 obstetricians/gynaecologists who answered all the assessment questions were included in this analysis. Analysis of pre- vs post-intervention responses demonstrated a significant improvement in overall knowledge of both physician learner groups, with 200% more oncologists answering all questions correctly after education (30% post- vs 10% pre-CME) and 113% more obstetricians/gynaecologists answering all questions correctly after education (17% post- vs 8% pre-CME). Specifically, the knowledge regarding the latest clinical data in HER2-low breast cancer increased from 45% pre- to 65% post-CME for oncologists (P<.001) and from 33% pre- to 48% post-CME (P<.01) for obstetricians/gynaecologists, respectively.
Additionally, 41% of oncologists and 31% of obstetricians/gynaecologists reported increased confidence in individualizing treatment plans for patients with HER2-low metastatic breast cancer, and that increase was, on average, 71% and 52% among the two physician groups, respectively.

Conclusions: This analysis demonstrates the success of online, interactive, case-based education in improving clinician's knowledge and confidence in managing patients with HER2-low metastatic breast cancer. However, there remains a need to provide ongoing education to clinicians in this growing field.

P5-10-20: Patient awareness of HER2-low in metastatic breast cancer: survey results in a patient-facing app

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Background: The emergence of trastuzumab deruxtecan has led to significant improvement in clinical outcomes for patients with HER2-low metastatic breast cancer, which accounts for about half (45-55%) of breast cancer diagnoses. However, little is known about patients' awareness of diagnostic testing requirements and treatment implications associated with HER2-low status. This study aims to better understand patients' knowledge of HER2-low.
Methods: This cross-sectional survey was completed virtually on the Outcomes4Me mobile app, a direct-to-patient digital application that empowers patients to take a pro-active approach to their care. Eligible patients included those with Stage IV breast cancer living in the United States. Participants were surveyed on awareness of their tumor's HER2 biomarker status and willingness to discuss more with their oncologists if their status was unknown. Educational content about HER2 biomarker testing was accessible on the app. Responses were analyzed descriptively and reported in aggregate.
Results: In total, we received responses from 527 patients, of which 362 met eligibility for inclusion. Of the evaluable patients, 41.9% (152/362) were diagnosed over five years ago, 35.4% (128/362) had Stage IV disease at diagnosis, 32.9% received treatment in a community setting (119/362), and 43.4% (157/362) had progressed on at least one prior therapy for metastatic cancer. Sixty-four (18%) patients did not know or recall their HER2 status at diagnosis; 14 (3.9%) provided no response. Of the 284 (78.5%) patients who were aware of their HER2 status at diagnosis, 24 (7%) reported having HER2-low breast tumors. Within the cohort of patients with known HER2 status, 119/284 (41.9%) of patients were not at all aware or not very aware of the HER2-low classification. Almost 43% (112/284) of patients with a known HER2 status reported HER2 testing within the last year, but 51.3% (57/111) of patients who were classified as HER2-negative following their most recent testing did not recall their oncologist discussing HER2-low as a possibility. For patients with an unknown biomarker status, brief education about HER2 testing was provided within the app. After engagement, 60.9% (39/64) of patients with unknown HER2 status felt very or somewhat likely to talk to their oncologist about testing for HER2-low.
Conclusions: Knowledge gaps in HER2 biomarker testing persist in patients with Stage IV breast cancer. For patients with a known HER2 status, many remain unaware of the HER2-low classification. Digital education resources such as the Outcomes4Me app can facilitate patient empowerment and provide targeted education outside of traditional clinical settings, enabling shared decision making. After patients with an unknown status received brief education within the app, the majority expressed willingness to discuss more about HER2 testing with their oncologist.

P5-10-21: Efficacy and safety of utidelone plus bevacizumab in the treatment of patients with Her2- metastatic breast cancer study

Shaohua Zhang, Tao Wang, Li Bian, Guohui Han, Xiangdong Bai, Zefei Jiang

Background: Despite the rapid development of targeted therapy and immunotherapy for breast cancer in recent years, Chemotherapy remains an important treatment for metastatic breast cancer (MBC), and currently there is no standard chemotherapy regimen for MBC patients previously treated with taxanes. Utidelone is a new microtubule inhibitor drug developed in China, which has demonstrated excellent efficacy for metastatic breast cancer. Historical studies have shown that chemotherapy combined with angiogenic inhibitors can improve PFS in advanced breast cancer patients compared with chemotherapy alone. This is a real world study to evaluate the efficacy of utidelone plus bevacizumab for Her2- MBC patients.

Methods: HER2- metastatic breast cancer patients who had been previously treated with taxanes and anthracyclines were given utidelone (35 mg/m2, iv, d1-5 of each3-week cycle) combined with bevacizumab (10 mg/kg, iv, d1 of each cycle) until intolerance or disease progression. Efficacy was evaluated every 2 cycles according to the Response Evaluation Criteria In Solid Tumors version 1.1.

Results: Between January 2023, and June 2024, a total of 34 patients with metastatic breast cancer were recruited, with a median age of 52.5 years (range, 24 to 72). Among them, 14 patients were with HR-/HER2- breast cancer and 20 patients with HR+/HER2- breast cancer (8 of the HR+ patients with metastatic lesions that were HR-/HER2-). Patients with HR+ had progressed after receiving at least one CDK4/6 inhibitor treatment. The median number of prior treatment lines was 4.
The overall ORR of this study was 47.1%, the median PFS (mPFS) was 5 months, while the median OS was not reached. In the HR-/HER2- group, the median number of prior treatment lines was 2, its ORR was 42.9%, the mPFS was 5 months. The median number of treatment lines for the HR+ group was 7, its ORR was 50%, the median PFS was 4 months. Of all enrolled patients, 6 had brain metastases with 1 being HR-/HER2 -, and 5 being HR+/HER2- (1 metastatic lesion was HR-/HER2-). Two of the brain metastatic patients had previously received radiotherapy for intracranial lesions, and the other 4 were patients with new brain metastases. After treatment with utidelone combined with bevacizumab, intracranial partial response (PR) was achieved in 4 patients and stable disease (SD) in 2 patients, the CNS-ORR was 66.7% and CNS-CBR was 100%, suggesting a very good efficacy for MBC brain metastasis.
The common adverse events (AEs) were peripheral neurotoxicity (PN, 100%) and myalgia/artealgia (52.9%). However, most of the PN were grade 1-2 and reversible. Other AEs greater than 10% included liver toxicity (14.7%), myelosuppression (11.8%) and hypertension (11.8%). Gade 3 AEs included 3 cases (8.8%) of grade 3 PN and 5 cases (14.7%) of grade 3 myalgia/artealgia.

Conclusion: This study showed promising efficacy and a manageable safety profile of utidelone plus bevacizumab in the treatment of Her2- MBC patients relapsed after taxanes therapy. This combination regimen could become a new choice for these patients, especially for asymptomatic brain metastasis patients. Further study is warranted with expanded number of patients.

P5-10-22: Exploring the landscape of locoregional therapy de-escalation in early breast cancer: a systematic review

Alan David McCrorie, Hilary Sobart, David Dodwell, Shelley Potter, Stuart A McIntosh

Background: De-escalation of locoregional treatment in early breast cancer aims to reduce treatment-related morbidity whilst maintaining optimum oncological outcomes but robust evidence from clinical trials is essential to change practice. This review aimed to describe the current landscape of de-escalation trials.

Methods: This systematic review was prospectively registered on PROSPERO (CRD42023487777). Databases and trial registries were searched for interventional clinical trials involving locoregional de-escalation in early breast cancer published 2019-2024, in progress, due to commence, or recently terminated. RCTs and prospective cohort studies were included. Non-interventional studies were excluded. Descriptive and methodological information was extracted. Mixed-methods analysis was performed.

Results: 114 trials (56 (49%) RCTs, 58 (51%) cohort studies) including 99,998 participants were included. Most studies were multicentre (n=81, 71%) and based in Europe or North America (n=87, 76%). RCTs were large with 62% (n=32) aiming to recruit >1000 participants whereas most cohort studies (n=51, 88%) reported sample sizes of <500 patients. Median trial duration was 9 years (range: 1-27, IQR: 6). Of the 62 surgical studies, 52% (n=32) evaluated de-escalation of axillary treatment with over a third (n=22, 35%) assessing reduction of surgery following completion of neoadjuvant therapy. Radiotherapy trials (n=52) were more likely to focus on de-escalating treatment to the breast (n=49, 94%) in older patients (RT >50, n=27, 52% vs. surgery n=6, 10%) and/or use biomarker stratification (RT n=12, 23% vs. surgery n=3, 5%, p=0.004). Primary outcomes were mostly oncological (n=94, 82%) and often based on recurrence (n=64, 56%) with patient-reported outcomes used in a minority of studies (n=7, 6%). Most studies were powered at 80% for either non-inferiority or based on a pre-defined acceptability threshold, but few studies described how this margin was selected. Less than 10% (n=10) reported involving patients in trial design.

Conclusions: De-escalation studies should be robust, timely and patient-focused, but few

current trials achieve these aims. Innovative studies designed in collaboration with patients will be essential to support future personalisation of locoregional treatments.

P5-10-23: The Impact of Body Mass Index on Pathological Complete Response Following Neoadjuvant Chemotherapy (NACT) in Operable Breast Cancer

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Introduction: Breast cancer is a significant global health issue, characterized by an incidence rate of 11.7% and a mortality rate of 6.9%. Achieving a pathological complete response (PCR) after neoadjuvant chemotherapy is linked to better survival outcomes for breast cancer patients. In the United Kingdom, 63.5 % of adult population has an elevated body mass index (BMI), a figure that has been rising over time.Objective: The objective of this study was to find out whether the body mass index of breast cancer patients had any impact on pathological response following neoadjuvant chemotherapy, and, if so, to compare the rate of achieving complete pathological response between normal body mass index (18- 24.9) and those with an elevated body mass index (≥25).Material and Methods: The study included all the female with biopsy-confirmed operable breast carcinoma who underwent surgery after receiving neoadjuvant chemotherapy between January 2018 and December 2022. Patients were categorized into two BMI groups: normal (18-24.9) and elevated (≥25). Pathological complete response was defined as ypT0/Tis ypN0, ypT0/Tis, or ypN0. Descriptive analyses were performed. Univariate and multivariate logistic regression analyses were conductedusing R software.Results: A total of 829 breast cancer patients were included in the study. Of these, 277(33.31%) had a normal BMI and 552(66.59%) had an elevated BMI. Patients with a normal BMI had the highest rate of pathological complete response (68.23%), whereas those with an elevated BMI had a lower PCR rate (32.43%) ($p<0.001$). Body mass index remained an independent predictor of pathological complete response (OR=1 for normal BMI and OR=0.21for elevated BMI; $p<0.001$).Conclusion: In this cohort, breast cancer patients with an elevated body mass index had a lower rate of pathological complete response following neoadjuvant chemotherapy. Further studies are needed to elucidate the underlying mechanisms.

P5-10-24: Real-world characteristics and BRCA testing among HER2- early breast cancer patients receiving olaparib or other adjuvant therapy in the US community oncology setting

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Background: One-third of breast cancer (BC) cases in BRCA1-mutated (BRCA1m) patients are classified as triple-negative breast cancer, an aggressive cancer prone to early onset and high risk of metastasis. 70% of BC cases in BRCA2-mutated (BRCA2m) patients

are classified as hormone-receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-). Differences in patients with BRCA1m and BRCA2m tumors suggest the need for targeted screening and treatment strategies based on genetic risk profiles. Olaparib, a poly ADP-ribose polymerase inhibitor, was approved in 2022 for adjuvant treatment in patients with germline BRCAm, HER2-, high-risk early BC (eBC) with prior chemotherapy, following the significant survival benefit seen in the OlympiA trial. It is a priority to understand how the introduction of targeted therapies like olaparib affects clinical management – including BRCA testing – and outcomes in eBC. With two years of post-approval real-world experience, we conducted a preliminary landscape assessment describing eBC patients prescribed olaparib or another adjuvant therapy with respect to patient characteristics and BRCA testing prevalence.

Methods: This was a retrospective observational cohort study characterizing HER2- eBC patients treated with olaparib or other adjuvant therapy in US community oncology clinics utilizing the iKnowMed electronic health record. Adult patients (≥18 years) diagnosed from 1 March 2018 to 31 January 2024 were eligible for inclusion and followed until last patient contact or death. Descriptive analyses evaluated patient characteristics at diagnosis, as well as BRCA testing characteristics, both overall and by treatment (initiation of olaparib or completion of non-olaparib adjuvant therapy).

Results: 250 patients (134 olaparib, 116 non-olaparib) were included and followed for a median (interquartile range [IQR]) of 26.4 (16.1, 37.9) months. Olaparib users were younger at initial diagnosis (mean (standard deviation [SD]) age of 45.8 [12.2] years versus 61.4 [14.4] years among non-olaparib users), and all patients were predominantly White (61.9% olaparib, 57.8% non-olaparib) and non-Hispanic (80.6% olaparib, 76.7% non-olaparib). Where pathologic stage was documented, most patients were diagnosed at Stage II or lesser disease (76.8% olaparib, 86.0% non-olaparib) with a histology of ductal carcinoma (87.3% and 85.3%, respectively). Notably, 68.7% of olaparib users were diagnosed with G3 high grade tumors, versus only 32.8% of non-olaparib users. All olaparib users received a BRCA test (98.5% of patients BRCAm and 1.5% negative). Among the 60.3% of non-olaparib users tested, 23.3% were BRCAm.

Conclusions: This study is one of the first to report the real-world experience of eBC patients immediately prior to and following olaparib approval. All olaparib users received a BRCA test, indicating promising results with respect to appropriate use of olaparib in only BRCA-tested patients. The analysis also found that while a high number of non-olaparib users received a BRCA test and were BRCAm, these results may indicate a potential lost opportunity for both universal BRCA testing and for BRCA-mutated patients to receive targeted therapy. However, future analyses will assess the timing of BRCA testing with respect to the availability of olaparib in this patient subgroup. Future analyses may characterize potential changes in BRCA testing patterns in the post-olaparib setting, which

will facilitate the optimization of targeted treatment plans. Lastly, as the ultimate goal is to assess impact of olaparib on eBC patient management, including improvements in survival, a formal comparative assessment of key clinical outcomes in olaparib versus other adjuvant therapies should be conducted once additional follow-up time is available.

P5-10-25: Exploring JAM2's Potential in Reducing Breast Cancer Invasiveness through IGF2BP2 Regulation

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Background:Metastatic breast cancer (MBC) presents a significant challenge in the field of oncology, with limited treatment options and generally poor survival outcomes. Therefore, the discovery of new biomarkers and therapeutic targets is crucial for improving the management of MBC. Our previous studies have shown that JAM2, as a tumor suppressor, can reduce the invasiveness and migration of breast cancer cells by inhibiting the epithelial-mesenchymal transition (EMT) pathway, thus playing a key role in tumor biology. These findings suggest the importance of further investigating the specific mechanisms of JAM2.
Methods:This study employed various techniques to comprehensively evaluate the expression and function of JAM2. First, we analyzed clinical samples from breast cancer patients using immunohistochemistry and quantitative PCR to determine JAM2 expression levels and its correlation with disease prognosis and metastasis. Additionally, we established a nude mouse model of breast cancer metastasis by injecting MDA-MB-231 cells with altered JAM2 expression to observe its effects on tumor growth and metastasis. We also used HA-tagged immunoprecipitation and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to screen for proteins interacting with JAM2 and further analyzed these interactions using Western blot, quantitative PCR, and Transwell invasion assays.
Results:The results showed that JAM2 expression was generally low in breast cancer tissues, and its low expression was closely associated with poor prognosis and high metastasis rates. In the nude mouse model, JAM2 overexpression significantly inhibited tumor growth and metastasis. In vitro studies further revealed a direct interaction between JAM2 and IGF2BP2, where JAM2 overexpression significantly reduced IGF2BP2 levels, decreasing cell invasiveness and migration. Conversely, JAM2 knockdown increased IGF2BP2 expression, enhancing the invasiveness of breast cancer cells. Moreover, JAM2 inhibits the expression of the key EMT transcription factor Snail2 by affecting IGF2BP2, further emphasizing its role in inhibiting EMT and metastasis.
Conclusion:The findings of this study highlight the important biological function of JAM2 as a metastasis suppressor in breast cancer, providing new insights into the key proteins and pathways it regulates during tumor progression. JAM2 may inhibit the EMT and the invasiveness and migration of breast cancer cells by downregulating the m6A reader protein IGF2BP2, thus reducing the stability of SNAI2 mRNA. This suggests that enhancing JAM2 expression could be a viable therapeutic strategy to combat breast cancer metastasis.

Future studies are needed to fully elucidate the mechanisms by which JAM2 exerts its effects and to explore its potential clinical applications.

P5-10-26: Adverse events in routine real-world clinical practice in patients with HER2-negative early breast cancer according to BRCA status: a retrospective observational study using artificial intelligence in Spain

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Background:Early breast cancer (eBC) survivors often face potential adverse events (AEs) from treatment, especially those with germline BRCA1/BRCA2 mutations (gBRCAm) who are diagnosed at younger ages and are candidates for specific and aggressive risk-mitigation strategies. This study assessed real-world AE patterns in Spanish HER2-negative (HER2−) eBC patients (pts) according to gBRCA status using natural language processing (NLP) on electronic health records (EHRs).
Methods:This was an observational, multicenter, retrospective study of adult pts with incident HER2− eBC (stages I–III) who received (neo)adjuvant therapy between July 2015 and December 2019 at three Spanish hospitals. Pts with synchronous/previous cancers, or in clinical trials, were excluded. We used EHRead®, a validated technology based on NLP and machine learning using the Systematized Nomenclature of Medicine Clinical Terms, to extract clinical information included in structured and free-text EHR data. Pts were stratified by gBRCAm and non-gBRCAm (wild type/unknown) status. We analyzed patient demographics and clinical characteristics at inclusion, as well as (neo)adjuvant treatment patterns, and potential treatment-related AEs during follow-up.
Results:A total of 1,610 eligible pts with HER2− eBC were identified after screening 5,114,548 EHRs (from 81,956 pts). Among these, 191 (11.9%) had gBRCAm and 1,419 (88.1%) had non-gBRCAm (1,403 unknown). Overall, 1,581 (98.2%) pts were female, 72.8% had hormone receptor-positive (HR+) and 27.2% had triple-negative eBC. Specifically, 67.5% of the pts with gBRCAm and 73.5% of pts with non-gBRCAm were HR+. Mean (SD) age was 48.2 (13.7) years in those with gBRCAm and 57.7 (14.2) years in those with non-gBRCAm. Mastectomy rates were 48.7% (gBRCAm) and 17.8% (non-gBRCAm), with radiotherapy administered to 82.7% and 79.1%, respectively. (Neo)adjuvant systemic treatments were given as follows for gBRCAm vs non-gBRCAm: any chemotherapy 67.0% vs 46.3% (anthracyclines 61.8% vs 40.7%, cyclophosphamide 61.8% vs 42.7%, taxanes 63.4% vs 40.3%, fluoropyrimidines 16.2% vs 5.8%, platinum compounds 14.1% vs 3.5%), tamoxifen 33.0% vs 31.0%, aromatase inhibitors 51.8% vs 48.4%, and GnRH antagonists 20.4% vs 4.4%. Median follow-up (interquartile range) was 54.2 (98.0–74.0) months. The most frequently reported AEs in both groups were hematologic complications, affecting 87.4% of pts with gBRCAm and 63.9% of pts with non-gBRCAm, predominantly neutropenia (59.7% vs 40.0%) and thrombocytopenia (59.7% vs 41.5%). Other observed AEs for gBRCAm vs non-gBRCAm were: heart failure 16.8% vs 12.9% and ischemic cardiac disease 5.2% vs 6.3%; local complications 66.0% vs 51.5%, including breast pain 43.5% vs 31.2%

and lymphedema 28.8% vs 17.2%; pneumonitis 6.3% vs 3.9%; osteopenia 13.1% vs 16.6%, and osteoporosis 9.4% vs 14.7%; anxiety 22.0% vs 14.9% and depression 7.9% vs 9.2%. Sexual dysfunction, cognitive impairment, and fear of recurrence were detected in <5% of pts.
Conclusions:Compared with non-gBRCAm pts, those with gBRCAm were, on average, 10 years younger and more frequently treated with mastectomy and chemotherapy, experiencing higher AE frequencies. Low detection rates of AEs such as sexual dysfunctions and psychiatric/psychological disorders suggests potential underreporting in routine clinical practice, highlighting the need for standardized EHR completeness. This would enhance richness and accuracy of data collection, especially given the availability of NLP-based extraction tools for massive data extraction. Future NLP-based studies are essential for evaluating AEs related to new eBC treatments, including gBRCAm-targeted therapy, and for informing clinical decision-making.

P5-10-27: Incidence, risk factors and survival outcomes of Breast Cancer Brain Metastasis: a population-level SEER analysis.

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Introduction: Metastasis is the leading cause of mortality in breast cancer (BC), and brain metastasis (BM) carries the most unfavorable prognosis. BMs are typically diagnosed based on symptoms, and the clinical outcomes varies depending on the number, location, and size. The aim of our study was to characterize the incidence of BM at the time of diagnosis and to identify the risk factors that are associated with a higher incidence of Breast Cancer Brain Metastasis (BCBM).

Method: Patients diagnosed with BC from 2010 to 2020 were identified using the Surveillance, Epidemiology, and End Results (SEER) database. We summarized clinicodemographic data by BM status to identify potential risk factors for developing BM. Multivariate logistic regression was performed to determine the risk factors associated with BM at diagnosis. Survival outcomes by BM status were analyzed by using cox-proportional hazard regression for the overall population and adjusted for gender, age (18-40 years old (y), 41- 61 y, 61-80 y, and 81+ y), race, marital status, residential area, and treatment modalities. The analysis was done using R version 4.4.1 and statistical significance was defined at $P< 0.05$.

Results: Of the 673,638 patients diagnosed with BC, 2,924 (0.4%) had BCBM. The majority were females (99%, n=2,891) and aged 41 y or older (93%, n=1429). 61% were White (n= 1,772), 16% Black (n= 459), 15% Hispanic (n= 447), 7.2% Asian (n=211) and 0.9% Native American (n=27). BC subtypes among BCBM were: 38% hormone receptor (HR) +/HER2 -, 15% HR+/HER2+, 11% HR-/HER2+, and 18% triple negative BC (TNBC). Other metastases

sites included 64% bone, 44% lung and 31% liver.
Several factors were found to be significantly associated with higher risks of developing BCBM on multivariate logistic regression analysis. The following factors were associated with increased risk of brain metastases: ages 41-61 y (OR 1.2, 95% CI: 1.1-1.4, P <0.01), Hispanic ethnicity (OR 1.2, 95% CI: 1.1-1.3, P<0.004), being single, widowed, or separated (OR 1.2, 95% CI: 1.1-1.3, P<0.001), AJCC stage T4 (OR 4.4, 95% CI: 3.7-5.1, P<0.001) and nodal status N1/2/3 (OR 2.1, 95% CI: 1.9-2.3, P<0.001), presence of bone metastases (OR 20.6, 95% CI: 18.8-22.6, P<0.001), and HR-/HER2+ (OR 3.1, 95% CI: 2.7-3.5, P<0.001) and TNBC subtypes (OR 3.0, 95% CI: 2.7-3.4, P< 0.001).
Patients with BCBM had a shorter median disease specific survival (DSS) compared to patients without BM (13 months (mo) vs. not reached (NR) (HR=22.5, 95% CI: 21.5-23.5, P <0.001). Moreover, they had shorter median overall survival (OS) compared to patients without BM (9 mo vs. NR) (HR=15.0, 95% CI: 14.4-15.7, P <0.001). TNBC patients with BMs have the worst OS (5 mo vs NR) (HR= 2.10 95% CI: 2.07-2.14, P<0.001) and DSS (7mo vs NR) (HR =2.71, CI 2.65-2.77 P<0.001). OS and DSS are also shorter among Black patients, with a median of 8 mo vs NR (HR=1.26, 95% CI: 1.24-1.28, P < 0.001) and (10mo vs NR) (HR =1.27, CI 1.25-1.30 P<0.001) respectively.

Conclusion: Our study shows that patients with HER2-positive and TNBC, Hispanic ethnicity, older age, higher stage T4, living without a partner, nodal and bone metastases are at higher risk of developing BCBM at the time of diagnosis. Prompt screening and higher care should be provided to the patients with these high-risk factors to identify brain metastases. More studies are required to establish risk criteria for brain MRI screening and even preventive intervention on high-risk patients to delay the development of brain metastases.

P5-10-28: A retrospective analysis of the association between body composition measures and relative dose intensity of (neo)adjuvant chemotherapy for elderly breast cancer patients

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Introduction: The use of (neo)adjuvant chemotherapy (CT) in certain subtypes of early-stage breast cancer (EBC) is associated with improvements in pathologic complete response rates (pCR) and in long term outcomes, including event-free and overall survival. Frail and elderly patients (pts) may be less likely to tolerate full doses of CT and may require dose and schedule adjustments in CT. Previous studies have demonstrated that pts with EBC who receive >85% relative dose intensity (RDI) of the planned CT regimen achieve improved outcomes. This study evaluates the association between body composition (BC) or physical functioning (PF) measures and receipt of (neo)adjuvant CT at RDI >85%.
Methods: This study included EBC pts over age 65 who received (neo)adjuvant CT for EBC,

were seen in the rehabilitation clinic of the Miami Cancer Institute between 2018 and 2023, had complete CT records, and had PF and BC data within 1 year of receiving CT were included. Pt demographics, tumor characteristics, PF, BC, CT regimen, RDI, and distant recurrence free interval (DRFI) were evaluated. Validated PF measures included grip strength, sit-to-stand (STS) test, timed up and go (TUG) test, and 4-stage balance test. BC was measured using a bioelectrical impedance analysis (BIA) machine, yielding total body water (TBW), skeletal muscle and fat mass, phase angle, and intracellular and extracellular fluid. RDI was calculated as amount of CT received per time elapsed divided by amount of CT planned per standard time to complete planned CT. Pt characteristics were compared between RDI categories (<85% versus ≥85%) using Chi squared test for categorical variables or Mann Whitney U test for continuous variables. Kaplan Meier test was used to assess DRFI.

Results: 49 pts were included in this analysis and 47/49 pts (95.9%) were female (F). 31/49 pts (63.3%) were of Hispanic (H) ethnicity, 40 (81.6%) were white (W), and 7 (14.3%) were black (B). Of the entire group, 30/49 pts (61.2%) and 29/47 (61.7%) of F pts achieved >85% RDI, while 0/2 male (M) pts achieved this metric (p=0.07). When evaluating pts who received RDI >85% by ethnicity, differences were noted with 17/31 (54.8%) H and 13/18 (72.2%) non-H pts received >85% RDI (p=0.23). By race, 26/40 (65.0%) W pts achieved >85% RDI while only 3/7 (42.9%) B pts were able to do so (p=0.3281). Among the pts who received anthracycline-based CT, 17/30 (56.7%) achieved >85% RDI compared to 13/19 (68.4%) of those pts who received a non-anthracycline based regimen (p=0.41). STS was statistically significantly higher among those who achieved >85% RDI than those who did not (11.0 vs 7.4; p=0.004). TUG was also statistically significantly better among those who achieved >85% RDI (8.6 sec vs 11.9 sec; p=0.002). TBW was significantly lower in those with >85% RDI than those with <85% RDI (33.2 L vs 36.6 L; p=0.04). There were no significant differences in STS, TUG, or TBW based on race or ethnicity. Median DRFI was non-significantly longer in those who received RDI >85% (67 vs 57 months, p=0.601).

Conclusions: This study demonstrates that pts with poorer physical functioning as measured by STS and TUG tests and those with elevated TBW were less likely to achieve RDI >85%, an established benchmark for superior outcomes. These tests are efficient and convenient for use in the clinical setting. There were differences in RDI by gender as well as race/ethnicity, with M, B, and H pts demonstrating a non-significant trend toward lower RDI than F, W, and non-H pts. The establishment of a scoring system to predict which pts are unlikely to achieve RDI >85% could help tailor CT regimens for elderly or frail pts. This information can also be used to improve the physical function and nutritional status of these pts by incorporating rehabilitative and nutrition programs before and during (neo)adjuvant CT.

P5-10-29: Comparative analyses of Van Nuys index and NCCN guidelines in ductal carcinoma in situ in a Brazilian hospital

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Objectives: To compare the recommendations of the National Comprehensive Cancer Network (NCCN) and the Van Nuys Index with the treatment adopted for DCIS in patients at the Hospital do Servidor Público Estadual de São Paulo (HSPE), relating the treatment employed to recurrence rates during follow-up. Additionally, this study aims to analyze the epidemiological profile of patients with DCIS in the same facility.
Methods: This is a retrospective cross-sectional observational study, conducted by reviewing the medical records from the Mastology Department at HSPE. The following variables were evaluated: age, menarche, parity, breastfeeding, duration of breastfeeding, menopause, family history of breast cancer, initial presentation, follow-up duration, diagnostic method, anatomopathological results, nuclear grade, tumor size, surgical margin, immunohistochemistry, Van Nuys Index score, NCCN recommendation, treatment administered, number of recurrences, and deaths.
Results: A total of 145 patients with DCIS were evaluated between 1996 and 2021, with a mean age of 60.1 years. Of these, 100 had no family history of breast neoplasia, and 88 were diagnosed through screening exams. Among those with clinical symptoms, a palpable nodule was the most prevalent (24.1%), and the most commonly employed treatment was conservative surgery with radiotherapy (43.1%). During follow-up, 18 (15.5%) recurrences were observed. According to the Van Nuys Index, 45 patients were classified as low risk, 62 as intermediate risk, and 9 as high risk. According to the NCCN classification, 15 patients were in the low-risk group, and 101 were in the high-risk group. Qualitative factors and their relationship with the presence of recurrences were evaluated, but no statistically significant relationships were found.
Conclusions: DCIS predominantly affects postmenopausal women, with the majority of diagnoses made through screening and the primary treatment was conservative surgery followed by radiotherapy, resulting in a low number of recurrences. No significant factors associated with recurrences were identified. When comparing the Van Nuys Index and the NCCN guidelines, the latter advocates for a more aggressive treatment approach and showed a 5.6% reduction in local recurrences.

P5-10-30: Developing microenvironment-based prognostic biomarkers for early breast cancer

Christina Kozul, Keith Naylor, Allan Park, Cameron Nowell, Metta Jana, Sandun Silva, Madawa Jayawardana, Bruce Mann, Belinda Parker

Problem statement: Ductal carcinoma in situ (DCIS) is a pre-invasive stage of breast cancer in which tumour cells have not invaded beyond the ductal system. Approximately 25% of

screen-detected breast cancers are now diagnosed as DCIS. Predicting local recurrence in patients with DCIS is imprecise, with standard histopathological features and intrinsic tumour cell markers failing to identify a low-risk group that could be spared therapy. Myoepithelial cells, the cells that surround breast ducts, are considered the gatekeepers where they not only act as a physical barrier between epithelial cells and the surrounding tissue/basement membrane but also secrete factors that suppress pro-invasive mechanisms such as proteolytic activity. Myoepithelial proteins offer an alternative pathway for biomarker development in DCIS.
Methods:Candidate myoepithelial markers were identified through a high-throughput siRNA 3D co-culture screen that was designed to identify myoepithelial cell-derived suppressor proteins. To investigate the functional role of candidate myoepithelial proteins, we utilised and modernised an established DCIS-like model, incorporating an immortalised myoepithelial cell line (N1ME) that has been shown to restrain tumour cell invasion in 3D culture when co-cultured with a triple-negative breast cancer cell line (MDA-MB-231). Knockdown and CRISPR-based knockout of candidate adhesion proteins and protease inhibitors allowed assessment of the importance of these proteins in the suppressive capacity of myoepithelial cells. We quantified this using a perimeter-to-convex hull ratio and high-resolution single organoid analysis, including organoid tightness. Characterisation of the expression of these proteins in normal, DCIS and microinvasive tissues was performed using immunohistochemistry.
Results: An adhesion protein and two cathepsin inhibitors were identified for their tumour-suppressing function. Targeted siRNA knockdown and CRISPR knockout in N1ME cells significantly blocked myoepithelial cell-mediated suppression of breast cancer cell invasion when compared to the controls using both perimeter-to-convex hull ratio and high-resolution adhesion analysis of the N1ME cells within the organoid. Despite the physical presence of myoepithelial cells in these cultures, loss of these proteins was sufficient to allow tumour cell invasion beyond this cellular barrier. Given the potential of these myoepithelial proteins as biomarkers of risk of invasion, we measured expression in normal and early cancer lesions and confirmed that their expression is highly abundant in myoepithelial cells surrounding normal ductal epithelium but reduced or completely lost in micro-invasive DCIS regions, the earliest phase of invasion. Current studies aim to evaluate the prognostic value of these markers in predicting the risk of invasive recurrence in a larger cohort.
Conclusion: We have identified novel myoepithelial proteins that suppress early tumour invasion. Our work may offer a new approach to patient stratification to ensure treatment is personalised and treatment is optimised for patients with DCIS.

P5-11-01: LAD Dose and Cardiotoxicity in Breast Cancer Patients Receiving Radiation

Colleen Conger, Nhat Minh Ho, Bryson Hill, Jacob Buatti, Alissa Herrera, Mohit Shiv Agarwal, Jonathan Gelfond, Neil Newman

Purpose: Although whole heart dose has been associated with cardiac toxicity in patients receiving radiation therapy for breast cancer, data detailing the clinical significance of cardiac substructures, particularly the left anterior descending artery (LAD), are limited. We investigated whether dose to both the LAD and whole heart correlates with adverse cardiac events and death in our cohort.
Methods and Materials: We identified 1 male and 493 female patients treated from 2004 to 2022 who received definitive breast or chest wall irradiation, with or without regional nodal irradiation. Cumulative radiation dose, treatment dates, and treatment type were obtained from Mosaiq. EPIC was queried to identify cardiac events after radiation therapy, as well as anatomic stage at diagnosis, surgery, chemotherapy, and hormonal therapy received, baseline cardiac problems, whether the patient took cardiac medications prior to treatment, and whether the patient was deceased. Mean and maximum LAD and heart doses, as well as V15LAD, were calculated using Pinnacle. Univariate and multivariate Cox regression analyses were performed in R to determine the association of the collected variables with cardiac toxicity and death. Cumulative incidence curves for death and cardiac events were also calculated.
Results: Median follow-up time was 39 months. Fifty-two patients experienced a cardiac event, with 17 of these being grade 3 or higher. Forty-two patients (8.5%) were deceased at the time of data collection, 5 of whom experienced a cardiac event prior to death. One death was of cardiac origin. On univariate Cox regression, the only significant predictor of any cardiac event or cardiac event grade 3 or higher was baseline heart problems (p=.026 and p=<.001, respectively). On univariate Cox regression, statistically significant predictors of death included anatomic stage (p=<.001), mean LAD dose (p=<.001), max LAD dose (p=.002), V15LAD(p=<.001), and mean heart dose (p=<.001), and receipt of DIBH treatment was protective against death (p=.02). Cumulative incidence curves predicted a 12.64% probability of a cardiac event 5 years after treatment and a 37.28% probability at 10 years after treatment. They also predicted an 8.40% probability of death at 5 years post-treatment, and a 16.24% chance of death at 10 years.
Conclusion: The only significant predictor of adverse cardiac events in this cohort was baseline cardiac problems. Mean heart dose, mean LAD dose, max LAD dose, and V15LAD were all significant predictors of death, but not adverse cardiac events. Additionally, anatomic stage was predictive of death, and DIBH was protective. Longer term follow-up may prove useful in discerning the relationship between LAD dosing and adverse cardiac events in this cohort, as median follow-up was only 39 months.

P5-11-02: Dosimetric Advantages of Prone 3DCRT APBI: A Comparison with Supine IMRT APBI from the Livi Study

Rufus Banks, Jose G. Bazan, Rolan Cesar Domingo, Priya Mitra, Erin Healy

Background: External beam accelerated partial breast irradiation (APBI) has emerged as an alternative to whole breast radiation (WBI) in appropriately selected patients who have undergone breast conserving surgery. It is associated with lower short- and long-term

toxicity compared with WBI due to smaller target volumes and increased sparing of normal tissues. However, there have been conflicting data regarding toxicity and cosmesis from the various APBI clinical trials. Since publication of the long-term results of the University of Florence study by Livi et al. in 2020, the field has shifted towards using the Florence regimen, 30Gy in 5 fractions, which utilizes inverse planned intensity modulated radiation therapy (IMRT) in the supine position. Prior randomized trials used twice daily fractionation and 3D conformal (3DCRT) technique. There is uncertainty whether it is the fractionation schedule (BID vs. daily) or technique (IMRT vs. 3DCRT) that resulted in more favorable cosmetic results. At our institution, we utilize prone positioning and 3DCRT technique to deliver APBI. We set to compare our dosimetric results to those reported in the Livi study.

Methods: We queried our institution from January 2022 to June 2024 for patients with early-stage breast cancer who had undergone BCS and were treated with APBI in the prone position. All patients treated with the same dose and fractionation used in the Florence regimen, 30Gy in five daily fractions delivered on non-consecutive days. We collected patient and tumor characteristics as well as a detailed dosimetric parameters. We focused on the key parameters in the Livi study including heart V3Gy, ipsilateral lung V10Gy, contralateral lung V5Gy, and uninvolved breast V15Gy. We also captured the volume of the planning target volume (PTV) that received 95% of prescription dose (V95%). We compared our results to those reported in the Livi study using an unpaired T-test with a P-value <0.05.

Results: During this time, there were 82 patients who met our criteria. The median age was 61 years (IQR 52-68). 72% had invasive disease, 48.8% were left-sided, and 95% were estrogen receptor positive. Our target coverage PTV V95% was significantly higher than that recorded in the Livi study (mean 98.7%, median 99.3%, range 95-100%, SD=1.5 vs. a mean of 96.6%, median 97%, range 88-100%, SD=2.8, $P<0.0001$). Despite this, we found that the dose to all OARs were significantly lower in our population compared to those in the Livi study. The heart V3Gy was significantly lower in our population (mean 1.1%) compared to the Livi study (mean 7.4%, $p<0.0001$). Our ipsilateral lung V10Gy mean=0.71%, median=0%, IQR=0-0.6%, range=0-10%, SD=1.76 vs. mean=10.3%, median=11.0%, range=0.0-22.0%, SD=4.9, $P<0.0001$. Our contralateral lung V5Gy mean=0%, median=0%, range=0-0%, SD=0 vs. mean=0.9%, median=0.0%, range=0-19.0%, SD=3, P=0.007. Lastly, our uninvolved breast V15Gy mean=26.5%, median=27.5%, IQR=18.9%-33.6%, range=9.7%-48.7%, SD=8.52 vs. mean=32.3%, median=31.0%, range=8.0-62.0%, SD=11.4, $P<0.0001$.

Conclusion: Our study demonstrates that prone 3DCRT APBI achieves superior target volume coverage and significantly lower dose to organs at risk (OARs) compared to supine IMRT APBI reported in the Livi study. This suggests that prone 3DCRT APBI may be a suitable alternative to IMRT for achieving good target coverage while potentially minimizing toxicity to surrounding healthy tissues. However, longer follow-up is necessary to assess cancer control and cosmetic outcomes.

P5-11-03: Optimizing Adjuvant Treatment Decisions for Patients With Ductal Carcinoma In Situ Using the Oncotype DCIS Score: An Analysis from the National Cancer Database

Jose Bazan, Stephanie Yoon, Katharine Schulz-Costello, Jamie Rand, Peter Wu

Background: The optimal treatment of patients with ductal carcinoma in situ (DCIS) that have undergone breast conserving surgery (BCS) remains controversial given the need to strike a balance between preventing the development of invasive disease while minimizing the risk of over-treatment and treatment-related toxicity. Until recently, personalized genomic assays to help estimate recurrence risks such as the Oncotype DCIS Score were not available. We sought to determine the frequency of DCIS Score use within the National Cancer Database (NCDB) and to determine if the DCIS Score was associated with use of adjuvant treatments after BCS.
Materials/Methods The NCDB began collecting the DCIS Score in 2018. We identified patients with DCIS that had undergone BCS with negative margins from 2018 to 2021 who were pathologically stage group 0. We then identified patients who had a resulted DCIS score and dichotomized these patients into a low-risk (LR) group (DCIS Score<39) or non-LR group (DCIS Score≥39). We collected information on age, grade, size of DCIS, hormone-receptor status, receipt of radiation therapy (RT) and receipt of endocrine therapy (ET) for the entire cohort and for patients with a DCIS Score. We compared rates of RT and ET use between the LR and non-LR groups and applied multivariable logistic regression to calculate odds ratios. We then created a propensity score matched (PSM) cohort balanced for age, race, performance status, size of tumor (<1cm, 1-2.5cm, 2.5cm+), grade, ER/PR status, insurance status, year of diagnosis, income, and compare rates of RT and ET use in patients with a DCIS Score versus those without.
Results: We identified 82,965 patients that met inclusion criteria, of which 2688 (3.2%) had a DCIS Score. The proportion of patients with a DCIS Score decreased from the first year to the last year of the study (4.5% vs. 2.4%, p<0.001). In patients with a DCIS Score, 69.3% (1,791) were in the LR group and 30.7% (794) in the non-LR group. In the DCIS Score cohort, 39.9% (N=301) of patients with grade 3 disease fell into the LR group and 18.5% (N=319) of patients with grade 1-2 disease were in the non-LR group. Rates of RT (71% vs. 36%, OR=3.2, 95% CI: 2.6-3.9, p<0.001) were significantly higher in the non-LR group compared to the LR group. ET did not vary significantly with respect to LR vs. non-LR group (65% vs. 59%, OR=1.2, 95% CI: 1.0-1.5, p=0.1). In the PSM cohort, the rates of RT use were 47% vs. 68% (OR=2.8, 95% CI 2.5-3.2, p<0.001) and rates of ET use were 63% vs. 65% (OR=1.1, 95% CI 1.0-1.3, p=0.1) in patients with DCIS Score vs. without DCIS Score. Patients with a DCIS Score had significantly higher rates of receiving no adjuvant therapy compared to those without a DCIS Score (22.3% vs. 15.0%, OR=1.6, 95% CI 1.4-1.9, p<0.001).
Conclusion: Overall, the adoption of the DCIS Score in patients within the NCDB since 2018 has been low and appears to be decreasing. However, the DCIS Score appears to have clinical utility regarding adjuvant RT in patients that have undergone BCS and DCIS Score receipt was also associated with higher rates of BCS alone. Use of personalized tools such as

the DCIS Score should be encouraged to help patients and physicians optimize adjuvant radiation therapy use for DCIS.

P5-11-04: SHORT-TERM ECOCARDIOGRAPHIC ALTERATIONS AMONG BREAST CANCER PATIENTS WITH HYPOFRACTIONATED RADIATION THERAPY

Ricardo Mendoza Coronado, Christopher Cerda Contreras, Ricardo Mendoza Coronado, Enrique Gonzalez Nava, Rafael Piñeiro Retif, David Hernandez Barajas, Oscar Vidal Gutierrez, Marco Antonio Gamez Ruiz, Melissa Emilia Rodriguez Ruiz

Radiotherapy is one of the mainstays of breast cancer (BC) treatment. In the adjuvant setting, either conventional or hypofractionated radiotherapy (HRT) eradicates subclinical disease (1). Nevertheless, long-term adverse effects such as secondary neoplasms, skin, pulmonary and cardiac toxicities are not infrequent. Radiation-induced cardiotoxicity (RC) involves valvopathies, early atherosclerosis, arrhythmias and heart failure due to acute left ventricle myocardial dysfunction (2). Early cardiac repolarization and mild subclinical myocardial disfunction are found along, 6-week or 9-month after RT by EKG and echocardiography, respectively (3-5).
Objective:
To identify subclinical cardiac changes immediately after hypofractionated RT using global longitudinal strain (GLS) measured by doppler and 2D-echocardiography (2DE).
Methods:
A prospective pilot-cohort study was conducted. Sociodemographic, clinical and paraclinical data of non-metastatic BC patients were collected by the end of last year. Postoperative breast RT was planned for all patients with 40 Gy in 15 fractions to tumor bed and/or chest wall with or without nodal irradiation, followed by sequential boost to tumor bed if required, using sequential helical tomotherapy. Cardiac radiation dose distribution was also registered. A 2DE was performed before starting and the day after completion of RT.
Results:
38 female BC patients were recruited. Median age was 57 years. 60% had hypertension and 39% diabetes. 42% had overweight & 50% were obese. III stage disease accounted for 44,7%. 71% were hormone receptor positive, 15,7% HER2 positive and 26,3% triple negative BC. Mean and maximum heart dose were 3,55 +/- 0,5 mSv and 23,47 +/- 9,2 msV, respectively. Measure of left ventricular ejection fraction with biplane Simpson's method before and after HRT were 59,4% +/- 5,1 and 59,21% +/- 4,9. GLS prior to RT was 19,26 +/- 1,97, and afterwards was 19,11 +/- 2,09. No statistical difference in LVEF (p=0,899) nor GLS (p=0,605) were found previously or following HRT. However, very subtle diastolic changes such as IVSd (interventricular septum thickness), PWd (left ventricular posterior wall) and mitral E wave velocity were statistically significant (p=0,030; p=0,033 and p=0,083). No myocarditis, pericarditis, pericardial effusion or any other significant echocardiographic finding were observed.
Conclusion:

Although no meaningful echocardiographic changes were found after HRT, this pilot study supports the theory that HRT is safe and does not induce relevant hyperacute cardiac anomalies in non-metastatic BC. 2D-echocardiography is a useful tool to diagnose and monitor subclinical disease before long-term radiation-induced cardiotoxicity is established. Middle and long-term cardiovascular changes of this cohort will be analyzed in future publications.

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P5-11-05: Phase II Trial of Ultrahypofractionated Image-Guided Partial Breast Irradiation Following Lumpectomy with Optional Oncoplastic Reconstruction for Early-Stage Breast Cancer

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Background & Purpose: Breast conservation surgery followed by adjuvant radiotherapy has shown more positive body image attitudes compared to mastectomy. This study investigates the use of a 5-fraction accelerated partial breast irradiation (APBI) radiation for early breast cancer with or without oncoplastic surgery with the aim to share subjective and objective outcomes, including toxicities and cosmetic results. Methods & Materials: Eligible patients for this single institution phase II trial (IRB 18-002) were aged 50 or older with unifocal stage 0 to 1 breast cancer measuring ≤ 3 cm undergoing breast conserving surgery or oncoplastic reconstruction with negative surgical margins (≥ 2 mm margin width), ER positive, negative nodes and no lymphovascular invasion. Presurgical MRI was routinely performed. Patients were treated to 30 Gy in 5 fractions via intensity-modulated radiation therapy (IMRT) using a QOD schedule on the Varian Edge radiosurgery system. The planning target volume (PTV) was defined as the tumor cavity + 1.5 cm cropped 5 mm off skin with the mean PTV30 of 146 cc (range 43-408). Clinical follow-up included history

and physical, breast imaging, and the validated Breast Cancer Treatment Outcome Scale (BCTOS) at 6 to 12 months intervals. Results: A total of 50 patients with 52 tumors were enrolled with a median age of 76 (range 51 to 89) between November 2018 and July 2022 with 23 patients (46%) undergoing oncoplastic breast surgery. Median follow-up was 37 months (range 9 to 64), the 4-year local control, regional control, distant control, and overall survival were 100%, 96%, 100%, and 94% respectively. There were 2 recurrences (1 regional nodal failure, 1 second primary triple negative breast cancer) and 3 deaths from other causes other than breast cancer occurred in this patient population. Treatment was well tolerated with 22% grade 1 to 2 acute toxicity and 4% late toxicity consisting of grade 1 pain. Physician-rated long-term cosmesis was rated as 83% excellent and 17% good. BCTOS scores at baseline were 58% excellent (91% lumpectomy, 13% oncoplastic), 42% good (9% lumpectomy, 88% oncoplastic), 0% fair to poor, and at ≥2 year follow up were 92% excellent, (100 % lumpectomy, 86% oncoplastic), 8% good (0 % lumpectomy, 14% oncoplastic), and 0% fair to poor. Conclusion: This trial demonstrated the safety and efficacy of a modernized Florence regimen in low-risk stage 0 to 1 breast cancer patients treated at a community hospital. Long-term cosmetic outcomes were excellent and oncoplastic reconstruction does not appear to be a contraindication for APBI.

P5-11-06: Adjuvant radiation therapy following pathologic complete nodal response to neoadjuvant chemotherapy; Real World Data

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Background and objectives: After neoadjuvant chemotherapy (NACT), the likelihood of loco-regional recurrence (LRR) within 10 years in locally-advanced breast cancer patients (cT1–3, N0–N1) is substantial, surpassing 10%. Adjuvant nodal irradiation (RNI) has been the standard of care in locally advanced breast cancer patients with affected lymph nodes to lower the chances of loco-regional recurrence of breast cancer despite the response following neoadjuvant chemotherapy for many years. However, it comes with the cost of significant skin, lungs, and heart toxicities. Moreover, post-mastectomy radiotherapy (PMRT) is associated with a high rate of implant-related complications.
Researchers have tried to address if regional nodal irradiation can be safely omitted after achieving lymph nodes pathologic complete response (ypN0) in breast cancer patients with lymph node involvement receiving neoadjuvant chemotherapy. In the recently presented abstract of the NSABP B-51/RTOG 1304 trial, with median follow up of 59.5 months, isolated Loco-regional recurrence was reported in 1.6% of the patients who did not receive RNI and in 0.7% of the patients who did, with invasive breast cancer recurrence reported as 8.2% and 7.3% in both groups, respectively.
This study aims to review treatment outcomes of the same cohort of patients at King Hussein Cancer Center (KHCC) and asses if RNI omission can by safely applied.

Research method: In this study, we will retrospectively analyse treatment outcomes of eligible women who are diagnosed at age of 18 years or older with non-metastatic breast cancer patients with cT1-3 disease, with biopsy proven lymph nodes involvement, received neoadjuvant chemotherapy and completely responded in the nodes as determined by sentinel-lymph-node biopsy or axillary-node dissection. They also should've been treated with regional nodal irradiation after mastectomy or breast-conserving surgery at KHCC. Patients with bilateral, de-novo metastatic disease or residual nodes are excluded. Primary Endpoint is Loco-regional recurrence in the ipsilateral breast or regional nodes. Secondary outcomes include distant Recurrence-free Interval, disease-free and overall Survival.
Result and findings: Of all patients who were retrieved from KHCC cancer registry, treated and followed up at KHCC between Jan, 2013 and Dec 2023, 209 Patients fulfilled the eligibility criteria. Median age was 48 years. Majority had clinical stage cT2. half of the patients had grade 3 and had ductal histology in 92.3%. 86.6% had LVI negative. All patients received radiation with mean dose 47Gy, one third received tumour bed boost. Only 7% were triple negative. 45% achieved complete tumour response while only 7.2% had ypT2-3. The median follow-up period was 55 months. Overall survival (OS) rates were 99.0% at 24 months and 96.6% at 48 months. Progression-free survival (PFS) rates were 97.1% at 24 months and 87.0% at 48 months. Locoregional control rates were 99.0% at 24 months and 93.2% at 48 months. Eleven patients experienced locoregional failure. Histologically, none of them below cT2, 63.6% were grade 2 and 36.4% were grade 3. Lymphovascular invasion was present in 36.4%, and none had perineural invasion. 63.6% received a tumor bed boost, and almost all had negative margins. Regarding receptor status, 36.4% were hormonal receptor-negative and 54.5% were HER2-positive. 18% had ypT2, the rest equally distributed among ypT0, ypTis, and ypT1.

Conclusions: The B-51/RTOG 1304 study indicates that omission of adjuvant radiotherapy in ypN0 isn't negatively impacting outcomes. We would anticipate a locoregional recurrence rate without radiation of 14.46% in our patients compared to 1.375% in the B51 cohort if we applied basic assumptions based on our recurrence rate. Careful selection is warranted.

P5-11-07: A longitudinal analysis of the specific types of anxiety towards radiotherapy in patients with breast cancer before, during, and after radiotherapy

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Backgrounds: Most patients have many concerns about radiotherapy (RT) because they know little about it. Radiotherapy Categorical Anxiety Scale (RCAS) was formulated to evaluate the specific types of anxiety among cancer patients receiving RT. This study aims to apply the RCAS to breast cancer patients in China and assess the reliability and validity of the Chinese version; and to investigate its longitudinal changes throughout treatment.

Methods: This prospective, longitudinal study enrolled 504 eligible breast cancer patients receiving RT in China. Patients completed questionnaires assessing the specific types of RCAS, depression (9-item Patient Health Questionnaire [PHQ-9]), and anxiety (7-item Generalized Anxiety Disorder [GAD-7]) before, during, and after RT. Psychometric properties of RCAS including internal consistency, construct validity, convergent validity, and discriminant validity, were assessed. Generalized estimating equations were performed to evaluate dynamic changes in RCAS total and sub-scale scores. Univariate and multivariate regression analyses were conducted to explore factors associated with RCAS score before and after RT.
Results: The Chinese version of RCAS demonstrated its excellent reliability (Chronbach's α 0.89 to 0.92) and construct validity (Bartlett sphericity test $P < 0.001$; Kaiser-Meyer-Olkin index = 0.95). Confirmatory factor analysis further demonstrated its adequate convergent and discriminant validity. RCAS scores decreased significantly during RT over time. RCAS-F3 (treatment effects of RT) remained stable, while RCAS-F1 (adverse effects of RT) and RCAS-F2 (environment of RT) decreased significantly over time. Patients presented higher scores in items about side effects, after-effects, and treatment effects of RT compared with other items in RCAS. Multivariate analysis revealed that only high PHQ-9 and GAD-7 scores were independent factors for high RCAS scores, no matter before or after RT.
Conclusions: The Chinese version of RCAS was eligible for quantitatively evaluating the specific types of anxiety in breast cancer patients receiving RT in China. Clinicians should pay more attention to dispelling patients' concerns of side-effect, after-effects, and treating efficacy of RT, especially for patients with depression and anxiety.

P5-11-08: Primary analysis of radiotherapy data from prospective randomized controlled phase III VENUS trial: sentinel lymph node biopsy Versus No axillary surgery in early breast cancer clinically and UltraSonographically node negative

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Background: Radiotherapy is an important treatment for breast cancer. The axilla possibly receives some dose of radiation from treatment in the breast field and could perhaps treat node-positive disease. Previous trials on de-escalation of axillary surgery, including omitting sentinel lymph node biopsy (SLNB), have not detailed or presented few radiotherapy data. VENUS is an ongoing trial that evaluates omission of SLNB in early breast

cancer clinically and ultrasonographically node negative. This is a partial report on the first interim radiotherapy data collected up to 4.5 years after the VENUS trial has started. The objective is to evaluate radiotherapy dose in axilla and whether radiotherapy is being uniform between VENUS groups.
Methods: prospective, multi-center, non–inferiority, phase III, randomized controlled clinical trial including T1-2 N0 (clinical/ultrasound) M0 breast cancer patients (>18 years old) randomized into: SLNB or no axillary surgery. The sample size is calculated at 800 randomized women, with patients undergoing mastectomy and neoadjuvant chemotherapy being allowed. Randomization (1:1) is being stratified by age and tumor size. Endpoints include disease free survival after 5 years of follow-up (primary endpoint), overall survival, regional recurrence free survival, axillary recurrence rate, axillary morbidity rate, ultrasound accuracy and cost-effectiveness. Adjuvant radiotherapy planning was based on local protocols adopted by each study center. In the no-surgery group, axilla status was considered N0 during planning. Radiotherapy features analyzed were: planning, site, number and location of fields, whole-breast/boost dose, fractioning and estimates of dose volumetry (total and 90%) distribution in axillary levels I-III. VENUS trial is registered at ClinicalTrials.gov (NCT05315154) and ReBEC (RBR-8g6jbf).
Results: Until June 2024, 372 women were randomized. Radiotherapy was performed in 249 (SLNB n=133 and no-surgery n=116). Two-D, 3D-IMRT and 3D-Conformational planning were applied for 7, 26, 200 patients, respectively, with no imbalance across study groups (p=0.34). Mean whole-breast dose was 3558.17cGy in SLNB and 3773.40cGy in no-surgery (p=0.22). Mean percentage of total prescribed breast doses distribution in axillary were: Level I 4.39% SLNB vs 2.34% no-surgery (p=0.21), Level II 0.31% SLNB vs 0.06% no-surgery (p=0.72), Level III 0.72% SLNB vs 0.00% no-surgery (p=0.08). Radiotherapy fields (axilla, supraclavicular fossa, breast and internal mammary) and boost are described and were all evenly balanced across study groups.
Conclusion: Breast radiotherapy has achieved an unintentional low radiation dose in the axilla of some patients, mainly at Level I. However, there was no difference between VENUS trial groups in radiotherapy parameters. So far, with more than 40% of the sample size achieved, there has been no violation of radiotherapy procedure protocol in the VENUS trial. Of the axillary surgery de-escalation trials, up to now VENUS trial presented 42% radiotherapy data.

P5-11-09: Evaluating Setup Accuracy via Daily CBCT of SGRT Based Tattooless vs Tattooed (Non-SGRT) Setup for Tangential Whole Breast Radiation

Mona Karim, Zhanrong Gao, Junsheng Cao

Introduction: Recently Tattooless patient positioning using Surface Imaging Guided Radiotherapy (SGRT) has become popular for the setup of breast cancer radiotherapy. However, the use of permanent skin tattoos in alignment with in-room lasers is still the standard setup approach. In this study, we compared the setup accuracy between

traditional tattoo based and tattooless approaches.

Materials and Methods: This study involved 27 whole breast cancer patients undergoing 3D planned tangent beam radiotherapy via Varian TrueBeam. They were randomly divided into two groups: 13 were positioned using traditional tattoo markers aligned with in-room lasers and without SGRT, and 14 were positioned tattooless using AlignRT surface imaging technology (London, UK). Each patient consented to participate in the study and the study received approval from our institutional IRB. Prior to radiation delivery in each fraction, patient setup for both groups of patients wasverified with onboard daily CBCT. Setup error was defined as position displacement from planning CT to daily CBCT. Wilcoxon rank sum test was used for statistical analysis.

Results and Analysis: A total of 171 daily patient setups were measured from 14 patients with Tattooless setup approach using SGRT while 226 were measured from 13 patients with Traditional Tattoo based setup approach. For tattooless setup, the median absolute shifts were 0.25cm in Anterior-Posterior (AP) direction (range 0~0.88cm), 0.2cm in Superior-Inferior (SI) direction (0~0.94cm) and 0.2cm in Lateral direction (0~0.83cm). For the tattoo based setup approach, the corresponding median shifts were 0.37cm for AP (range 0~2.14cm), 0.25cm for SI (range 0~1.15cm), and 0.2cm for Lateral (0~1.22cm). We found that the setup error was smaller in the AP direction for Tattooless by using the Wilcoxon rank sum test ($p<0.05$) while there was no significant difference in Lateral and SI directions between Tattooless and Tattooed approaches ($P=0.93$ and 0.37 respectively). For Tattooless setup, 10% of absolute shifts exceeded a magnitude of 8mm, while for traditional Tattoo based setup, a corresponding 16.8% exceeded 8mm.

Discussions and Conclusions: Tattooless setup via AlignRT may have a better setup accuracy in the AP direction, and has an equivalent accuracy in SI and LR directions compared to a tattoo-based method. In addition, Tattooless method can reduce the frequency of large shifts from 16.8% to 10% as compared to traditional tattoo based setup approach.

P5-11-10: Analysis of Daily Setup Errors for Patients Undergoing Left Prone Whole Breast Radiotherapy by BMI

Jared Hunt, Lamia Choudhury, Patrick Beagen, Xiao Zhao

Purpose/Objective: For women with early stage left-sided breast cancer who undergo breast conserving surgery, whole breast radiation therapy is often recommended as adjuvant treatment. The most concerning late toxicity in these women is heart toxicity. In recent years, prone treatment techniques have helped reduce heart dose by positioning the breast further from the body. However, this comes with risks as the setup is more unstable and difficult to replicate. Setup uncertainties can lead to higher-than-expected heart doses

or under-dosing of breast tissue. Our objective was to investigate the daily setup errors and potential dosimetric benefit of utilizing a low dose cone beam CT (CBCT) for prone left breast radiation.

Materials/Methods: We performed a retrospective review of consecutive patients treated at our institution with left breast cancer between April 2019 and June 2021 in the prone position. Patients were set up to clinical marks and a custom low dose partial arc CBCT was acquired with each fraction. Daily shifts were recorded for each fraction, with vector displacements calculated for both total magnitude and angular displacement in both the transverse and coronal plane. Using the daily shifts, we calculated the delivered dose if these shifts were not performed. Differences between planned and pre-CBCT shift doses were recorded for the heart and breast tissue clinical target volume (CTV). Patient demographics were also collected. Patient were placed into three groups based on BMI, normal (<25), overweight (25-30), obese (>30). After checking for normality using Shapiro-Wilk tests, statistical comparisons were made using Mann-Whitney, Kruskal-Wallace, Dunn's multiple comparisons and one sample Wilcoxon tests.

Results: 37 patients were included, with 581 daily CBCT shifts and an average of 15.7 CBCTs per patient (13-25). The mean age was 60.8 (39-80), and the mean BMI was 27.6 (19.5 – 45). The median magnitude of daily vector displacement was 8.1 mm (1.0-33.4) and was found to be correlated to BMI ($p = <0.0001$). Obese patients had a significantly higher daily displacement magnitude than normal BMI patients, 10.3 vs. 7.7 mm ($p = <0.0001$). Patients with high BMI showed no consistent pattern in their angular movement in either plane, while normal BMI patients displayed consistent setup errors in both the coronal ($p=<0.0001$) and transverse ($p=<0.0001$) planes.

Obese patients showed significantly larger differences in planned vs received breast CTV coverage compared to normal BMI patients ($p=0.0054$). For heart dose, patients with a normal BMI had significantly higher differences between the planned heart dose and delivered heart dose when compared to overweight patients ($p=0.0020$).

Conclusion: Overall, our study shows significant variation in daily setup for prone breast radiation. All patients demonstrated a degree of error and variability during daily setup. However, the magnitude of error increased with BMI. For patients with lower BMI, the setup error was more consistent and systematic, while higher BMI patients had more randomness to the daily variation. The random error caused some treatments to over-treat the heart where-as other treatments would have under-treated the breast tissue. This highlights a potential danger of not using daily imaging and using previous shifts for future treatments in higher BMI patients. In lower BMI patients, however, we saw that consistent systematic errors could lead to an overdosing of the heart if not corrected properly.

Overall, this data provides more clarity on the importance of imaging for patients undergoing prone left breast radiation. Our data suggests that patients with lower BMI may

not need as frequent imaging due to consistent systematic shifts whereas higher BMI patients would benefit from daily imaging given the daily randomness of shifts.

P5-11-11: Dual–responsive nanoprobe with oxygen economization capacity for potentiating synergistic–checkpoint-blockade–and–radiotherapy-radiodynamic therapy in breast cancer

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Radio-immunotherapy is a critical therapeutic approach for patients with advanced breast cancer, especially for triple-negative breast cancer (TNBC) patients. However, its efficacy is constrained by inaccurate assessment of the tumor locations, as well as poor sensitization and hypoxia of tumor. To address these clinical dilemmas, we developed a pH/GSH dual-responsive NIR-II fluorescenc nanoprobe, which was designed by co-coordinated indocyanine green (ICG) and Hafnium (Hf, Z = 72) within a glutathione (GSH)-responsive tetra-sulfide-bridged porous organosilica, and afterward enclosed by a pH-responsive catechol-borate crosslinking layer. As the nanoprobe accumulated in tumor regions, the layer dissociated under tumor acidity, which resulted in a charge reversal and facilitated cellular endocytosis. Subsequently, the core of the nanoprobe reacted with the intracellular GSH, decomposing to release ICG-Hf. Upon X-ray irradiation, high Z metal Hf could not only capture X-ray photoelectrons to catalyze the production of hydroxyl radicals (-OH) for enhanced RT, but also transfer X-ray energy to coordinated ICG to initiate the generation of singlet oxygen (1O2) and superoxide anion (-O2-) for RDT. In addition, the generated H2S from tetra-sulfide cleavage by GSH could reprogram oxygen metabolism to intensify RT-RDT efficacy and trigger a robust anti-tumor immune response by induction of immunogenic cell death (ICD). When combined with checkpoint blockade therapy, the nanoprobe mediated a significant inhibition of primary and distant tumors via the stimulation of DCs maturation and T cells infiltration, leading to a robust abscopal effect in vivo. Collectively, this novel nanoprobe exhibited tremendous potential for precise tumor localization and potentiating immunogenic RT-RDT.

P5-11-12: “Less is More”: Multi-institutional Experience with Fast-Forward Dose and Fractionation and the Impact of Dosimetric Parameters on Toxicity in Breast Cancer Patients

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Background: In 2020, the Fast-Forward trial demonstrated the non-inferiority of a radiotherapy dose of 26 Gy in 5 fractions over 1 week compared to 40 Gy in 15 fractions over 3 weeks for local tumor control and safety for up to 5 years. Additional data is required to assess its side effects and the impact of dosimetric factors on toxicities across diverse

patient populations. Our primary objective is to describe our institutional experience with this dosing schedule in terms of observed breast toxicity and to explore the impact of dosimetric parameters on toxicities as a secondary objective.

Method: This is a multicenter study involving 100 breast cancer patients treated between June 2020 and June 2023 with an adjuvant radiotherapy dose of 26 Gy in 5 fractions at King Faisal Specialist Hospital & Research Center and King Abdulaziz University Hospital in Saudi Arabia. Patients who did not complete their planned radiotherapy treatment course and patients with stage IV breast cancer were excluded.
Data were collected retrospectively from medical records and during follow-up visits, with at least one visit occurring at least 6 months post-treatment. Patients who were lost to follow-up were contacted by phone.
Statistical analysis includes descriptive statistics, bivariate analysis, and regression analyses. Independent variables include breast CTV, boost, plan's Dmax, breast V107%, breast V105%, breast separation, BMI, age, neoadjuvant and adjuvant chemotherapy, and patient comorbidities. Dependent variables include breast pain on a pain scale of 0-10, breast shrinkage, telangiectasia, breast edema, breast discomfort, skin dermatitis graded as per RTOG, breast hardness, hypo/hyperpigmentation, and change in breast shape.

Results: Our study, with a median follow-up of 19 months, revealed that 5% of patients experienced Grade III dermatitis and 15% reported breast pain rated ≥5 out of 10 on the pain scale. Breast pain showed a statistically significant correlation with larger breast CTV in cc and breast V107% (p. 0.013 and p. 0.012, respectively). Furthermore, dosimetric analysis revealed significant correlations between breast dermatitis and breast CTV in cc, BMI, and breast separation (p. 0.0001, p. 0.015, and p. 0.001, respectively).

Conclusions: This radiotherapy dose schedule is tolerable in our patient population. Our dosimetric analysis highlights an association between specific dosimetric parameters and toxicity, suggesting the need to optimize these parameters to minimize treatment-related morbidity and improve the patient toxicity profile.

P5-11-13: A Method for Quickly Estimating Lung V20 for Forward-Planned Breast Patients

Michael Karp, Jonathan Klein, Michael Silver

Purpose/Objective(s): Prolonged dosimetric planning can delay radiotherapy (RT) start time and affect a clinic's efficiency. Planning time may be prolonged when dose constraints are found to be exceeded and repeat planning is required. If a radiation oncologist can reliably predict dose constraints without formal dosimetric planning, then a need for repeat planning may be avoided. The volume of lung receiving ≥20 Gy (V20) is commonly used to

estimate potential lung toxicity and is often exceeded during planning and therefore is a common source of replanning. We developed a novel method to predict V20 for breast cancer (BC) radiotherapy.

Materials/Methods: The method was tested on two groups of BC patients treated at a single center with forward-planned 3D conformal RT: "Group TAN" patients received whole breast RT using tangent fields and "Group RNI" received RT to the breast/chest wall and regional lymph nodes. Using Eclipse planning software, we outlined the boundaries of the RT field. We then created an "Overlap" structure consisting of the portion of the RT field that overlaps the ipsilateral lung. We divided the volume of the Overlap structure (VO) by the total ipsilateral lung volume (TILV) to calculate Percent Overlap Volume (POV). We then subtracted POV from the final plan's calculated V20 (cV20) to determine the difference between the two parameters (V20D). Univariate analysis via SPSS v.29.0 assessed correlation between planning characteristics and V20D.

Results: A total of 92 patients were included, 46 in each group. Within each group, patients were evenly divided between right- vs. left-sided RT and single (6MV) vs. mixed (6/23 MV) energies. For left sided patients in the TAN group, 9 (20%) patients were treated with free breathing and 28 (61%) with deep-inspiration breath holding (DIBH) technique; in the RNI group 15 (33%) were treated using free breathing and 7 (15%) with DIBH.

In Group TAN, mean POV was 10.4% (SD 4.3%) and mean V20D was 0.88% (SD 0.3%). In Group RNI, the mean POV was 28.1% (SD 5.7%) and mean V20D was 4.2% (SD 1.1%). cV20 was larger than POV for all patients, meaning that our method consistently overestimated V20 compared with the actual planned value.

On univariate analysis of Group TAN patients, left-sided RT ($p<0.001$), DIBH ($p=0.026$), cV20 ($p=0.012$), and VO ($p=0.06$) correlated with a lower V20D, while energy group and TILV were not predictive of V20D ($p>0.05$). Within Group RNI, cV20 ($p=0.003$) was correlated with a lower V20D, while no correlation was found between V20D and laterality, DIBH, energy group, TILV, or VO.

Around 95% of the time, the difference between POV and the corresponding cV20 will be equal or less than the mean V20D plus 2SD. For Group TAN, V20D mean+2SD = 1.5% and for Group RNI, V20D mean+2SD = 6.4%. Therefore, to estimate whether a V20 constraint will be met for a tangents-only plan we recommend calculating the estimated V20 as: V20est = POV + 1.5% and for RNI, V20est = POV + 6.4%

Conclusion: Using our method, the POV was consistently larger than the calculated V20 and the differences between these two fell within a predictable range, creating a reliable method to predict ipsilateral lung V20. We found that a radiation oncologist may estimate the

ipsilateral lung V20 by contouring the beam overlap with the lung (POV) and adding 1.5% or 6.4% for tangent-only and RNI plans, respectively, and can safely assume that actual the actual V20 value will be lower than this estimate.
Taking an extra few minutes to estimate the V20 using our method may speed radiation planning and increase clinic efficiency by minimizing the need for replanning. Similar methods may be developed to estimate other dose parameters for both RT planning for breast and other cancers.

P5-11-14: Survival Outcomes of Breast Cancer Patients Underwent Neoadjuvant Radiotherapy vs. Adjuvant Radiotherapy: A Population-based Observational Study

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Objectives: Neoadjuvant radiotherapy (Neo-Rad) improves locoregional control, down-stages the tumor, and increases the success rate of breast-conserving surgery. However, there remains controversy regarding its effect in long-time survival. The aim of this research was to, combined with various clinicopathologic characteristics, compare the survival outcomes of breast cancer (BC) patients underwent Neo-Rad versus adjuvant radiotherapy (Adj-Rad), and to determine whether the radiation sequence was the independent prognostic factor for BC.
Methods: A total of 128113 BC patients from Surveillance, Epidemiology, and End Results (SEER) database were identified in our study, of whom 127707 patients underwent Adj-Rad and 406 patients underwent Neo-Rad. The Kaplan-Meier function and the COX proportional hazards model were applied to compare overall survival (OS), the Nelson-Aalen cumulative hazard method and competing risk model were applied to compare breast cancer specific survival (BCSS) between two groups.
Results: The OS and BCSS of Neo-Rad group were significantly prolonged than Adj-Rad group ($P<0.001$) in general. The radiation sequence was as an independent prognostic factor for OS (HR 0.61, 95% CI 0.49-0.75, $p<0.001$). Neo-Rad group also showed better survival outcomes than Adj-Rad cohort in stage Ⅰ-Ⅱ patients, LN positive/negative patients, patients with different molecular subtypes, and patients underwent partial/ total Mastectomy ($p<0.05$). By contrast, in stage Ⅲ patients, the OS and BCSS were comparable between two groups ($p>0.05$).
Conclusions: Neo-Rad may be applicable not only for patients with advanced, inoperable, or aggressive subtype of BC, but also in the setting of early-stage, luminal subtype of patients, and it may significantly improve OS and BCSS in most subgroups of patients. These finding warrants more prospective clinical trials to clarify the optimal radiotherapy timing and radiation technologies for corresponding type of patients, and to improve local control and survival outcomes at the same time.

P5-11-15: Application of in vitro 3D models to study the effect of hypofractionated radiation therapy for breast cancer

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Breast cancer (BCa) is a heterogeneous disease and the most common cancer in women. Approximately 50% of BCa treatment is breast-conserving surgery followed by adjuvant radiation therapy (RT). Recently, a study published in The Lancet (Brunt et al, 2021), suggested that a hypo-fractionated RT of 5.2 Gy per fraction (for a total dose of 26 Gy in 1 week) is a safe and effective regime in controlling disease for up to ten years. However, there is a lack of knowledge of cellular responses to hypofractionated RT using relevant preclinical models. Thus, this study utilised a semi-synthetic hydrogel (gelatin methacryloyl; GelMA), which mimics the human tumour microenvironment, to examine cancer cell response when applying hypofractionated RT.
Patient-derived BCa (ER+/PR+/HER2-) cells and Human BCa cell lines MCF-7 (ER+/PR+/HER2-) and MDA-MB-231(ER-/PR-/HER2-) cells were grown in 20 µL GelMA hydrogels for five days and then treated with the hypo-fractionated radiation dose of 26 Gy (5 fractions for 5 days) continuously. Cell viability, metabolic activity, and radiosensitivity gene expression were examined by utilising FDA/PI staining, Prestoblue assay and Q-PCR after RT respectively.
Cell viability decreased on day 7 in MCF-7 cells, and on day 21 post RT in MDA-MB-231 and patient cells when compared to the untreated condition. Cell metabolic activity showed a significant decrease in fractionated RT when compared to control on day 21 in patient and MCF-7 cells, but on day 14 in MDA-MB-231 cells. The fractionated RT reduced the levels of gene expression of the cancer stem cell markers in patient-derived and MCF-7 cells, in contrast to increased expression in MDA-MB-231 cells. The DNA damage repair marker was significantly decreased in both patient-derived and MCF-7 cells but not changed in MDA-MB-231 cells.
This project suggests that patient-derived BCa cells shared similarities with MCF-7 cells in their response to hypofractionated RT for both cell metabolic activity and DNA damage repair, but not in MDA-MB-231 cells. These findings also suggest the GelMA hydrogels can be provided as a tool for pre-clinical models for RT studies. For further investigation, GelMA hydrogels will be utilised to examine the effects of combined radiation-chemotherapy and may provide an opportunity for personalised treatment for patients based on their own BC subtypes.

P5-11-16: Outcomes in Elderly Patients with Triple-Negative Breast Cancer Receiving Neoadjuvant Chemo-immunotherapy and Chemotherapy Alone.

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Introduction: Neoadjuvant chemo-immunotherapy with pembrolizumab (NAC+P) has become the standard care for stage II-III triple-negative breast cancer (TNBC) following the KEYNOTE-522 (KN522) trial, which showed improved pathologic complete response (pCR) rates and event-free survival compared to chemotherapy alone (NAC). We aimed to describe pCR rates by age and treatment in TNBC patients receiving either NAC+P or NAC alone at our institution.

Methods: Women aged ≥65 years with stage I-III TNBC treated with NAC+P from 6/2021 to 9/2023 were compared to patients aged <65 years treated with NAC+P and to patients ≥65 who received NAC alone from 03/2010 to 9/2019. Patients with metaplastic breast cancer and neoadjuvant radiotherapy were excluded. Clinicopathological characteristics and pCR rates (ypT0/is pN0) - were compared between groups using Chi-square tests. Logistic regression for univariate (UVA) and multivariable analysis (MVA) was used to evaluate the association between age, treatment, and pCR.

Results: 481 patients were included: 69 NAC+P ≥65 years (median age 69, IQR: 67-73); 338 NAC+P <65 years (median age 48, IQR: 38-56); and 74 NAC ≥65 years (median age 69, IQR: 66-73).

Compared to younger patients, older patients treated with NAC+P were more likely to have public insurance (87% vs 13%, p<.001), lobular and other non-ductal histologies (6% vs 1.2% and 10% vs 5%, respectively, p= .009), cT1 and cT4 stages (22% vs 11% and 13% vs 3.9%, respectively, p< .001) and cN+ disease at presentation (58% vs 45%, p=.04). In both age groups, 81% received the KN522 backbone regimen with carboplatin, and the remaining mostly received NAC with cyclophosphamide, anthracycline, and taxane. There was no difference in treatment completion rate - defined as the completion of all preplanned neoadjuvant therapy cycles regardless of interruptions, regimen changes, or dose reductions - and in immune-related adverse events (33%) between age groups. However, older patients had a longer time from NAC+P to surgery (38 vs 32 days, p= .002). Breast surgery was similar between age groups, but axillary dissection (ALND) was more common in older patients (32% vs 19%, p= .023). The pCR rates did not differ between older and younger patients (49% vs 58%, p=.2). On MVA, high tumor grade (OR 13.9, 95% CI 4.54-61.7, p< .001) was positively associated with pCR, whereas other histologies (OR 0.13, 95% CI 0.04-0.39, p< .001), cT4 stage (OR 0.10, 95% CI 0.02-0.36, p<.001) and cN+(OR 0.60, 95% CI 0.38-0.95, p= .029) were less likely to facilitate pCR.

In patients ≥65 years, those receiving NAC+P, compared to NAC alone, more frequently had lobular and other histologies (6% vs 0% and 10% vs 4.1%, respectively, p= .028), intermediate tumor grade (15% vs 1.4%, p=0.003), and cT1 and cT2 stages (22% vs 16% and 61% vs 46%, respectively, p= .045).NAC+P patients more frequently received carboplatin (81% vs 6.8%, p<.001). There was no difference in NAC completion and time to treatment start, but the NAC+P group had longer time to surgery (38 vs. 30 days, p = 0.039). More NAC patients underwent ALND (56% vs 32%, p= .005), with no difference in cN+ disease and breast surgery. The pCR rates were significantly higher in the NAC+P group (49%) compared to NAC (23%, p=.001). On UVA, NAC+P significantly improved overall pCR (OR 3.26 p=.001). However, on MVA, after adjusting for tumor histology, grade, stage, neoadjuvant carboplatin, and completion rate, the use of immunotherapy was not

independently associated with pCR.
Conclusions: Within the NAC+P cohort, pCR rates did not differ by age, and no significant increase in irAEs was seen in elderly patients, though we acknowledge a possible selection bias toward healthier elderly patients. Compared to NAC only, NAC+P showed potential for improving pCR in older individuals and could be a viable treatment option for elderly TNBC patients who are likely to tolerate it.

P5-11-17: Clinicopathological characterized by HER2-low-expressing breast cancer in triple-negative breast cancer

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Background: Human epidermal growth factor receptor 2 (HER2)-targeting antibody-drug conjugates have been developed and a new treatment option for breast cancer patients, including those with HER2-low-expressing breast cancer. Previous reports have shown that HER2 crosstalk with the estrogen receptor, and that there is a high expression group of genes involved in androgen/estrogen metabolism in the molecular classification of triple negative breast cancer (TNBC). We have been performed to assess basal-like TNBC in clinical practice to immunohistochemical staining of EGFR, CK5/6 and androgen receptor (AR) since 2014. In this study, we evaluated the clinicopathological features of the differences between HER2-negative and HER2-low TNBC and hypothesized that HER2-negative TNBC would have more basal-like features and HER2-low TNBC would have more non-basal-like features.

Methods: From April 2014 to May 2023, 195 patients diagnosed with triple-negative breast cancer (TNBC) through needle biopsy and immunohistochemical staining for EGFR, CK5/6, and AR were investigated. BRCA mutations, which are considered common in basal-like types, were also assessed. BRCA mutations were evaluated in germline-derived samples. PD-L1 and tumor-infiltrating lymphocytes (TILs) were evaluated for immunogenicity assessment. TILs were assessed by averaging the area occupied by lymphocytes and plasma cells (not hotspots) in the interstitial area within the invasive cancer area, as proposed by the International TILs Working Group. They were classified into four levels: 0%, 1-9%, 10-49%, and ≥50%.
PD-L1 was assessed using the SP142 antibody, and positive cells were defined as those with 1% or more positive tumor-infiltrating immune cells. PD-L1 was evaluated at 0%, 1-9%, and ≥10%.

Results: Among the TNBC biopsies, 74 cases (38%) were HER2-low (ITC 1+ or ITC 2+ and FISH negative or not performed), and 121 cases (62%) were HER2-negative. Significant differences were observed between HER2-low and HER2-negative for histological classification (P=0.011), BRCA mutation (P=0.032), and PD-L1 (P=0.008).

Invasive ductal carcinoma (IDC) was found in 65% (48/74) of HER2-low and 79% (95/121) of HER2-negative (P=0.011). Apocrine carcinoma was found in 32% (24/74) of HER2-low and 15% (18/121) of HER2-negative. There was a correlation between apocrine carcinoma and AR expression (P<0.001). AR expression was 50% (37/74) positive and 50% (37/74) negative in HER2-low, while it was 20% (25/121) positive and 80% (96/121) negative in HER2-negative (P<0.001). BRCA mutations were found in 15% (5/34) of HER2-low and 55% (21/58) of HER2-negative. PD-L1 was positive in 43% (43/89) of HER2-low and 63% (54/86) of HER2-negative.
There was a correlation between PD-L1 and TILs (P<0.001).There was no difference in Ki-67 (P=0.100), EGFR (P=0.363), CK5/6 (P=0.452), nuclear grade (P=0.490), neoajuvantchemotherapy treatment response (P=0.468) and TILs (P=0.546) between HER2-low and HER2-negative.

Conclusion: AR is higher in HER2-low-expressing breast cancers and apocrine carcinoma is significantly more frequently classified. BRCA mutation and PD-L1 positivity are higher in HER2-negative TNBC. Our study revealed that HER2-negative breast cancer has more basal-like clinicopathologic features, while HER2 Low TNBC has non-basal features.

P5-11-18: Treatment Strategies and Pathologic Responses in Triple-Negative Breast Cancer: A Retrospective Analysis

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Triple-negative breast cancer (TNBC) is a highly aggressive subtype characterized by the absence of estrogen receptors, progesterone receptors, and HER2 amplification. This unique molecular profile limits treatment options, as TNBC does not respond to hormonal or HER2-targeted therapies, making chemotherapy the cornerstone of systemic treatment. Achieving a complete pathologic response (pCR) after neoadjuvant chemotherapy has been associated with improved long-term outcomes and survival rates in TNBC patients. However, pCR rates can vary significantly depending on the chemotherapy regimen used, as well as the inclusion of other modalities such as immunotherapy.
A retrospective, single-center study was conducted at Chihuahua's State Cancer Center, where medical records of patients aged ≥ 18 years diagnosed with TNBC between January 2012 and June 2023 were reviewed to evaluate pCR after neoadjuvant treatment. The study compared the effectiveness of three chemotherapy regimens: anthracyclines and taxanes (AC-T), anthracyclines combined with taxanes and carboplatin (AC-Tcarboplatin), and any chemotherapy regimen paired with immunotherapy (IT). Fisher's exact tests were used to compare pCR rates across these groups, with p-values calculated to determine clinical significance and identify the most effective treatment strategies.
In total, 1279 breast cancer patients were included, with 182 (14.23%) diagnosed with

TNBC. Among these, 92 (50.55%) received neoadjuvant treatment, resulting in 35 (19.23%) achieving pCR. Upon observing the pCR rates among different regimens, we found clinical relevance in the variations. Statistically significant differences were confirmed when comparing neoadjuvant treatments in relation to pCR rates (p=0.029). Sixty patients received the AC-T regimen, achieving a pCR rate of 26.67%. Thirteen patients received AC-Tcarboplatin, with a pCR rate of 46.15%. Thirteen patients received chemotherapy combined with immunotherapy, showing a high pCR rate of 92.31%. Finally, 6 patients received other chemotherapy regimens without immunotherapy or carboplatin, with only 1 (16.67%) achieving pCR.
This analysis highlights significant differences in pCR rates depending on the administered treatment, emphasizing firstly the clear benefits of adding carboplatin to the neoadjuvant regimen. Additionally, although the number of patients treated with immunotherapy was limited, the results unequivocally indicate its benefit in significantly enhancing complete pathological responses compared to other treatment groups. While each therapeutic group demonstrated specific advantages in pCR rates, stressing the critical importance of early access to advanced therapeutic options such as immunotherapy from the time of TNBC diagnosis is crucial. Finally, it is recommended to establish better public policies to improve access to these treatments and explore the potential additional benefits of low-dose immunotherapy in future research to further optimize clinical outcomes.

P5-11-19: Single arm prospective study evaluating the relationship of MRD via ctDNA and pCR in a diverse early TNBC patients receiving neoadjuvant chemotherapy.

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Background: Chemotherapy plus pembrolizumab in the neoadjuvant (NAC) and adjuvant settings (Keynote 522 (KYN)) is standard of care in early-stage II and above triple negative breast cancer (TNBC), because of improvement in pathologic complete response(pCR), event-free survival, and possibly overall survival. Circulating tumor DNA (ctDNA) has the potential to be used to assess response to chemotherapy, guide de-escalation of chemotherapy and detect early recurrence. Herein, we report the results of a pragmatic, single-center, prospective, observational pilot study to evaluate the ability of tumor-informed, quantitative ctDNA to assess early response in the neoadjuvant setting.
Methods: A total of 31 new breast cancer patients (pts) were enrolled. The inclusion criteria were having early-stage TNBC (ER/PR < 10%, HER2 negative) and meeting criteria for NAC. Serial ctDNA, using a commercial tumor-informed test, was obtained at baseline and prospectively every three weeks (wks) while on NAC and then every 4-12 weeks in the adjuvant setting. Blood was collected immediately before the next dose of chemotherapy. The primary endpoint was the concordance of ctDNA clearance (molecular complete response (mCR)) and pCR. Secondary endpoints include the rate of mCR at 3, 6, and 9 wks, the correlation of imaging CR (iCR) with mCR and pCR, and the rate of pCR in this real-

world diverse population. For the initial treatment, 61% of patients received weekly paclitaxel, carboplatin, pembrolizumab (tcP) prior to doxorubicin, cyclophosphamide, and pembrolizumab (ACP), and 38% received ACP followed by tcP. The change happened to accommodate the carboplatin drug shortage.
Results: Thirty-one female pts signed consent but only 29 had sufficient tissue for tumor informed ctDNA analysis: 20 (64%) African American (AA), 10 (34.4%) Caucasian (CA), and 1 (3.4%) Asian. At baseline 86.2% (25/29) tested positive for ctDNA. The four pts with negative ctDNA at baseline had stage II disease, and one of these had significant residual disease (RCB 2) upon resection. Four pts had not received surgery at time of data cutoff. Two died prior to surgery unrelated to disease progression and 2 experienced surgical delays. Based on ITT, 48% (14/29) of pts experienced pCR. Taking into consideration the 4 censored pts, the pCR rate was 54% (14/26). The mCR at 3 weeks was 38% (11/29), 6 weeks 59% (17/29), and 9 weeks was 65% (19/29). After completion of NAC (24 weeks), 79% (23/29) achieved mCR and 35% (11/31) achieved iCR with all 11 having iCR's having an mCR. Only 6/28 (21%) had mCR, iCR and pCR; all 14/29 who had pCR also had mCR by week six. None of the non-mCR pts at 6 weeks obtained a pCR. In the adjuvant setting, 76% (22/29) continued to have mCR at 4 weeks. Three patients had detectable ctDNA at 4 weeks with 67% (2/3) experiencing progression. Overall, 66%(19/29) pts had RCB 0 or 1 and all of these patients had sustained mCR at 6 weeks. Of the AA patients who received surgery, 75% (12/16) achieved mCR and 43% (7/16) achieved pCR; for CA pts it was 77% (7/9) and 60% (6/10) respectively. The one Asian patient achieved mCR and pCR.
Conclusion: Pre-operative ctDNA measurement was feasible in this pilot study. mCR correlated with but was not predictive of pCR in the diverse population. Lack of mCR at 6 weeks was highly predictive of non- pCR and post-surgery detectable ctDNA resulted in rapid disease progression.

P5-11-20: Impact of Immune-Related Adverse Events on Response to Neoadjuvant Chemoimmunotherapy in Triple Negative Breast Cancer

Michelle Sterpi, Yungtai Lo, Susan Fineberg, Harjot Gill, Della Makower

Background: Addition of the PD-1 inhibitor pembrolizumab to neoadjuvant chemotherapy improves pathologic complete response (pCR) rates and event-free survival in triple-negative breast cancer (TNBC). Immunotherapy may lead to immune-related adverse events (irAE), which can be life-threatening and permanent. Presence of tumor-infiltrating lymphocytes (TILs) in TNBC is also associated with improved prognosis and treatment response. Little data exists regarding associations between irAE and pCR, and between TILs and irAE, in TNBC. This study evaluates associations between irAE, clinicopathologic factors (including TILs), and pCR after neoadjuvant chemoimmunotherapy (NCIT) in a diverse single-institution cohort of TNBC patients (pts).
Methods: Pts who received NCIT at our institution between 2021 and 2023 were identified from pharmacy database. Details of tumor stage, NCIT treatment, response to therapy, and presence or absence of irAE were obtained by chart review. IrAE were graded using

Common Terminology Criteria for adverse events (CTCAE). Stromal TILs were estimated within the borders of the invasive carcinoma as per International TILs Working Group guidelines. Associations of age, race, ethnicity, tumor size (T stage), node involvement (N stage), grade, HER2 IHC status, TILs, and completion of planned NCIT with irAE incidence and pCR were assessed by Wilcoxon rank-sum tests for continuous variables and chi-square or Fisher's exact tests for categorical variables. P-values <0.05 were considered statistically significant.
Results: 46 pts were treated with NCIT [27 (58.7%) Black; 14 (30.4%) Hispanic]. Median age was 60.5 (range 33-88). All NCIT regimens included pembrolizumab and a taxane. 35 pts (76.1%) received doxorubicin, and 25 (54.3%) received carboplatin. 31 (67.4%) pts received 80% or more of their planned NCIT regimen. Reasons for early discontinuation were chemotherapy toxicity (8 pts), irAE (4), progression of disease (POD) (2), unknown (1). 13 pts (28.2%) developed at least 1 irAE, including hypothyroidism (3 pts), rash (3), adrenal insufficiency (2), hepatitis (2), arthritis (2), myositis, pneumonitis, pericarditis, panniculitis, and encephalitis (1 each). irAE was Grade 1-2 in 6 pts, Grade 3 in 5 pts, Grade 4 in 1 pt, and Grade 5 in 1 pt. 41 pts underwent surgery. Reasons for no surgery were POD (2 pts, included in response analysis), fatal irAE (1 pt), pt refusal (1 pt), and unknown (1 pt). Of 43 evaluable pts, 24 (55.8%) achieved pCR. Development of irAE (p=0.039), younger age (p=0.028), and Hispanic ethnicity (p=0.005) were associated with pCR. Black pts were less likely to achieve pCR compared to non-Black pts (p=0.003). T and N stage, tumor grade, HER2 IHC status, and completion of planned NCIT were not associated with pCR. TILs were associated with achievement of pCR (p=0.002), but not with presence of irAE (p=0.341). No associations between irAE and age, race, ethnicity, T or N stage, tumor grade, HER2 IHC status, and completion of planned NCIT were seen.
Conclusion: In this diverse cohort of TNBC pts, TILs and irAE, as well as age, race, and ethnicity, were associated with achievement of pCR to NCIT, while tumor stage and grade, and completion of planned NCIT, were not. No predictive factors for development of irAE were seen.

P5-11-21: Pembrolizumab impact on surgery and radiation therapy in non-metastatic triple negative breast cancer treatment

Alizée Camps Maléa, François Peyre-Pradat, Angelica Conversano, Layal Rached, Julia Dixon Douglas, Chayma Bousrih, Barbara Pistilli, Joana Ribeiro, Sofia Rivera

Background: In recent years, the prognosis for women with non-metastatic triple-negative breast cancer (TNBC) has improved. However, few data are available on the impact of adding immunotherapy to neoadjuvant chemotherapy (NAC) on locoregional treatment toxicity. In this study, we aimed to investigate perioperative and radiotherapy (RT)-related toxicities when incorporating pembrolizumab into standard NAC regimens for non-metastatic TNBC.

Methods: This monocentric retrospective study included patients with non-metastatic

TNBC treated with pembrolizumab and NAC, followed by surgery and adjuvant RT. The safety profiles after surgery and RT were evaluated using CTCAE v4.0. RT items were extracted from the end-of-treatment reports. Patient demographics, clinical and pathological staging, NAC treatment regimen, initiation and completion, as well as date of surgery and postoperative complications were collected via medical reports. Data was analyzed using SAS software version 9.2.

Results: From February 2022 to April 2023, 70 patients with complete treatment data available were selected. The median age of the cohort was 51 years, 98.5% ECOG performance status 0-1, mainly non-smokers, 38.6% premenopausal with a median BMI of 26. Most patients presented T2-T3 invasive breast cancer (91.4%), 47.1% were N0 and 81.2% grade 3, with a median Ki67 of 70% and median TILs of 20%. All received NAC with pembrolizumab. The pathologic complete response rate was 61.4% and 84.1% received adjuvant pembrolizumab.
The median time between the end of neoadjuvant treatment and surgery was 22 days. Surgery consisted of mastectomy in 51.4% of cases and breast conserving surgery in 48.6%. Immediate reconstruction was performed in 24.3% of cases, all using prosthetic implant. Axillary lymph node dissection (ALND) was performed in 52.9% patients and sentinel node biopsy in 47.1%.
Post-operative complications, occurring between 3 days and 2 months after surgery, were reported in 26 patients (37.1%) and identified 29 events, as hematoma (1), necrosis (3), and infections, inflammatory reactions or abscess (4). In 21 patients (80.8%), post-operative complications were lymphatic disorders (lymphangitis/lymphorrhea/lymphoedema/lymphocele/lymphatic cord) and in 18 patients (69.2%) it was not associated with other event.
Five surgical re-interventions were performed with 2 hematoma within 3 days after surgery, 2 necrosis (at 11 and 22 days) and 1 infected periprosthetic seroma (at 15 days) in patients whom were mostly, first operated by mastectomy (80%) with immediate reconstruction (60%).
In 77.1% of patients, RT was concomitant as authorized per Keynote 522 study. RT was performed in 49 patients (70%) with VMAT and 30% with fixed IMRT or conformal 3D. Breath hold was used in 75.7% of RT. A dose of 40.05 Gy in 15 fractions was prescribed in 97.1% of patients and 2.9% patients received 26 Gy in 5 fractions. A tumor bed boost and nodal RT were delivered in 18.6% and 61.4% of cases respectively.
Radiation dermatitis was reported in 51 patients, 98% of which were grade 1. Dysphagia was reported in 14 patients, 92.9% of which were grade 1. No grade >2 RT-related toxicities were reported in our series.

Conclusion: Safety data after surgery and adjuvant RT in women treated with pembrolizumab for non-metastatic TNBC are reassuring regarding the combination of pembrolizumab with locoregional therapy. Despite mostly concomitant pembrolizumab-RT delivery, no grade > 2 RT-related toxicity was reported. Postoperative complications included mostly lymphatic disorders. This is a frequent event after surgery regardless of

concomitant treatment. Despite the limitations of our analysis, the findings suggest that no specific safety concerns were observed regarding the impact of pembrolizumab on locoregional treatment or the concomitant combination of pembrolizumab and RT.

P5-11-22: Neoadjuvant chemotherapy and survival outcomes in triple-negative breast cancer: a population-based study (Queensland, Australia).

Larissa Vaz-Goncalves, Louise Marquart-Wilson, Melinda Protani, Jodi Saunus, Julie Moore, Michelle Morris, Marina Reeves

Background: Assessing the survival benefit of neoadjuvant chemotherapy (NAC) in triple-negative breast cancer (TNBC) patients is typically evaluated through clinical trials. Clinical trials are limited by their highly controlled context, often limiting the generalisability of findings. Globally, there is a lack of population-based evaluations of 'optimal care' for those diagnosed with TNBC, the most aggressive breast cancer type with no targeted treatment. The main advance in clinical practice for TNBC treatment has been the use of NAC, with its use now recommended even for small triple-negative tumours. In this study, we use a Queensland (Australia) population-based study to identify TNBC patients who received NAC and achieved pathological complete response (pCR) to examine associations with overall survival (OS) and breast cancer-specific survival (BCSS).
Methods: All women diagnosed with invasive TNBC between 2017 to 2019 in Queensland, Australia, were identified from the Queensland Oncology Repository (a state-wide database) and followed up to Feb 17, 2024, for OS and up to Dec 31, 2021, for BCSS. Stage IV and patients who had not undergone any treatment were excluded. pCR was defined as no residual invasive breast cancer after NAC regardless of lymph node status. Multivariable logistic regression models were developed using Directed Acyclic Graphs (DAGs) to identify confounding variables to be included in models and were used to assess the likelihood of being treated with NAC and achieving pCR. Survival rates for OS and BCSS were estimated for no NAC versus NAC with pCR and NAC without pCR using Kaplan-Meier, and adjusted hazard ratio (HR) were estimated using flexible parametric survival models to account for time-varying hazards; at one-year, two-year, and four-year survival, adjusting for age, geographic region, prognostic stage, and hospital type (public vs private).
Results: A total of 1,021 TNBC cases were included in the analysis. Overall, 21.5% had received NAC, with NAC more common in 2019 (27.3%) compared to 2017 (16.1%) and more common in those living in major cities (24.4%) and outer regional/rural areas (21.9%) than those in inner regional areas (12.6%). Patients who received NAC (versus those who did not) were younger (mean ± SD 49.0 ± 13.0 vs. 61.4 ± 14.6 years) and were more commonly diagnosed with stage IIIa-IIIc (70.5% vs 53.1%). Among those who had NAC, 42.4% achieved pCR, with higher rates of pCR in 2019 (53.8%) compared to 2017 (31.5%). pCR was more common in those with stage IIa-IIb (62.5%) versus stage IIIa-IIIc (34.9%). The median follow-up was 4.96 years (range, 0.01-7.12) for OS and 3.21 years (range, 0.14-4.99) for BCSS. Compared to those who did not receive NAC, those who did and achieved pCR had better OS (HR 0.27 to 0.41; $p>0.05$) at 1-, 2- and 4-years post-diagnosis,

whereas those who did not achieve pCR had significantly poorer OS (HR 2.22 to 3.57; $p<0.05$) at all follow-up years. Similar trends were observed for BCSS.
Conclusion: The best survival was observed in patients who received NAC and achieved pCR. In this population-based sample, survival differences in those with pCR versus those without were similar to that observed in clinical trials. Receipt of NAC however, differed by age and geographical region, with those living in inner regional areas being less likely to receive NAC. Equitable access to current optimal care is essential for addressing survival disparities, while continued research into tailored treatments for TNBC is vital for improving outcomes for those who do not benefit from current treatments.

P5-11-23: Sociodemographic Factors Associated with Adjuvant Chemotherapy Receipt in Stage I Triple Negative Breast Cancer (TNBC)

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Background: Although the National Comprehensive Cancer Network (NCCN) clearly recommends systemic therapy for those with Stage II and Stage III TNBC, guidelines for Stage I disease are less clear, with recommendations such as “consider adjuvant therapy” for many patients. Even without adjuvant therapy, five-year breast cancer-specific survival rates of T1a/1bN0 TNBC exceed 95%. Despite this favorable prognosis, the existing literature reveals that up to 83% of individuals in this group receive chemotherapy. A better understanding of factors associated with receipt of chemotherapy in T1N0 TNBC is helpful for clinical decision making. In this study, we assessed the sociodemographic factors associated with use of adjuvant therapy in the T1N0 TNBC patient population.

Methods: This observational study used the Oncoshare database which integrates electronic medical record and California Cancer Registry data for patients treated in Northern California in the Stanford University Health Care Alliance. We included patients treated for T1N0 TNBC from 2000-2024, obtaining sociodemographic data from our database. Standardized mean differences (SMD) were used to preliminarily analyze imbalances between patients who received adjuvant chemotherapy and those who did not. Descriptive statistics are reported, and a multivariate analysis is anticipated.

Results: The study population included 252 patients with T1aN0/T1bN0 disease and 392 with T1cN0 disease. Approximately 73% of patients with pT1cN0 TNBC and 56% of patients with T1a or T1b disease received adjuvant chemotherapy. We collected extensive data on sociodemographic factors, including age, race and ethnicity, neighborhood socioeconomic status (reported as statewide quintiles), distance traveled for care, rurality, Charlson comorbidity scores, and insurance status.

Initial analysis was completed to determine the SMDs between those who received adjuvant systemic therapy and those who did not. For those with T1aN0/T1bN0 TNBC, there were

small differences between those who received adjuvant therapy and those who did not for SES quintile (0.208), rurality (0.238), and distance to Stanford (SMD 0.254). There were moderate differences between those who received adjuvant therapy and those who did not by race and ethnicity (SMD 0.331) histology (SMD 0.433), BMI (SMD 0.466), and insurance type (SMD 0.545). Of those who received adjuvant chemotherapy, 58% had private insurance, comparatively, of those who did not undergo chemotherapy, 68% had government or other insurance types.

There were large differences between T1aN0/T1bN0 treatment groups for age (SMD 0.658) and grade of the tumor (SMD 0.750). Of those who received adjuvant therapy, 38% were below the age of 50, whereas 14% of those who did not receive adjuvant therapy were below the age of 50.

For T1cN0 TNBC, we found small differences between those who received chemotherapy and those who did not for BMI (SMD 0.307). We observed moderate differences for insurance type (SMD 0.483), age (SMD 0.509), and race and ethnicity (SMD 0.371). Of those who received adjuvant chemotherapy, 49% had private insurance and 74% of those who did not receive chemotherapy had government issued or other types of insurance. We observed larger differences for tumor grade (SMD 0.631); 77% of those who received adjuvant chemotherapy were grade 3 compared to 51% of those who did not receive chemotherapy.

Conclusions: Most patients with T1N0 TNBC still received adjuvant chemotherapy despite studies showing excellent outcomes. Initial data demonstrates that differences exist in clinical and sociodemographic factors between those receiving and not receiving adjuvant systemic therapy, especially regarding age, insurance status, and tumor grade. Multivariate analysis will help us to better understand the association between sociodemographic factors and receipt of adjuvant therapy.

P5-11-24: OUTCOMES IN EARLY-STAGE TRIPLE NEGATIVE BREAST CANCER WITH DOSE-DENSE AC AS PART OF A PEMBROLIZUMAB-BASED REGIMEN

Danielle Roman, Mikaila Eross, Mitchell Mirabile, Rachelle Nadour, Christie Hilton

Background: The KEYNOTE-522 trial established the current standard treatment of high-risk early-stage triple-negative breast cancer (TNBC) with perioperative chemotherapy and immunotherapy. This regimen utilized every-3-week doxorubicin and cyclophosphamide (AC); however, AC given every two weeks (dose-dense) has been historically preferred over every-3-week dosing due to improved disease-free survival (DFS) and overall survival (OS). This study was designed to compare the rate of pathological complete response (pCR) between patients receiving dose-dense AC versus AC administered every 3 weeks within the KEYNOTE-522 regimen for early-stage TNBC and to evaluate the toxicity of dose-dense AC administered with pembrolizumab.

Methods: This single-center, retrospective, cohort study was conducted in patients with newly diagnosed, early-stage TNBC receiving neoadjuvant treatment with the KEYNOTE-522 regimen including AC given dose-dense or every 3-weeks between August 1, 2021 and December 1, 2023. The primary endpoint was pCR defined as ypT0/Tis ypN0 and secondary endpoints included toxicity rates.
Results: A total of 77 patients were included in this analysis (16 in the dose-dense AC group and 61 in the every 3-week AC group). There was no difference between pCR rates in the dose-dense and every 3-week AC groups (62.5% vs 65.6%; p=.82). Safety profiles were similar to previous reported trials, with a higher incidence of dose reductions (62.5 % vs 21.3%) and grade ≥ 3 toxicity (62.5% vs 26.2%) in the dose-dense AC group. Patients receiving dose-dense AC had higher rates of pembrolizumab discontinuation, with a median of 4.5 neoadjuvant cycles compared to a median of 8 neoadjuvant cycles with standard AC.
Conclusion: Use of dose-dense AC within the KEYNOTE-522 regimen did not improve pCR rates for patients with early-stage TNBC compared to every 3-week AC. There was a greater incidence of toxicity in the dose-dense AC group. A more robust comparison with a larger sample size is necessary to confirm a difference in pCR and toxicity between the two groups.

P5-11-25: Impact of Cardiometabolic Comorbidities on Outcomes in Early-Stage Triple Negative Breast Cancer Patients Treated with Neoadjuvant Chemo-immunotherapy (Keynote-522 regimen): A Retrospective Analysis

Asfand Yar Cheema, Jasmin Hundal, Olga Lytvynova, Serhan Unlu, Baidehi Maiti

Background: The presence of cardiometabolic comorbidities, such as hypertension (HTN), obesity, diabetes mellitus (DM), dyslipidemia, and chronic kidney disease (CKD), have been associated with increased mortality and poor prognosis in breast cancer. This study aims to investigate the influence of these comorbidities on overall survival (OS) and pathologic complete remission (PCR) in patients receiving neoadjuvant pembrolizumab plus chemotherapy. Understanding the role of these comorbidities in this context could inform personalized treatment approaches and improve outcomes.
Methods: We conducted a retrospective analysis of 107 early-stage triple negative breast cancer patients treated with neoadjuvant pembrolizumab and chemotherapy, Keynote-522 regimen. Cardiometabolic disease and its components CKD, HTN, obesity (BMI>30), DM, low high-density lipoprotein (HDL) levels (<50 mg/dL), and high triglycerides (TAGs) (>150 mg/dL)) were assessed. PCR and overall survival rates at 1 and 2 years were calculated, and the log-rank test was used to determine the statistical significance of differences in survival between patients with and without cardiometabolic disease. Fisher's exact test and Wilcoxon rank sum test were used to compare factors between cardiometabolic disease status groups as appropriate. Overall survival was estimated by the Kaplan-Meier method and compared using the log-rank test.
Results: Among the 107 patients, 33 (31%) had cardiometabolic comorbidities. The distribution of comorbidities was as follows: CKD (15%), HTN (45%), obesity (48%), DM (31%), low HDL levels (34%), and high TAGs (20%). The median age at diagnosis was 61

years, with a range of 28 to 87 years.
PCR rate was significantly worse in patients with cardiometabolic comorbidities (30% vs 69%, p-value = 0.03) and patients with CKD by subgroup analysis (14% vs. 85%, p-value 0.04). The median follow-up time was 15.0 months (3.4-47.6 months). The median OS was not reached; however, the 1-year and 2-year OS rates were 94% (95% CI: 89-99%) and 92% (95% CI: 87-98%), respectively. Patients with cardiometabolic comorbidities had significantly worse OS compared to those without (1-year OS: 82% vs. 99%, 2-year OS: 77% vs. 99%, p-value=0.0072;). Subgroup analysis revealed that patients with HTN (p-value 0.0099), obesity (p-value 0.03), and DM (p-value 0.03) had significantly poorer survival outcomes. The presence of other factors such as CKD, low HDL levels, and high TAGs did not show statistically significant differences in OS although a trend towards a worse outcome was observed. Additionally, there were no significant differences in survival based on smoking status, ethnicity, ECOG performance status, or cancer stage.
Conclusion: This study shows that the presence of cardiometabolic syndrome is associated with significantly worse OS and PCR rates in triple negative breast cancer patients treated with neoadjuvant chemo-immunotherapy. On subgroup analysis, HTN, obesity, and DM are associated with significantly worse overall survival. These findings underscore the importance of recognizing and managing specific cardiometabolic comorbidities, particularly hypertension, obesity, and diabetes, to potentially improve treatment outcomes and tailor survivorship care plans for this patient population. While other factors such as CKD, low HDL levels, and high TAGs did not show statistically significant differences, their role should not be entirely disregarded. Further research and prospective studies are needed to elucidate this association, underlying mechanisms and explore potential interventions to mitigate the negative impact of these comorbidities on patient outcome.

P5-11-26: Stage-Based Survival Analysis in the TRIple-Negative Breast Cancer PrOspective Registry in MiddLe East AfrIca (TRIPOLI) Study

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Background: Globally, triple negative breast cancer (TNBC) accounts for ~15% of all breast cancer cases and some retrospective studies have suggested an advanced presentation and poorer outcomes in the Middle East and North African countries. This study aims to address the lack of reliable treatment and efficacy data in this region.
Methods: TRIPOLI is a prospective multinational study from 17 institutions in 9 Arab countries including newly diagnosed patients with early TNBC (eTNBC) followed for a total of 3 years and patients with locally advanced/metastatic TNBC (la/mTNBC) followed for a total of 2 years. Herewith, we report survival analyses by stage in all patients and by

pathological complete response (pCR) in eTNBC patients that received neoadjuvant chemotherapy (NAC) and surgery. Survival analyses include overall survival (OS), real-world progression-free survival (rwPFS) and real-world event-free survival (rwEFS). RwEFS was defined as the time from treatment initiation for eTNBC until the date of recurrence or death by any cause.
Results: We report the data of 702 patients recruited between 2017 to 2019. Breakdown of patients by stage is as follows: 57 (8.1%) with stage I, 349 (49.7%) with stage II, 228 (32.5%) with stage III and 68 (9.7%) with stage IV disease. Of the 573 evaluable patients with eTNBC, the 3-year rwEFS rate was 96.3% in patients with stage I, 81.0% in patients with stage II and 66.5% in patients with stage III (excluding stage IIIc) ($p<0.0001$). Of the 99 evaluable patients with la/mTNBC, rwPFS was reported at 37.4%. The estimated 3-year OS rate for eTNBC patients was 100% for stage I, 94% for stage II and 87% for stage III (excluding stage IIIc). The median OS for patients with stage IIIc/IV was 20 months. As reported in Interim Analysis-2, 205 (39.5%) of 519 patients with eTNBC received NAC and surgery, with a pCR rate of 33.2%. The rwEFS rate was 82.4% in patients that achieved pCR versus 57.6% of patients that did not achieve pCR ($p=0.0004$). Deaths were reported in 9 (13.2%) patients that achieved pCR and in 16 (11.7%) that did not achieve pCR. At 3-year follow-up, OS did not achieve the pre-specified boundary for statistical significance.
Conclusion: Among patients with early and locally advanced/metastatic TNBC, the survival outcomes decrease with higher stages. In patients who received NAC and surgery, achieving a pCR was associated with a higher statistically significant rwEFS compared to those who did not achieve pCR. These findings highlight the importance of early detection and effective treatment strategies for TNBC in the Arab countries, with a particular emphasis on the neoadjuvant approach to improve outcomes.

P5-11-27: Analysis of Nectin-4 and its predictive value of pathological response in early Triple negative (TN) breast cancer

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Background
Early TN breast cancer is a very heterogeneous disease, where neoadjuvant treatment represents the fundamental therapeutic strategy. The complete pathological response allows predicting those patients who will obtain a better chance of cure, and allows redirecting the treatment of those who do not obtain it. However, we do not have tumor biomarkers that allow us to predict this pCR.Nectin 4 is a protein involved in maintaining cell adhesion and stability, a mechanism that is highly altered in tumor processes. There is overexpression in some aggressive tumor subtypes such as TN breast cancer, where some authors point out its unfavorable prognostic role. Our study seeks to describe the expression of Nectin 4 and other variables in a cohort of TN patients, candidates for neoadjuvant therapy in routine clinical practice, evaluating its role as a predictor of response.

Methods: This is a retrospective study of 50 patients with triple negative breast cancer, diagnosed in the General Hospital of Segovia, who received neoadjuvant treatment following standard clinical practice. Patients´ demographics and clinical variables were gathered from medical records. Expression of Nectin-4 in tumor cells was performed by two independent pathologists with immunohistochemistry technique, using a monoclonal antibody against Nectin-4 (Abcam ab 192033), considering Quick score > 100 (high expression) or < or = 100 (low expression). Complete pathological response to treatment (pRC) was evaluated in 45 patients in definitive surgery and its correlation with nectin and tumor Infiltrating Lymphocytes (TILs) expression.

Results: The majority of patients were postmenopausal (70%), with a body mass index greater than 25 (62%), T2 tumors (56%), with lymph node involvement at diagnosis (54%). The 86% received treatment with platinum and 24% also received immunotherapy. The complete pathological response rate was 36%.Patients who receive platinum and tumors with greater infiltration of TILS achieve greater pRC (0,032 y 0,007) respectively.
The 60% of the tumors had a Nectin-4 expression greater than 100, presenting greater infiltration of TILS and CD8 but it is not significant. The 75% of them (High expression Nectin-4) expression had complete pathological response, versus the 48% (0,082).

Conclusions: This study shows a possible prediction of a favorable pathological response in triple negative breast tumors with greater expression of nectin-4. It is necessary to expand this study with a larger number of patients to continue investigating this association.
Funding
Scientific Foundation of the College of Physicians of Segovia Table: Nectina-4 Expression and its correlation with pathological response.

P5-11-28: Management of triple-negative breast cancer in renal transplant patients: A case report and multidisciplinary approach

Alexandra Montenegro, Diana Cardoso Simão, Leonor Fernandes, Sónia Duarte Oliveira

Introduction: In renal transplant (ReT) patients, the incidence of breast cancer (BC) is similar to the general population, albeit with a less favorable prognosis. The literature lacks guidelines for the diagnostic and therapeutic approach to BC in ReT patients, thus recommendations are based on those for the general population. It is crucial to manage the oncological disease while preserving the function of the transplanted organ by preventing treatment toxicity and drug interactions between immunosuppressants and oncological treatments.

Case Description: This report describes a 45-year-old woman who detected a painful lump in her left breast (LB), prompting further investigation. The patient underwent ReT in 2017

due to end-stage chronic kidney disease and is on immunosuppressive therapy (tacrolimus 3.5 mg daily and mycophenolate mofetil 250 mg twice daily). Her family history of cancer includes a brother diagnosed with gastric cancer at age 55.
Further investigation of the LB mass revealed a suspicious nodular formation at the transition of the upper quadrants of the LB, measuring 18x14 mm and no suspicious axillary lymphadenopathy (LB BI-RADS 5). Breast MRI revealed a 50mm unilateral atypia. An ultrasound-guided core needle biopsy was performed, which showed a left breast carcinoma of no special type, grade II, estrogen receptor (ER) negative, progesterone receptor (PR) negative (both 0%), HER2 negative (0), and Ki67 60%. Contrast-free CT scan, abdominal ultrasound, and bone scintigraphy showed no suspicious secondary lesions. The patient was referred to Medical Oncology and Genetics consultations, given her young age and diagnosis of triple-negative breast cancer (TNBC) cT2N0M0.
Neoadjuvant chemotherapy (NACT) based on taxanes and anthracyclines was proposed, followed by local therapy. During a nephro-oncology meeting, it was decided to conduct weekly monitoring of renal function (pre-NACT creatinine 1.82 mg/dL). The patient completed the planned NACT without dose reductions, delays or unexpected toxicities. Dose adjustments of immunosuppressants due to subtherapeutic levels were done. Post-NACT, renal function remained stable (post-NACT creatinine 1.72 mg/dL).
The patient underwent a total mastectomy with sentinel lymph node biopsy due to large tumor-to-breast-size ratio. Complete pathological response (pCR) was achieved. At 12 months post-surgery, the patient remains free of disease recurrence.

Discussion and Final Comments: We share a case of a ReT patient presenting a stage IIB TNBC diagnosed five years after the ReT. The patient underwent taxane-anthracycline NACT. There was renal graft function preservation and a pCR was achieved. This was facilitated by regular renal function monitoring and a multidisciplinary approach. However, nowadays, a combination of immunotherapy (IO) and chemotherapy is the standard of care.
Lessons Learned
There is a lack of literature addressing how to manage breast cancer in ReT.
Breast Cancer Treatment in ReT patients can be extrapolated from the general population.
A multidisciplinary approach is crucial and regular renal function monitoring is needed.
Immunotherapy's role in solid organ transplant receipts is being debated.

P5-11-29: Single center experience of the usage of KEYNOTE-522 regimen in patients with high-risk early stage triple negative breast cancer (TNBC)

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Introduction: Chemoimmunotherapy, carboplatin/paclitaxel/pembrolizumab followed by adriamycin/cyclophosphamide/pembrolizumab (AC) (KEYNOTE-522 regimen), has shown a remarkable pathological response rate (pCR) of 68% and became the new standard of

care for high risk stage II/III triple negative breast cancer (TNBC). The neoadjuvant use of anthracycline remains an area of debate with added short-term toxicities and well established long-term cardiac toxicity and leukemia risk. Long-term outcomes of the ABC Trial questioned whether anthracyclines are necessary for every patient with TNBC as the inclusion of anthracyclines in older pre-immunotherapy regimens did not show an overall survival (OS) benefit. Additionally, the NeoPACT trial had an encouraging pCR of 58% with 6 cycles of carboplatin/docetaxel/pembrolizumab, providing an alternative regimen for patients with TNBC who are not eligible for anthracycline-based chemoimmunotherapy. A phase 3 SWOG SCARLET trial is ongoing for head-to-head comparison of the efficacy and safety of the NeoPACT and KEYNOTE522 regimen. Therefore, we performed a single center retrospective review on patients with early stage TNBC treated with either the KEYNOTE-522 regimen or a truncated KEYNOTE-522 regimen with weekly carboplatin paclitaxel pembrolizumab without AC. The primary goal is to describe the pCR rate, usage of anthracycline, and tolerance.

Methods: The study was conducted using an IRB approved institutional protocol through Cedars-Sinai Medical Center (CSMC). Inclusion criteria included adults with early stage (I-III) TNBC who were intended to be treated following Keynote 522 with curative intent and were post-surgery by the data cutoff date 06/8/2024 at CSMC or Huntington Health. 70 patients met this criteria. Patients were considered to be treated with AC if they had any exposure neoadjuvantly. We retrospectively recorded baseline patient age, stage, BRCA status disease, and treatment characteristics. “Clinical complete response” (CCR) was defined by the treating physician using imaging and physical exam. We used the D’Agostino & Pearson method for normality testing and the Mann-Whitney test for between-group comparisons. Ninety five percent confidence intervals were calculated with the Wilson-Brown method. Categorical comparisons used Fischer’s exact test. Toxicity was graded according to CTCAEv.5.

Results and Discussion: Most patients (49, 70%) received AC neoadjuvantly. Those who did not receive AC (21, 30%) were further sub-divided by the reason why. The most common reasons were CCR (11, 52%), namely no signs of disease on imaging or physical exam after treatment with carboplatin, paclitaxel, and pembrolizumab or toxicities such as colitis or septic shock due to prior therapy. There were no significant differences between groups in race, initial clinical stage, or BRCA1/2 mutation status. Germline testing data availability (83%-100%) was not significantly different between groups. Those with CCR had an astounding pathological pCR of 91% [62%, 98%] but was not significantly different compared to those treated with Keynote-522 (63% [49%, 75%], p=0.14). These patients with CCR were also significantly younger than those who did not receive AC due to toxicity ($p<0.01$) but not when compared to those who received AC (p=0.09). OS data is not currently mature enough to be significant. Patients who received AC had significantly more grade 3 or 4 cytopenia (34, 72% vs 8, 38% [p=0.01), and more episodes of febrile neutropenia (8, 17% vs 0, 0%, p=0.05). Only two patients who omitted AC initially went on

to receive AC adjuvantly. Based on these initial observations, it appears that AC can be omitted from carefully chosen subpopulation of patients with non-metastatic TNBC to avoid the toxicities of anthracyclines.

P5-11-30: Residual Cancer Burden (RCB) with Dose-Dense versus Every 3-Week AC regimen during Neoadjuvant Chemoimmunotherapy for Early-Stage Triple-Negative Breast Cancer – The Neo-Real Study

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Background: Dose-dense chemotherapy regimens have demonstrated survival benefits in early-stage breast cancer treatment. However, it remains unclear whether dose-dense anthracycline and cyclophosphamide (ddAC) offers additional benefits during neoadjuvant pembrolizumab plus chemotherapy (P+CT) for triple-negative breast cancer (TNBC).

Methods: The Neo-Real study is an ongoing multicenter effort evaluating real-world data of patients with TNBC undergoing neoadjuvant P+CT since July 2020. This analysis compares ddAC versus every 3-week (q3w) AC, focusing on updated pathologic complete response (pCR) and residual cancer burden (RCB), with subgroup analyses based on disease stage.

Results: Among the patients included in the Neo-Real study to date, 349 have undergone surgery and had pathology reports available. RCB classification was described for 305 patients. Only 3 patients had a disease progression during the neoadjuvant therapy that precluded surgery; two were in the q3w group and one had the progression during carboplatin and paclitaxel (before AC). The AC regimen was done as ddAC in 56.2% and q3w AC in 42.4% of the cases. Baseline characteristics were similar for the ddAC and q3w AC groups, with the exception of the Ki67 index (Ki67 ≥ 50%: 84.8% in ddAC and 72.5% in q3w AC, P=0.008); 73.2% of the patients had stage II and 26.8% had stage III disease.
In line with our previous report, a pCR was observed in 65.3% with ddAC versus 59.5% with q3w AC (P=0.311), with a numerically higher difference in the stage III disease cohort (pCR 58% with ddAC and 42.1% with q3w AC, P=0.196). In stage II, the pCR rate was 66.9% with ddAC and 65.4% with q3w AC (P=0.455).
Among patients with a RCB description, RCB 0-1 was higher with ddAC (82.8%) than q3w AC (73.3%) in the overall cohort (P=0.049). RCB 0-1 occurred in 71.7% vs 50.0% in the stage III group (P=0.066), and 85.9% vs 82.2% in the stage II group, respectively (0.566). The distribution of patients in each RCB category according to AC regimen was as follows: 65.3% RCB 0 (pCR), 8.7% RCB 1, 13.8% RCB 2, 1.5% RCB 3, and 10.7% residual disease without RCB description with ddAC, and 59.5% RCB 0 (pCR), 5.4% RCB 1, 17.6% RCB 2, 6.1% RCB 3, and 11.5% residual disease without RCB description with q3w AC.

Conclusion: Dose-dense AC was associated with a higher rate of RCB 0-1 compared to q3w AC during neoadjuvant P+CT for TNBC. The absolute difference in RCB 0-1 favoring ddAC was higher in stage III disease. Longer follow-up is warranted to evaluate the impact of AC regimen on long-term outcomes.

P5-12-01: Prognostic implications of tumor size and nodal involvement in invasive lobular carcinoma of breast

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Background: Invasive lobular carcinoma (ILC) represents the second most prevalent histological subtype of breast cancer, comprising 3-15% of all diagnosed cases. Despite its unique clinical and pathological profile when compared to invasive ductal carcinoma (IDC), there remains significant debate regarding the prognosis and optimal management strategies for ILC. This study aimed to investigate the differences in clinical and pathological features, survival between ILC and IDC and their impact on prognosis.

Methods: We reviewed the data of the 15,489 IDC patients and 966 ILC patients who were treated between January 2005 and December 2022 at Seoul National University Hospital. The clinical and pathological characteristics of ILC and IDC were compared, and survival outcomes were analyzed using cox proportional hazard models. We conducted a parallel analysis using the Surveillance, Epidemiology, and End Results (SEER) database. We extracted data from 247,290 breast cancer patients diagnosed between 2010 and 2015, comprising 219,404 cases of IDC and 27,886 cases of ILC.

Result: ILC had a larger tumor size, higher proportion of HR+/HER2- subtype, lower tumor grade, and lower Ki-67 index compared to IDC. Kaplan-Meier survival analysis with log-rank tests showed no statistically significant differences in breast cancer-specific survival (BCSS) or overall survival (OS) between patients with ILC and IDC ($p = 0.52$, 0.27, respectively). After adjusting for histologic grade and subtype, we found distinct patterns in survival determinants between ILC and IDC. In IDC, both tumor size (T stage) and nodal status (N stage) showed consistent prognostic significance. Compared to T1, T2 and T3 stages had 2.5-fold (95% CI: 2.00-3.00) and 4.4-fold (95% CI: 3.40-5.72) higher breast cancer-specific death risks, respectively. N1, N2, and N3 stages showed 2.5-fold (95% CI: 2.08-3.06), 3.8-fold (95% CI: 3.06-4.74), and 6.0-fold (95% CI: 4.66-7.67) increased risks compared to N0. Interestingly, in ILC, tumor size did not significantly impact survival ($p=0.38$ for T2, $p=0.12$ for T3 vs T1). However, advanced nodal involvement in ILC showed markedly higher risk, with N2 and N3 stages having 5.2-fold (95% CI: 1.66-16.57) and 26.1-fold (95% CI: 10.54-64.69) increased risks, respectively.
SEER data analysis corroborated these findings. For IDC, T2 and T3 stages showed 2.2-fold

(95% CI: 2.14-2.29) and 3.4-fold (95% CI: 3.20-3.51) increased risks, while N1, N2, and N3 stages had 1.7-fold, 2.8-fold, and 4.2-fold higher risks, respectively. In ILC, the impact of tumor size was less pronounced, with T2 and T3 stages showing 1.9-fold (95% CI: 1.69-2.04) and 2.43-fold (95% CI: 2.17-2.71) increased risks. However, nodal involvement in ILC again showed higher risk, with N2 and N3 stages having 3.5-fold (95% CI: 1.66-16.57) and 6.18-fold (95% CI: 5.53-6.90) increased risks, compared to 2.83-fold and 4.17-fold in IDC for N2 and N3, respectively.

Conclusion: In conclusion, our study highlights that ILC exhibits distinct characteristics and prognostic patterns compared to IDC. Although ILC is often detected at a larger size, the impact of tumor size on survival differs from that of IDC. In ILC, tumor size alone may not be as strong a predictor of survival as it is in IDC. Conversely, nodal involvement in ILC, especially at advanced stages, carries a particularly high risk for breast cancer-specific mortality. These findings underscore the need for tailored prognostic assessments and potentially different treatment strategies for ILC and IDC.

P5-12-02: Is BRCA2 a marker for worse prognosis in Breast Cancer?

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Background: Data regarding survival outcomes in BRCA1/2 Breast Cancer (BC) patients (pts) is controversial. Better chemosensitivity and survival was associated with triple negative BRCA1/2 BC, while positivity for the estrogen receptor (ER) was associated with worse prognosis. In BRCA2-BC, chemotherapy and exposure to ovarian hormones were described as modifiers of this ER effect. The objective of this national study is to analyze prognosis (overall, (OS) and breast cancer specific survival, (BCSS) of BC associated with the germline BRCA2 Founder variant c.156_157insAlu (BRCA2-P)
Methods: National retrospective case control study. From 224 BC pts from 16 Portuguese Hospitals 198 (F-184; M-14) pts diagnosed between 09/1982-12/2021 with a BRCA2-P, were included in this study. Survival cutoff date: 31/12/2022. Controls: 3 BC pts per case, matched by gender, stage, year of birth and cancer diagnosis, from the national oncological registry. Kaplan-Meier method was used to estimate OS and BCSS. Cox proportional hazards regression analyses to compute hazard ratios for all-cause and breast cancer mortality with the corresponding 95% CIs. Statistical analysis was performed using the software R (v4.3.3).
Results: Cases had a median age of 44 yrs (26 – 78) at BC diagnosis and 50 yrs at genetic testing. Eleven of the 198 pts had bilateral BC and 29 developed contralateral BC. Staging: I+II (145; 73%), III (48; 24%) and IV (5; 3%). Most (178) were invasive carcinomas NST. Subtype: 19 (9.6%) triple negative, 15 HER2+ (7,6%) and 168 (84.8%) were Luminal. Treatments: Surgery: 193 pts (137 primary); chemotherapy: 166 (83.8%);

hormone therapy: 167 (84.3%); radiotherapy: 141 (71.2%); anti-HER2 16 (8.1%). Uptake of Risk Reduction (RR) surgeries: mastectomy (RRM): 87 females (35 simultaneous and 52 after a previous therapeutic surgery); Bilateral salpingo-oophorectomy (BSO): 121 (117 after and 4 before BC diagnosis). Second cancers: BC: 29/198 pts (15%); Ovarian Cancer: 10/184 (5.4%); prostate cancer: 4/14 (28.6%); pancreatic cancer: 3/198 pts (1.5%); GIST, thyroid, melanoma, lung cancer, MDS: 2 cases each (1%). At the cutoff date for survival analysis, 62 pts died: 50 (80.6%) from cancer, 5 (8.1%) from other causes, 7 (11.3%) unknown. BC was the leading cause of cancer-related death (39; 78%) followed by ovarian (4; 8.7%) and pancreatic (3; 6%) cancer.
OS and BCSS: Median survival was 20.85 and 34.3 yrs for the BRCA2-P and Control group (p=0.12), respectively. To minimize immortality bias, OS and BCSS were analyzed separately for diagnoses after 2006: 88.7% of cases were alive at 5 yrs (vs 88.4% of controls) while at 10 and 15 yrs this difference was 72% vs 78.4% and 68% vs 73.8%, respectively (p=0.44).
Multivariate analysis: variables associated with lower risk of death (OS and BCSS) were adjuvant chemotherapy (HR 0.24 CI: 0.08-0.69, p=0.01 and HR 0.21 CI: 0.06-0.82, p=0.03) and RR mastectomy* (HR 0.37 CI: 0.16-0.88, p=0.02 and HR 0.34 CI: 0.12-0.94, p=0.04), respectively. BSO* was associated with a better BCSS (HR 0.36 CI:0.13-0.93, p=0.35), but not with OS (HR 0,45 CI: 0.19-1.05, p=0.06). Staging III and higher were associated with worse OS and BCSS (HR 6.27 CI: 1.37-28.58, p=0.02 and HR 24.16 CI: 2.39-244.32, p=0.01, respectively).The association of ER/PR with OS and BCSS was not significant: (HR 0,52 CI 0.15-1.73, p=0.3 and HR 0,27 CI 0.07-1.06, p=0,06).
Conclusions: No significant differences in OS and BCSS were observed for BRCA2-P BC when compared to sporadic BC controls. Variables with a positive impact on OS for BRCA2-P pts were stages under III, adjuvant chemotherapy and RR mastectomy. BSO was associated with better BCSS. In this study, where virtually all Luminal BRCA2-P BC pts were treated with hormonotherapy and 65% underwent RRBSO, we did not observe a negative association of ER and survival.

*Cox proportional hazards regression model with time co-variates

P5-12-03: Influence of antibiotic use on the efficacy of neoadjuvant chemotherapy in breast cancer

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Introduction: The rediscovery of the microbiota has revealed its influence on carcinogenesis and response to treatment. Antibiotic use is common in cancer patients, potentially causing

intestinal dysbiosis, disturbing the tumor microenvironment, intratumoral microbiota and affecting pharmacokinetic interactions with chemotherapy. These factors seem to influence cancer treatment benefits, but this is not well understood. Small studies suggest antibiotic use may reduce neoadjuvant treatment benefits in breast cancer.

Objectives: This real-life, retrospective study includes a broad series of women diagnosed with breast cancer between 2009 and 2023, candidates for neoadjuvant chemotherapy based on anthracyclines, cyclophosphamide, taxanes +/- antiHER2. We analyze the influence of antibiotic use during neoadjuvant treatment or within 30 days prior to its initiation on pathological complete response (pCR/RCB-0) and achieving a good response defined as RCB-0 and RCB-1, according to residual cancer burden (RCB) index.

Methods: The analysis included 1314 patients, divided into two groups: those who did not receive antibiotics during neoadjuvant treatment (60.6%) and those who did while or within 30 days prior to it (39.4%). Clinical and pathological variables were compared using the chi-square test, with a significance level set at <0.05. The average relative dose intensity (RDI) was calculated using the Hryniuk method for each type of chemotherapy administered. To identify the most significant predictors for our logistic regression model, we employed a stepwise selection procedure. Variables were selected based on the Akaike Information Criterion (AIC), which balances model fit and complexity by penalizing less significant predictors.

Results: The clinico-pathological characteristics were balanced between groups, with no significant differences. 66% were in stage II and 29% in stage III (p 0.92), 54% and 56% were premenopausal (p 0.66), and 54% and 57% were grade 3 tumors (p 0.22) in both groups respectively. Regarding phenotypic subtypes, there were no differences between the two groups (p 0.85). Among patients without antibiotics, 35% were luminal tumors, 39% HER2+, and 26% triple-negative. Among those with antibiotics, 34% were luminal, 39% HER2+, and 27% triple-negative.
Significant differences were observed in achieving an optimal RDI >85%. 95% of patients without antibiotics had an RDI >85% compared to 89% with antibiotics. However, the frequency of patients with an average RDI ≤85% was low in both groups: 5% without antibiotics and 11% with antibiotics (p 0.00026).
The effect of antibiotics on neoadjuvant therapy efficacy was explored based on the pathological evaluation of residual disease using the RCB index. Significant associations were observed, indicating patients who received antibiotics were less likely to achieve optimal pathological responses. Complete pathological responses (RCB-0) were higher without antibiotics: 33% vs 28% (p 0.04). For RCB 0/I vs RCB II/III, 49% of patients without antibiotics achieved RCB 0/I vs 37% of those with antibiotics (p <0.0001). This trend was significant too in luminal tumors: 21% without antibiotics achieved RCB 0/I vs 13% with antibiotics (p 0.03) and in HER2+ and triple-negative tumors (63% vs 50%,

p<0.0001).
The multivariate analysis revealed antibiotic use had a negative effect on achieving pCR (p 0.03) and RCB 0/1 (p<0.0001), along with other predictive variables. Our model demonstrated the RDI did not influence the pathological response.

Conclusion: This study suggests that antibiotic use during neoadjuvant treatment may negatively impact the pathological response in breast cancer. These results highlight the need to consider antibiotic use during chemotherapy to optimize outcomes and the need for prospective studies to explain the mechanism of this influence.

P5-12-04: BRCA genetic testing and treatment patterns for patients with early-stage HER2-negative breast cancer in the United States (U.S.) community setting, 2016–2022

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Background: The presence of a germline pathogenic variant in BRCA1/2 (gBRCAm) markedly increases the risk of breast cancer and its recurrence after standard therapy. Adjuvant olaparib received regulatory approval in the U.S. in March 2022 for treating adult patients with gBRCAm early-stage, high-risk HER2-negative breast cancer who had received neoadjuvant or adjuvant chemotherapy and who were selected for therapy based on an approved companion diagnostic test. This retrospective cohort study aimed to describe BRCA testing and subsequent treatment patterns with olaparib.
Methods: We studied adults (≥18 years) with initial diagnosis (1-Jan-2016 to 30-Dec-2022) of HER2-negative and clinical stage I, II, or III breast cancer who were included in a longitudinal, real-world dataset from the Syapse Learning Health Network, drawn from large community US health systems. Patients with unknown hormone receptor (HR) status and those enrolled in a clinical trial or with lobular carcinoma in situ, lymphoma, or other primary cancer were excluded. Testing for BRCA status was summarized by year. Patient characteristics were described by BRCAm status.
Results: Of the 15,784 eligible patients, 5,821 (37%) had a BRCA test (>99% for gBRCA, <1% somatic) before a diagnosis of metastatic disease, including 4,762 (35%) of 13,748 with HR-positive tumors and 1,059 (52%) of 2,036 with triple-negative breast cancer (TNBC). The timing of BRCA testing, when recorded, was on/before surgery for 74% of patients and after surgery and before recurrence/metastasis for 26%. Testing rates rose by year from 28% in 2016 to 46% in 2022. The percentages of patients tested across all years ranged from 36% to 39% by known race: namely, 37% of White patients (n=12,588), 36% of Black/African American patients (n=2,323), 39% of Asian patients (n=591), and 39% of American Indian/Alaska Native patients (n=49) had a BRCA test. Sampling for BRCA was primarily derived by blood (86%) followed by saliva (12%). Overall, among the 5,821 patients tested, BRCAm were identified in 205 cases (3.5%), including in 117 (2.5%)

patients with HR-positive tumors and 88 (8.3%) patients with TNBC, respectively. Of the tested patients, surgery at the primary site was conducted for 97% with BRCAm vs. 96% non-BRCAm, including mastectomy (84% vs. 51%), and lumpectomy only (12% vs. 44%), respectively. Radiation to the primary site was administered to 44% with BRCAm vs. 60% non-BRCAm. Among patients tested for BRCAm, seven were treated with olaparib and 6 of 7 patients had TNBC.
Conclusions: The results of this study indicate that BRCA genetic testing rates have increased from 2016 to 2022 in the US, but fewer than half of eligible patients received a BRCA test. We observed that, for patients with early-stage, HER2-negative breast cancer, BRCA testing rates are greater among patients with TNBC than among those with HR-positive tumors (52% vs. 35%), indicating an opportunity for HR-positive tumors. Further, roughly 26% received a BRCA test after surgery, which suggests an opportunity to improve timing of testing to before surgery. The percentages of patients tested were similar across racial groups. A more contemporary cohort may extend our understanding of olaparib use in this patient population. Future work will investigate use of olaparib for early-stage HER2-negative breast cancer in the U.S. in subsequent years.

P5-12-05: Current delivery and challenges in breast cancer care in the UK in 2023

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The National Health Service (NHS) in the UK is committed to providing consistent quality of care and to achieving equality objectives, however variation in care remains a significant issue. The treatment of Early Breast Cancer (EBC) has seen an influx of additional therapeutic agents that intend to improve patient outcomes. We hypothesized that the rapidly changing and increasing demands in the EBC landscape may have resulted in significantly heterogenous development of services. Understanding and highlighting these differences may encourage proactive service evaluation and improvement. Additionally, this may provide rationale for standardised approaches to reduce variation in both care and patient outcomes.
This study aimed to identify the current state of the nation and the challenges facing UK oncology teams. Healthcare Professionals (HCPs) from across the UK involved in EBC patient management (n=70, Oncologists, Surgeons, Pharmacists, and Nurses) were surveyed from August to December 2023.
Most (90%) MultiDisciplinary Team meetings (MDTs) were weekly, utilised local guidelines (81%) with Clinical Oncologists, Medical Oncologists, Surgeons, Radiographers and Breast Care Nurses present (>60%). Of note, other roles such as Pathologists (53%) and Pharmacists (13%) were less consistently reported in attendance. Only 34% had separate MDTs for Early and Advanced Breast Cancer (ABC) despite the different workforce involved in each pathway. Each MDT discussed up to 110 cases (average 53) for 1-15 minutes each with some (39%) using a triage system to streamline the MDT process.

The majority (77%) reported conducting joint (ABC and EBC) clinics with 40% indicating they have a separate EBC clinic, which may be in addition to a joint clinic. A variety of HCPs were involved in the follow up care of EBC patients, such as Pharmacists, Nurses, Surgeons, Radiographers and Physician Associates. Patients were prioritised to be seen in clinic in 57% of responses, largely based on risk factors such as nodal status, time from surgery, inflammatory Breast Cancer and suitability for Neoadjuvant Chemotherapy. Comparatively 43% of responders did not prioritise patients when scheduling for clinics.
Key challenges described in systemic adjuvant therapy provision were staff shortages (71%), clinic capacity for increased patient numbers (68%) and the increased time required to discuss new therapies (41%). The introduction of CDK4/6 inhibitor therapy to the adjuvant setting was noted to have had various impacts with patients requiring more intensive Medical Oncologist involvement than those on endocrine therapy alone.
In conclusion, changes in the treatment landscape have resulted in high levels of inconsistency between services delivered across the UK. These differences include varieties of MDT composition and process streamlining, HCP deployment and patient follow-up models. Despite these innovations in approach patient volume, clinic and staff availability are still significant issues in meeting the delivery demands of new therapies. Together these findings emphasize that proactive service evaluation and adaptation may be urgently required to accommodate increased demand and to reduce variation in care quality.

P5-12-06: Elucidating the role of NEAT1_2 in DCIS malignancy

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Background: Ductal carcinoma in situ (DCIS) is the most common form of non-invasive breast cancer where the epithelial cells are cancerous but have not spread beyond the breast milk tubes or ducts. A large fraction of human DCIS (>50%) may not need the multimodality treatment currently offered to most patients. More importantly, while we may be overtreating many, challenges remain in the identification of those most at risk for invasion/metastasis. Revealing the cellular and molecular mechanisms by which some DCIS remain indolent while others advance to invasive and metastatic breast cancers is currently an unmet need. To address this gap and to study molecular and cellular mechanisms associated with DCIS invasive and metastatic progression, we developed the Mouse-INtraDuctal (MIND) model, in which patient-derived (PDX) DCIS epithelial cells are injected into mouse mammary glands by the intraductal method and allowed to progress naturally, forming in situ lesions that, in some cases, progress to invasive ductal carcinoma. Methods: Single cell RNAseq was performed on 17 DCIS samples, including nine progressors and eight non-progressors. Progressors are patient-derived DCIS cells that show invasive progression, while non-progressors are patient-derived DCIS cells that remain non-invasive in our MIND model. Single cell RNAseq analysis identified differentially expressed genes that were upregulated in progressors compared to non-progressors in stroma and epithelial cells. CellPhoneDB was utilized to identify receptor-ligand interaction pairs in progressors vs. non-progressor DCIS MIND models. Results: We observed a significant upregulation of

NEAT1_2 expression in our progressed patient samples as well as our progressed xenografts compared to non-progressors. NEAT1_2 is a long non-coding RNA that is a major rate-limiting factor in paraspeckle formation. Paraspeckles are believed to promote tumor progression by retaining double-stranded RNA (dsRNA) and microRNA. Retention of dsRNA by paraspeckles may prevent the expression of interferon alpha (IFNa) and suppress anti-tumor immune responses. Indeed, analysis of the breast cancer TCGA dataset revealed that NEAT1_2 expression showed a significant upregulation with MYC targets and downregulation of IFNa signaling. The MYC pathway is associated with high expression of dsRNA. Upregulated MYC expression and downregulated IFNα in NEAT1_2 high samples suggest a link between MYC, increased expression of dsRNA, dsRNA retention by the paraspeckles, and a subsequent decrease in anti-tumor immune responses. To study the role of NEAT1_2 in DCIS invasive and metastatic progression, a NEAT1_2 antisense oligo (ASO) was used. We showed that NEAT1_2 ASO was an effective strategy for NEAT1_2 knockdown (KD) in DCIS cell lines. Based on preliminary data, we hypothesize that NEAT1_2 may play a role in DCIS invasive progression through the retention of dsRNA by paraspeckles to restrain an anti-tumor immune response. NEAT1_2 KD may provide an effective strategy for the induction of anti-tumor immunity and prevention of DCIS invasive and metastatic progression. Future Directions: Immunocompetent mouse mammary precancerous progression models will be used to test the efficacy of NEAT1_2 ASO in preventing DCIS invasive and metastatic progression. We will also study the mechanism by which NEAT1_2 remodels the tumor immune microenvironment to drive DCIS malignancy.

P5-12-07: Increase of repeated biopsies in metastatic breast cancer over time: Results from the Austrian AGMT_MBC-Registry

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Background: Predictive biomarkers have become increasingly important to guide treatment decisions for metastatic breast cancer (MBC). Some of these biomarkers, especially estrogen receptor (ER), HER2, and ESR1 mutations, are known to change over time due to therapeutic pressure and tumor evolution. Consequently, an increase in biopsies of metastatic sites at diagnosis of metastatic disease and during the course of the disease is expected. To test this hypothesis, we analyzed data from the Austrian Study Group of Medical Tumor Therapy (AGMT) MBC-Registry.
Patients and methods: The AGMT_MBC-Registry is a multicenter nationwide ongoing retrospective and prospective registry for MBC patients in Austria. For this analysis, only patients with sufficient data quality were included. All biopsies, fine needle aspirations or surgical procedures of metastatic sites were evaluated. The relative frequency of

biopsies/surgeries per patients on antitumoral treatment per year and per year category were calculated (2010-2012, 2013-2015, 2016-2018, 2019-2021, 2022-2024) and categorized according to the line of treatment (before first-line, before second-line etc.). In cases with reported ER and HER2 status, differences between the most recent primary tumor and samples from metastatic sites were investigated.
Results: As of 26-June-2024, 2,850 patients were included in the registry. Out of 2,630 evaluable patients, 1,437 (54.6%) had at least one histologic confirmation of a metastasis during the course of disease: 1,124 (42.7%) via core needle biopsy, 116 (4.4%) via fine needle aspiration, and 352 (13.4%) via surgery. The ratio of biopsies/surgeries per patients per year significantly increased over time with 0.32, 0.30, 0.40, 0.54, and 0.56 in the years 2010-2012, 2013-2015, 2016-2018, 2019-2021, and 2022-2024, respectively (Pearson's Chi-squared test P <0.001). Most of the biopsies (66.9%) and surgeries (63.3%) were performed before first-line (65.7% in total), with decreasing numbers from line to line (14.2% before second-line, 5.4% before third-line, 2.5% before forth-line and 2.6% before fifth-line). Only 20.7% of patients had more than one biopsy of a metastatic site with an increasing ratio over time (0.049, 0.067, 0.077, 0.128, and 0.165 in the years 2010-2012, 2013-2015, 2016-2018, 2019-2021, and 2022-2024, respectively Pearson's Chi-squared test P <0.001). The discrepancies between ER (ER- vs ER+) and HER2 status (HER2 0 vs. HER2-low vs. HER2+) between the primary breast cancer and at least one metastatic lesion were 6.4% and 14.4%, respectively.
Conclusion: The rising number of biopsies, before first- and second-line treatments reflects the increasingly stratified treatment strategies in MBC. It can be assumed that less invasive measures, such as liquid biopsies, will further increase the number of repeated biomarker assessments to guide treatment decisions.

P5-12-08: Single-center experience in antibody drug conjugate sequencing in HER2-low metastatic breast cancer

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Introduction: Two antibody-drug conjugates (ADCs), trastuzumab deruxtecan (T-Dxd) and sacituzumab govitecan (SG), were approved by the FDA for HER2 low and HER2 negative metastatic breast cancer (MBC) respectively and the current data of the optimal sequencing of ADCs is limited. This study evaluated the efficacy of the second ADC (ADC2) following the first ADC (ADC1) in HER2-low MBC patients (pts) who have received both ADCs of T-Dxd and SG.

Methods: This study represents an IRB approved retrospective cohort study of adult pts (age ≥ 18 years) at Moffitt Cancer Center between December 1, 2019, to January 31, 2024 with HER2-low MBC treated with both ADCs. Data was obtained via abstraction of the electronic medical record. The primary objective was to evaluate progression free survival

(PFS) after ADC 1 (PFS1) and after ADC2 (PFS2). PFS is defined as the time from first dose of ADC to time of imaging showing progression or death. The Kruskal-Wallis test was applied to assess the association between continuous and categorical variables, while the Chi-square test or Fisher's exact test was used to evaluate the association between two categorical variables. All statistical tests were two-sided, with a significance level set at $p < 0.05$.

Results: Overall, 34 pts met inclusion criteria. Seventeen pts received T-Dxd as ADC1 and 17 patients received SG as ADC1. The cohort included one male patient, 41.2% (14/34) were hormone receptor (HR) positive, and 58.8% (20/34) were triple negative breast cancer (TNBC) from metastatic biopsy. Two of the patients with TNBC MBC had ER or PR positive ≤ 10%. Two of the 34 patients were still receiving ongoing therapy with ADC2 at the time of the data cutoff. All of the HR positive subgroup received T-Dxd first followed by SG, and most TNBC patients (85%) received SG first followed by T-Dxd. Overall median PFS1 (mPFS1) with ADC1 in all comers was 5.8mo, and median PFS2 (mPFS2) with ADC2 was 2.4mo. PFS1 and PFS2 by drug sequence can be seen in table 1. Eight patients had significantly longer PFS2, defined as having received at least 8 cycles of therapy, and six of these patients received T-Dxd as ADC2. Five of the eight pts with this longer PFS2 were TNBC at the initial diagnosis and remained TNBC at time of ADC treatment. The other three pts were TNBC at initial diagnosis and changed to HR positive disease at time of ADC treatment.
67.6% (n=23) of pts received ADC therapies back-to-back with no lines of therapy in between, while 20.6% (n=35) of pts received one line of therapy in between ADCs. mPFS1 for those who received back-to-back therapy was 5.8mo while those with one line of therapy in between had a mPFS1 of 4.3mo.
Of the 7 pts with brain metastases at start of ADC1, mPFS1 was 8.5mo. Of the 10 pts with brain metastases at the start of ADC2, mPFS2 was 2.9mo. Ten (29.4%) pts experienced any grade 2 or above adverse events (AEs) with ADC1, while 12 (35.3%) pts experienced any grade 2 AEs with ADC2. Further description of AEs will be discussed at the presentation.

Conclusion: Our data demonstrates that mPFS is shorter following ADC2 which is consistent with limited available data to date. Interestingly, there were 8 pts with prolonged PFS2 of over 6 months, having received 8 or more cycles of therapy prior to progression. Overall, our study is limited by its retrospective nature and small sample size.

P5-12-09: Pathologic complete response (pCR) rate in patients with a germline pathogenic variant in ATM and early breast cancer

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Background: The incidence of ATM (ataxia telangiectasia mutated gene) pathogenic variants in breast cancer patients is estimated at ~ 1%. ATM is a serine/threonine protein kinase that is activated by double strand DNA breaks and, upon activation, ATM phosphorylates several downstream proteins integral to double strand DNA repair. Hereditary loss of function mutations in ATM (gATM) moderately increase the risk of female breast cancer by ~2-4x that of the general population. Higher rates of pathologic complete response (pCR) after pre-operative chemotherapy have been reported in patients with breast cancer and germline pathogenic variants in other DNA damage repair associated genes such as BRCA1, BRCA2, and PALB2. We sought to determine the rate of pathologic complete response in patients with early-stage breast cancer and gATM.

Methods: Patients with invasive breast cancer and gATM who were treated at our institution between 1994 and 2022 were identified from prospectively maintained research databases. Data analyzed included breast cancer subtype and stage at diagnosis, treatment, and residual cancer burden status. pCR was defined as the absence of residual invasive cancer on hematoxylin and eosin evaluation of the complete resected breast specimen and all sampled regional lymph nodes.

Results: We identified 86 patients with invasive breast cancer and gATM. Pre-operative chemotherapy (anthracycline and/or taxane-based with or without anti-HER2 therapy) was utilized in 31 of the 86 (36%) patients. The pCR rate was 5/31 (16.1%). Of the 5 patients with pCR, 2 had triple positive subtype and 1 patient each had ER-/HER2+, ER+/HER2 neg, and triple negative breast cancer subtype.

Conclusion: The pCR rate was modest (16.1%) in our cohort of patients with invasive breast cancer and gATM. This suggests that altered ATM may not augment chemosensitivity in breast cancer, however larger studies are needed. Additional pre-operative agents including immunotherapy, platinums, new anti-HER2 therapies, and ATM inhibitors may improve the pCR rate.

P5-12-10: Overall survival in patients with recurrent breast cancer in stage II-III using surveillance imaging detection vs symptomatic disease at a reference hospital in Mexico

Rocio Crystal Grajales Alvarez

Background: Breast cancer is the most common cancer worldwide, the annual risk of locoregional recurrence is 1-1.5% for at least 15-20 years. The relationship between an early recurrence detection and the impact on quality of life and overall survival (OS) has been analyzed for decades and yet according to international guidelines, the only approved image study for follow-up is the annual mammography because there hasn't been enough

evidence for additional imaging surveillance that impact in OS nor disease free survival (DFS). Most of this evidence comes from studies prior the implementation of the intrinsic subtypes which are the main prognostic factor and current therapeutic guide. There is a recent retrospective protocol which demonstrated a benefit in OS of asymptomatic detection of distant recurrences in patients with HER2+ and triple negative breast cancer (TNBC) compared with symptomatic detection.
Methods: The aim of this study is to determine the OS in patients with stage II-III breast cancer whose recurrence is documented by symptomatic disease vs asymptomatic detection by surveillance imaging. A retrospective observational cohort study was performed. Patients diagnosed with stage II-III breast cancer, treated at the UMAE Hospital de Oncología of the CMN Siglo XXI from January 2018 – December 2022 were included. OS and DFS were calculated with Kaplan- Meier method with Log Rank, Breslow and Mantel tests. The prognosis analysis of the complete models was performed using Cox proportional hazards model. The analysis was stratified according to intrinsic subtype and site of recurrence (local and distant).
Results: A total of 618 patients were included. Local recurrence (31.1%) was less common than distant recurrence (68.9%). Most of the patients were luminal (75.2%), 22.4% HER2-positive, and 15.3% TNBC. The median OS was 83 months. A total of 434 patients (70.2%) were symptomatic detections while 184 patients (29.8%) were detected by surveillance imaging. The median OS (mOS) in the asymptomatic group and the symptomatic group were 92 months and 80 months, respectively (p= 0.895). In those with distant recurrence, the subgroup analysis by breast cancer subtype showed no difference between the luminal and TNBC groups, according to symptomatic vs asymptomatic detection (mOS 111 months vs 95 months, p= 0.37 in luminal; and 45 months vs 36 months, p= 0.73 in TNBC) but there was a statistically significant association in the HER2-positive group by the Breslow test with improved survival in those with imaging detection (53 months vs 95 months, p=0.034) which means the survival benefit is seen during the first follow-up months. There was no difference in OS in patients with locoregional recurrences by intrinsic subtype.
Conclusions: In patients with breast cancer stage II-III there is no benefit in OS with an asymptomatic surveillance imaging recurrence detection vs a symptomatic disease. Those with HER2-positive breast cancer showed a significant benefit in survival of an asymptomatic detection during the first months of follow-up, probably related to antiHER2 therapy available for the first-line treatment for this population.

P5-12-11: Differences in overall survival between patients with fungating and non-fungating breast cancer with distant metastases at the time of diagnosis

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Background: Giant fungating breast cancers (FBCs), characterized by exudative discharge, foul odor, massive tumor formation, and extensive ulceration, often require palliative

treatment, even without distant metastasis. However, our results presented at SABCS2023 indicated that multimodal therapy, including surgery, results in equivalent overall survival (OS) in patients with FBC and stage III non-fungating breast cancers (non-FBCs). Notably, reports on the treatment and outcomes of patients with stage IV FBC with distant metastases are limited. Therefore, we tested the hypothesis that the OS of patients with stage IV FBC at diagnosis is equivalent to that of patients with stage IV non-FBC.

Methods: We identified 112 patients diagnosed with stage IV breast cancer at initial presentation between June 2012 and March 2020, of whom 24 had FBC and 88 had non-FBC. We analyzed the clinicopathological characteristics, metastatic sites, and treatment modalities of both groups. We further subcategorized the patients into two groups based on the presence of single or multiple visceral metastases and compared their survival rates. We used Kaplan-Meier curves and log-rank tests d for statistical analyses, while a Cox proportional hazards model was used to determine predictors of OS.

Results: The mean ages of the FBC cohort and non-FBC cohort were 61.5 years (range: 41–92) and 65.0 years (range: 31–89), respectively. There were no statistically significant differences in subtype distribution or nuclear grades between the two cohorts. The number of patients receiving initial chemotherapy was similar in both groups (58.3% vs. 61.4%, p=0.817). In the non-FBC cohort, isolated bone metastases were observed in 19 cases (21.6%), whereas in the FBC cohort, they were observed in only one case (4.2%). However, there was no difference in the proportion of cases with visceral metastases between the FBC and non-FBC cohorts, both for single (50.0% vs 56.8%, p=0.350) and multiple visceral metastases (28.6% vs. 21.6%, p=0.484). The 2-year survival rate tended to be lower in patients with FBC than in patients without FBC (75.0% vs. 85.0%, p=0.235). At 3 years, the survival rate for the FBC cohort was significantly lower (52.5% vs. 80.0%, p=0.015), and this difference became more pronounced at 5 years (29.5% vs. 73.9%, p<0.001). The median OS time was also significantly shorter in patients with FBC than in those without FBC (3.1 years vs. 7.0 years, p=0.003). Among cases with visceral metastases excluding those with isolated bone metastases, the FBC cohort exhibited markedly lower 3-year survival rates in both the single (63.5% vs. 81.3%, p=0.061) and multiple organ metastases subgroups (33.3% vs. 73.7%, p=0.012). In a multivariate Cox model involving 112 patients, the diagnosis of FBC was a significant independent predictor of a poorer OS (hazard ratio=2.264, p=0.011).

Conclusion: While the OS of patients with stage III FBC was comparable to that of non-FBC, survival rates were significantly lower for patients with stage IV FBC than those with non-FBC. These findings provide an important reference to help clinicians determine treatment strategies for patients with stage IV breast cancer.

P5-12-12: Refining Sentinel Lymph Node Biopsy Decisions for Clinically Node Negative Microinvasive DCIS

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Background: With improved screening and detection of breast cancer, we are diagnosing breast cancer at early stages. Consequently, there is a need to better understand how to manage early diseases such as Ductal Carcinoma In Situ with Microinvasion (DCISM). DCISM is defined as an invasion ≤ 1 mm in size and is typically found in 5-10% of ductal carcinoma in situ (DCIS) cases. The appropriate management of DCISM remains debated, as it is unclear whether these lesions should be treated similarly to DCIS or as a small invasive disease. As we advance towards precision medicine and emphasize enhancing patients' quality of life, there has been a concerted effort to further stratify patients to identify those at low risk, allowing for the de-escalation of morbid treatments such as axillary surgery during initial surgical intervention. However, optimal axillary management of DCISM remains elusive due to limited literature on this topic. This study aimed to identify pre-operative predictive variables that increase the risk of upstaging to invasive cancer on final pathology, enabling stratification of patients into low- and high-risk groups and guiding the de-escalation of sentinel lymph node biopsy (SLNB).
Methods: A retrospective chart review was conducted of women over the age of 18 who had DCIS with confirmed or suspected microinvasion on initial breast biopsy and were clinically node-negative at Montefiore/Einstein Comprehensive Cancer Center from 2013 to 2023. A chi-squared analysis of pre-operative factors associated with the histopathologic upgrade to invasive carcinoma on final pathology was conducted, including hormone negativity, mass size on mammogram and MRI, palpable mass, high grade, comedonecrosis on pathology, guidance method, biopsy device, biopsy needle gauge size, and number of cores obtained.
Results: A total of 68 women were identified with DCISM on initial biopsy. Among them, 40% (n=27/68) were upstaged to invasive carcinoma on final pathology, and 66% (n=45/68) underwent SLNB at the time of surgical resection. For those who underwent SLNB and were upstaged on final pathology, 19% (n=4/21) had positive lymph node metastasis, whereas those who underwent SLNB and did not upstage had 0% (n=0/24) lymph node metastasis. The time from the initial biopsy to the final surgery did not differ significantly between those who upstaged and those who did not (p = 0.2446). Pre-operative variables that predicted higher odds of upstaging included a palpable mass (OR 7.62, 95% CI 2.06-28.18; p=0.002) and a size on mammogram ≥ 3.5 cm (OR 4.12, 95% CI 1.22-13.95; p=0.020). During the initial biopsy, using a 9-gauge needle and obtaining ≥ 4 cores resulted in a sensitivity of 80% for the final diagnosis of DCISM, compared to a sensitivity of only 18% when using a smaller 14-gauge needle and obtaining < 4 cores.
Conclusion: Patients with a diagnosis of DCIS with microinvasion who are clinically node-negative, and have pre-operative characteristics including size on mammogram < 3.5 cm and lack of a palpable mass, may benefit from the de-escalation of SLNB, as these patients have low odds of upstaging to invasive carcinoma and potentially, low rate of lymph node metastasis. These findings underscore the importance of thorough pre-operative

assessment in patients with DCISM to guide surgical management. Identifying predictive factors for upstaging can help stratify patients into low- and high-risk groups, potentially guiding the de-escalation of SLNB and reducing the morbidity associated with more invasive procedures. As we move towards improved early detection and diagnosis of early-stage disease, there is a need for guidelines addressing these early lesions and larger prospective studies.

P5-12-13: Does Ductal Carcinoma in Situ have metastatic potential? A nationwide cancer registry-based study of Ductal Carcinoma in Situ with sentinel lymph node positivity

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Background: The current clinical paradigm around Ductal Carcinoma in Situ (DCIS) is that it consists of malignant cells confined to the breast ducts, and therefore cannot metastasize. Nonetheless, several studies have reported DCIS with metastasis in the sentinel lymph node. For accurate risk communication and management, we aimed to assess to what extent registered “metastatic spread” in DCIS could be explained by limitations in registration or missed invasive breast cancer at time of diagnosis.

Methods: Nationwide data on women diagnosed with DCIS and a positive sentinel node (DCIS SN+) between 2005 and 2021 were obtained from the Netherlands Cancer Registry (NCR) and the Dutch Nationwide Pathology Databank (Palga). The incidence of registered DCIS SN+ and the size of the metastasis was determined with data from NCR. Pathology data of the primary DCIS diagnosis was thoroughly reviewed, with all known corresponding history pathology reports of in-situ or invasive breast cancer or unknown primary. Cases were excluded from further analysis if PALGA data indicated registration errors, DCIS mixed with other types of lesions, or diagnostic uncertainties such as suspicion of microinvasion, uncertain sentinel node status, poor tissue quality or positive surgical margins.
Next, hematoxylin and eosin stained tissue slides of eligible DCIS SN+ cases were independently reviewed by two expert breast pathologists to assess the presence of (micro)invasion and sentinel node status. Presence of invasion and sentinel node positivity was scored as yes, uncertain, no or not applicable. For cases scored as uncertain, additional immunohistochemical (IHC) stainings with cytokeratin 5/6 or 8/18 were used. Agreement between the two pathologists was assessed using the linearly weighted Kappa statistic.

Results: A total of 30,863 patients were identified with a DCIS diagnosis between 2005 and 2020, of which 16,070 (52.1%) underwent sentinel lymph node biopsy according to NCR data. SN+ was identified in 454 (2.8 %) patients: 47 (10 %) had macrometastases (>2 mm), 78 (17%) had micrometastases (>0.2 - <= 2 mm), and 329 (73%) were positive for isolated tumor cells (ITCs) (<= 0.2 mm). Out of the 454 registered DCIS SN+ cases, 273 (60%) were

excluded from further investigation based on pathology data from Palga, based on registration errors (n=44), due to the presence of other lesions (n=147), or diagnostic uncertainties (n=82). There was no significant difference in reasons for exclusion between micrometastases, macrometastases or ITCs (p = 0.36). Tissue material of 47 out of 181 DCIS cases with macro- and micrometastases were reviewed by the pathologists. Initial agreement on sentinel node status was weak with a kappa statistic of 0.33 (95% CI 0.15 – 0.52; p = 0.003. Agreement on the presence of an invasive component was minimal with a kappa statistic of 0.14 (95% CI 0.08 – 0.34 ; p = 0.12). The most frequent discrepancies were between 'uncertain' and 'no' scorings. After reaching consensus, three cases were scored as having an invasive component, and additional IHC staining was requested for 40 cases due to suspicion of the presence of (micro)invasion. Review results of these 40 cases will be presented at the conference.

Conclusions: Our study offers a nuanced understanding of DCIS SN+ cases, suggesting that while these cases pose diagnostic challenges, the metastatic potential of pure DCIS remains low.

P5-12-14: IMPACT OF HER2-LOW STATUS ON SURVIVORSHIP IN HER2-NEGATIVE EARLY BREAST CANCER PATIENTS UNDERGOING NEOADJUVANT TREATMENT WITHOUT PATHOLOGICAL COMPLETE RESPONSE

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Purpose: Despite recent improvements in the preoperative management of early breast cancer (eBC), many patients still do not achieve pathological complete response (pCR) and have inadequate treatment outcomes. Our analysis aims to investigate the role of human epidermal growth factor receptor 2 (HER2)-low status in predicting survival outcomes in patients not achieving a pCR.

Methods: We retrospectively identified patients with stage I-III HER2-negative breast cancer receiving neoadjuvant treatment and who did not achieve pCR at The Royal Marsden NHS Foundation Trust, London, between 2013 and 2023. Disease characteristics including HER2-low status, defined as HER2 1+ on immunohistochemistry (IHC) or 2+ on IHC without in situ hybridization (ISH) gene amplification, were collected. We analysed relapse-free survival (RFS) and overall survival (OS) based on HER2 status in the overall population and in patients with oestrogen receptor (ER)-positive (ER+) and ER-negative (ER-) cancers.

Results: Overall, 565 patients were included in the analysis. The mean age of the study

population was 50.0 ± 11.1 years. Median follow-up time was 44.9 months (interquartile range 13.8-69.8).
370 patients (65.5%) had stage II disease, 390 (69.0%) had grade 3 disease, and 502 (88.8%) had ductal histopathology. 321 patients (56.8%) had ER+ BC, while 244 patients (43.2%) had ER- BC. Overall, 254 patients (45.0%) had HER2-low disease, while 174 (54.2%) ER+ BC patients and 80 (32.8%) ER- BC patients had HER2-low BC.
HER2-low BC patients had significantly improved OS [HR 0.65 (95% CI, 0.45-0.95, p=0.026)] and a trend towards improved RFS compared to HER2-zero BC patients. Multivariable analysis did not confirm any impact of HER2, whereas ER status and BC stage impacted survival outcomes.
In the ER- population, HER2-low status did not have any impact on RFS nor on OS.
In the ER+ population, HER2-low BC patients had significantly improved RFS [HR 0.65 (95% CI, 0.43-1.00, p=0.047)] and OS [HR 0.50 (95% CI, 0.29-0.85, p=0.009)] compared with HER2-zero disease. In the same cohort, multivariable Cox-regression model confirmed the positive impact of HER2-low status on OS independently from stage, age, grade and ER-low/high positivity [HR 0.51 (95% CI, 0.30-0.88, p=0.015)].

Conclusions: The study highlights the potential relevance of HER2-low status as a predictive factor for survival outcomes in HER2-negative early BC patients, particularly in the context of ER+ disease. These findings underscore the importance of considering HER2 status (including HER2-low status) alongside traditional prognostic factors when assessing the prognosis and treatment strategies for this patient population.

P5-12-15: Survival Nomogram Including Lymph Node Ratio for Patients with Invasive Micropapillary Breast Cancer

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Objectives: Invasive micropapillary carcinoma (IMPC) stands out as a distinct and notably aggressive variant of breast cancer, and is known for its propensity for spreading to regional lymph nodes. The variability in the number of lymph nodes removed during surgery complicates assessment of the metastatic risk, an issue that is even more pronounced in IMPC due to its tendency for lymphatic spread. In this study, we evaluated the role of the lymph node ratio (LNR) in forecasting the outcomes of IMPC and developed a predictive nomogram for breast cancer-specific survival (BCSS) in patients with IMPC.
Methods: We analyzed data from 1,697 female patients diagnosed with IMPC, gathered from the Surveillance, Epidemiology, and End Results (SEER) database spanning 2010 to 2020. Participants were randomly assigned to either a training set or an internal validation set in a 7:3 ratio. An additional group of 176 patients from our institution was an external validation cohort (SZSPH cohort). Key prognostic indicators were identified by univariate analyses and the least absolute shrinkage and selection operator (LASSO) regression technique. Then we crafted a novel nomogram that integrates all independent prognostic indicators and a thorough validation of the nomogram's predictive capability was

conducted.
Results: A significant observation was the prevalence of high-grade (II-III) and undifferentiated tumors across both datasets, accounting for 93.5% in the SEER cohort and 95% in the SZSPH cohort, highlighting the aggressive nature of IMPC. Lymph node metastasis was notably prevalent among the patients, with 54.4% in the SEER dataset and an even higher rate of 71% in the SZSPH group, underscoring the propensity for IMPC to spread to lymphatic structures. From the LASSO regression analysis, six factors emerged with nonzero coefficients, signifying their statistical significance in relation to BCSS. Of note, LNR emerged as the most influential prognostic factor, followed by T-stage, radiotherapy, surgery, ER status, and chemotherapy, respectively. By incorporating these six significant variables, we developed a nomogram aimed at predicting 3- and 5-year BCSS for IMPC patients, which underwent the calibration and validation phases successfully. Its concordance indexes (C-indexes) for the training, internal validation, and external validation sets were 0.838, 0.871, and 0.758, respectively. The time-dependent receiver operating characteristic (ROC) curves also affirmed its clinical utility. The DCA curves illustrated that our nomogram provided greater net clinical benefits for predicting 3- and 5-year BCSS across all three cohorts. In the training set, the nutritional risk indexes (NRIs) for 3- and 5-year BCSS were 0.37 (95% CI 0.286–0.545) and 0.295 (95% CI 0.226–0.418), respectively, while the integrated discrimination improvement (IDI) values for the same time intervals were 0.043 (95% CI 0.035–0.235, $P < 0.001$) and 0.163 (95% CI 0.025–0.197, $P < 0.001$). These findings were corroborated in both the internal and external validation sets. In the final step, we computed risk scores for each patient utilizing the developed nomogram and proceeded with risk stratification: low risk (points <90.4) and high risk (points ≥90.4). Kaplan–Meier survival curves exhibited pronounced discrimination between the low-risk and high-risk subgroups, underscoring the nomogram's ability to effectively stratify patients based on their prognosis.
Conclusions: In the context of treating patients with IMPC, the LNR has emerged as a robust prognostic indicator. Utilizing LNR as a key parameter, we developed a nomogram designed to offer a practical and dependable method for predicting BCSS in patients with IMPC. This innovative tool serves to facilitate the identification of patients at high risk of adverse outcomes, providing clinicians with valuable insights to guide decision-making in patient care.

P5-12-16: Impact of Metastatic Sites Including Brain and Liver in Overall Survival Among Patients With Breast Cancer

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Background: The development of Brain Metastasis (BM) among patients (pts) with Metastatic Breast Cancer (MBC) is generally considered to have a worse prognosis than metastasis at other sites. However, BM often develops later than metastasis at other sites. Hence, the perceived poor prognosis with BM may be confounded by prior treatments,

more advanced tumors and frailer pts. Here we compare median overall survival (mOS) of de novo MBC pts stratified by metastatic sites (brain, liver, both, neither) at the time of diagnosis.

Methods: The National Cancer Database (NCDB) was screened to identify pts with de novo MBC, aged 18 or older, with available brain and liver metastasis (LM) information. Pts were divided into 4 groups (grp) based on metastatic sites at diagnosis: 1. With BM without known LM 2. With LM without known BM 3. With BM and LM 4. Without known BM or LM. mOS among grp were assessed with Kaplan Meier method, compared with Log Rank test. Multivariate analyses were done using Cox proportional hazard model. In secondary analyses, mOS among these 4 grp were individually analyzed in 3 subsets: ER or PR (ER/PR) +, HER2- BC, HER2+ BC and triple negative BC (TNBC).

Results: 98503 pts from 2010 to 2019 were included in the analyses. Among grp 1 through 4 respectively: number of pts (%) included were 5227 (5.3), 21661 (22.0), 2420 (2.5), and 69195 (70.2); mean age (standard deviation) were 61.55 (12.60), 60.18 (14.39), 58.58 (12.76), 63.60 (14.01) years; % female >98% in each group; % of white pts were 76.7, 75.4, 74.9, 77.9; % of non-Hispanic pts were 89.6, 91.5, 90.9, 91.2; % of total Charlson Deyo score (for comorbidity) 0 were 80.2, 81.1, 81.0, 79.6; % of lung metastasis at diagnosis were 38.3, 32.9, 59.2, 27.2; % of ER+ tumors were 58.0, 60.6, 54.0, 75.0; % of HER2 + tumors were 21.7, 33.3, 34.2, 16.9. mOS in years (95% CI) for grp 1 through 4 were 1.05 (0.98-1.10), 1.68 (1.64-1.73), 0.61 (0.54-0.69), 3.14 (3.10-3.18); $p<0.001$. In multivariate analysis, grp 1, 2, 3 were associated with increased risk of death vs grp 4 [Hazard ratio (HR), 95% CI: 1.84 (1.78-1.90), 1.64 (1.61-1.67), 2.51 (2.39-2.62) respectively; $p<0.001$]. Secondary Analyses: 1. ER/PR+ HER2- BC: 54271 pts included, 2335 (4.3%), 8908 (16.4%), 884 (1.6%), 42143 (77.6%) in grp 1 through 4. mOS in years (95% CI) in grp 1 through 4: 1.51 (1.36-1.66), 1.91 (1.84-1.97), 0.87 (0.71-1.02), 3.58 (3.54-3.63). In multivariate analysis, grp 1, 2, 3 were associated with increased risk of death vs grp 4 [HR, 95% CI: 1.86 (1.77-1.95); 1.81 (1.76-1.86); 2.68 (2.49-2.89)]; $p<0.001$. 2. HER2+ BC: 20863 pts included, 1133 (5.4%), 7219 (34.6%), 828 (3.9%) and 11683 (55.9%) in grp 1 through 4. mOS in years (95% CI) in grp 1 through 4: 1.77 (1.49-1.94), 3.13 (3.0-3.3), 1.17 (0.96-1.37), 4.49 (4.32-4.64). In multivariate analysis, grp 1, 2, 3 were associated with increased risk of death vs grp 4 (HR, 95% CI: 2.0 (1.86-2.16); 1.46 (1.40-1.52); 2.47 (2.27-2.68)), $p<0.001$. 3. TNBC: 12221 pts included, 1034 (8.4%), 3035 (24.8%), 433 (3.5%), 7719 (63.1%) in grp 1 through 4. mOS in years (95% CI) in grp 1 through 4: 0.54 (0.49-0.59), 0.81 (0.76-0.86), 0.29 (0.24-0.35) and 1.21 (1.18-1.26). In multivariate analysis, grp 1, 2, 3 were associated with increased risk of death vs grp 4 (HR, 95% CI: 1.76 (1.64-1.89), 1.41 (1.34-1.48), 2.42 (2.18-2.69)), $p<0.001$.

Conclusion: Among de novo MBC pts in NCDB, those with BM and LV, or with BM without LM or with LM without BM, all had significantly worse OS compared to pts with neither BM or LM. The risk of death was highest for those with BM and LM, followed by those with BM without LM, then those with LM without BM. Similar results were seen in TNBC and HER2+ subsets. In ER/PR+ HER2- MBC also, having both LM and BM and having neither were

associated with the worst and best survival, however, the risk of death between pts with BM without LM vs LM without BM were not statistically different.

P5-12-17: Breast MRI and FDG-PET/CT for Evaluation of Pathologic Response to Neoadjuvant Chemotherapy in Patients With Breast Cancer

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Introduction: This study compared the diagnostic test accuracy of magnetic resonance imaging (MRI) with that of 18F-fluoro-2-glucose-positron emission tomography/ computed tomography (FDG-PET/CT) imaging in assessment of response to neoadjuvant chemotherapy (NAC) in breast cancer.

Methods: A total of 202 eligible patients diagnosed with stage II and stage III breast cancer were given neoadjuvant chemotherapy at Mersin University Faculty of Medicine Hospital from 2019 to 2023. When performing data statistics in a continuous structure, mean and standard deviation, median minimum and maximum values of the features; Frequency and percentage values were used when defining categorical variables. Breast MRI and FDG-PET/CT were evaluated to evaluate response to treatment. After surgery, pathological complete response (pCR) was determined based on the final pathology reports. Tumors were divided according to hormone receptor (HR) and human epidermal growth factor receptor (HER2). As the gold standard treatment response method, evaluation was made according to pathological response status. Diagnostic test statistics, sensitivity, specificity, positive predictive value, negative predictive value and accuracy statistics, 95% confidance interval values are given. The statistical significance level of the data was taken as $p < 0.05$. IBM SPSS 21 version was used to evaluate the data.

Results: The total age was minimum 30, maximum 75, mean age was 50.4±11.2 and median age was 50. At the time of diagnosis, the disease stage was stage 2 in 24.8% of the patients and stage 3 in 75.2%. Histology was Invasive ductal carcinoma in 65.3%, invasive lobular carcinoma in 13.5%, other in 21.2%. Minimum Ki-67 was 3, maximum was 95, average was 31.9±18.8, and median Ki-67 was 30. Hormon receptor status (estrogen or progesteron positivity) was evaluated. While ER (estrogen reseptor) was positive in 59.4% of the patients, PR (progesteron receptor) was positive in 52.5%. Human epidermal growth factor receptor 2 (HER2) positive was 38.1%. HR+HER2- was in 42.7%, HR+,HER2- in 21.1%, HR+,HER2+ was in 17.6%, and triple negative in 18.6%. Grade 1 was in 11.9% of the patients, grade 2 in 43.6%, and grade 3 in 44.6%.
When postoperative pathology reports was evaluated, pathological complete response was achieved in 69 of 202 patients(%34.1). When FDG-PET/CT was evaluated determining complete response; Sensitivity 90% (95% CI: 0.83-0.97, $p<0.05$), Specificity 51% (95% CI: 0.42-0.59, $p<0.05$), PPV value 48% (95% CI: 0.40-0.57 , $p>0.05$), and NPV was 91% (95% CI:

0.84-0.97, p<0.05), and the accuracy rate was low and significantly 64% (95% CI: 0.57-0.71, p<0, 05). As a result of these meaningful statistical calculations; FDG-PET/CT diagnostic method was not reliable in terms of specificity and positive predictive value.
When magnetic resonance was evaluated, in determining complete response; Sensitivity 72% (95% CI: 0.63-0.82, p<0.05), Specificity 90% (95% CI: 0.85-0.95, p<0.05), PPV(positive predictive value) 78% (95% CI: 0.68-0.88 , p<0.05), and NPV(negative predictive value was 86% (95% CI: 0.80-0.92, p<0.05), and the accuracy rate was high and significantly 85% (95% CI: 0.78-0.89, p<0, 05). As a result of these meaningful statistical calculations; Response to treatment status (complete response/non-response) MRI diagnostic method was reliable and can be used instead of the currently used Gold Standard pathology diagnostic method.

Conclusion: Our study showed that breast MRI is superior to PET CT in predicting pathological complete response. This study showed that MRI is very important in evaluating treatment response in patients diagnosed with breast cancer receiving neoadjuvant chemotherapy.
Keywords
Breast cancer, magnetic resonance imaging, positron emission tomography/ computed tomography, neoadjuvant chemotherapy, pathologic complete response

P5-12-18: Characteristics, treatments and outcomes of localized synchronous bilateral breast cancers in the CANTO French prospective cohort study

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Background: Synchronous bilateral breast cancer (sBBC) is a rare occurrence. There is currently no consensus on the optimal management strategy for this condition, particularly when there is discordance between the clinical or biological presentations. Some studies have reported that sBBC is associated with poorer outcomes compared to unilateral breast cancer. The objective of this study was to describe the incidence, clinical, pathological and biological presentations, treatments and outcomes of sBBC in the prospective CANTO cohort.

Methods: We reviewed and included patients (pts) diagnosed with sBBC in CANTO, a French prospective cohort study which enrolled 12012 women with localized invasive breast cancer. Data on clinical and pathological patterns, locoregional and systemic treatments were collected. Pts characteristics were compared with those of patients with unilateral cancers. Survival endpoints were event free survival (EFS) and overall survival (OS).

Results: Of the 11341 analyzable pts of the cohort, 259 had sBBC (2.3%). Median age was 60.4y, vs 56.4y in pts with unilateral BC (p<.0001). Of those, 30.9% (79 pts) had a first-degree family history of breast cancer, vs 22.2% in pts with unilateral BC (p=.001). However, the proportion of BRCA mutations was similar in both groups (7.8% out of 90 tested pts vs 10% out of 2592 tested pts, p=.48). Median body mass index was slightly higher in the sBBC pts (25.7 vs 24.8, p=.003).
Concordant bilateral stage I and stage II disease was observed in 32% and 13.7% of sBBC pts, respectively. Discordant stage I/II, II/III and I/III disease was observed in 36.3%, 7.8% and 5.5% of pts, respectively. Conversely, pathological subtypes were predominantly concordant: 75.6% of pts had bilateral carcinoma of non-specific type (NST) while 10.5% had bilateral invasive lobular carcinoma. Bilateral ER+ RP+/HER2-, HER2 overexpressed/amplified and triple negative subtypes were identified in 77.4%, 2.8% and 1.6% of pts, respectively. Molecular subtype was discordant in 18.2% of cases. Tumor grade was concordant in 66.9% of pts (grade 2 or 3: 58.4%), while Ki67 was ≤30% in 80.5% of pts.
Locoregional and systemic treatments were tailored to stage and pathological subtype of each side. Overall, 22.2% of pts underwent a bilateral mastectomy, 73.6% had bilateral radiotherapy and 64.7% received neoadjuvant and/or adjuvant chemotherapy.
After a median follow-up of 60 months, median EFS and OS were not reached: OS and EFS at 5y were approximately 90% and 80%, respectively, regardless of discordance in stage, molecular subtype, or grade. Furthermore, EFS and OS were identical whether patients had bilateral or unilateral BC.

Conclusions: Women with sBBC were more likely to be overweight and older, and to have a family history of breast cancer than pts with unilateral BC. However, there was no evidence of a higher frequency of genetic predisposition. Approximately half of sBBC pts had discordant stages at diagnosis, in contrast to the majority of cases, which exhibited similar pathological and biological presentations. It can be observed that the locoregional and systemic treatments were adapted to the stage and presentation of the disease. It is of note that the prognosis was not influenced by stage or pathological discordance, or bilaterality.

P5-12-19: Patient-reported outcomes from CIPHER study evaluating patient attitude about ctDNA testing in early-stage breast cancer

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Background; CIPHER study (NCT05333874), a pilot, single-institution study, previously reported that ctDNA dynamics during neoadjuvant therapy (NAT) can serve as an early indicator of treatment response and inform disease management in the adjuvant setting among patients with early-stage breast cancer (eBC). However, the perceived utility of ctDNA testing and the impact of ctDNA results on pts' anxiety about cancer recurrence are

not well known.

Methods: This study included 30 patients with stage II-III triple-negative (TN) and HER2+ eBC receiving neoadjuvant therapy, who underwent longitudinal ctDNA testing (SignateraTM, Natera, Inc.) during standard of care treatment. The primary aim of the study was to evaluate the role of ctDNA in treatment decisions in the adjuvant setting. Two optional patient surveys were administered by paper at 6 (n=24) and 12 (n=17) months (mos) post-surgery: (1) Fear of Cancer Recurrence (FCR-4), and (2) ctDNA utility questionnaire (perceived utility and impact of ctDNA test results on pts' anxiety about recurrence). The score ranges were: FCR-4: 0 (minimal) to 20 (maximum) fear of cancer recurrence; ctDNA utility: 5=all the time, 4=a lot, 3=sometimes, 2=a little, 1=not at all.

Results: A total of 168 responses for the FCR-4 questionnaire and 118 responses for the ctDNA utility questionnaire were received from 24 patients. The survey revealed a marginal numerical difference in the mean FCR-4 score at 6 mos vs 12 mos (12 vs 11.4). Regardless of the ctDNA result, 88% (21/24) of responders at 6 mos and 100% (17/17) at 12 mos said they valued the additional information received from ctDNA results, 91% (20/22) at 6 mos and 94% (15/16) at 12 mos said they would continue using the ctDNA test to monitor cancer, and 82% (18/22) at 6 mos and 88% (15/17) of pts at 12 mos reported feeling they were receiving the right treatment after receiving their ctDNA results. ctDNA results reduced anxiety about cancer recurrence in 32% (7/22) at 6 mos and 29% (5/17) of pts at 12 mos. Notably, a patient whose ctDNA+ result after surgery triggered a change in therapy reported (1) an anxiety score decrease from 20 to 14 while maintaining a high degree of reassurance in receiving correct treatment, and (2) the intention to use ctDNA testing in future.

Conclusions: In this study, most pts with eBC valued the information they received through the personalized, tumor-informed ctDNA test and would continue ctDNA testing. Monitoring ctDNA led to minimal to no change in average anxiety scores even after a positive result.

P5-12-20: Clinicopathological Correlates of Immunohistochemistry Subtypes in Ductal Carcinoma In Situ: A Retrospective Analysis

Je Hyun Chin, Doo Reh Kim, Young Joo Lee, Chang Ik Yoon, Woo Chan Park, Soo Youn Bae

Introduction: Ductal carcinoma in situ (DCIS), an early neoplastic lesion confined within the mammary ductal system, exhibits considerable molecular heterogeneity, reflected in distinct immunohistochemistry (IHC) subtypes such as hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status. It's well-established that while DCIS represents a non-obligate precursor to invasive breast cancer (BC), a subset of cases

harbors the potential for progression to invasive disease. Understanding the intricate molecular landscape of DCIS, particularly the interplay between IHC subtypes and the propensity for invasive progression, holds paramount importance in clinical decision-making. This study aims to investigate how IHC subtypes such as ER, PR, and HER2 in DCIS correlate with the tumor's clinicopathological characteristics.
Methods: We analyzed data from the Korean Breast Cancer Society registration database, focusing on patients aged 20 and older who underwent surgery for DCIS between 2000 and 2019. Patients with confirmed lymph node metastasis or distant metastasis were excluded from the analysis.
Results: A total of 11,812 patients were analyzed. Among them, 7,427 (62.9%) had HR+/HER2- breast cancer (BC), 611 (5.2%) had triple-negative breast cancer (TNBC), 1,904 (16.1%) had HR-/HER2+ BC, and 1,870 (15.8%) had HR+/HER2+ BC. At the time of diagnosis, the median age was higher in HR-BC compared to HR+ BC (HR+/HER2-: 48years, HR+/HER2+: 49years, TNBC: 53years, HR-/HER2+: 53 years). Family history was more prevalent in HR+ BC (10.5%) compared to HR-BC (8%). The proportion of asymptomatic cases was higher in HR+ BC (HR+/HER2-: 39.9%, HR+/HER2+: 45.1%) compared to HR-BC (TNBC: 34.2%, HR-/HER2+: 35.5%). Among HR-BC, the percentage of cases with a palpable lump was higher (TNBC: 30.3%, HR-/HER2+: 26.3%). Bilateral BC was more common in HR+ BC (0.5%). Tumor size (median) was larger in HER2+ BC (HR+/HER2-: 1.1 cm, TNBC: 1.2 cm, HR+/HER2+: 1.5 cm, HR-/HER2+: 1.9 cm). Mastectomy was more frequent in HER2+ BC (HR+HER2+ 40.4%, HR-/HER2+: 51.6%). Regarding nuclear grade, HR+/HER2- BC had the lowest proportion of high-grade tumors (12.5%), while HR-/HER2+ BC had the highest (73.6%).
Conclusion: This study reveals the diverse clinicopathological features of DCIS based on IHC subtypes, emphasizing the importance of molecular characterization in guiding clinical management decisions. Understanding these distinct profiles can enhance prognostication and facilitate tailored treatment approaches for patients with DCIS.

P5-12-21: The utility of antiestrogen therapy in early-stage, pure mucinous carcinomas of the breast

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Background: Pure mucinous breast tumors are rare and characterized by cluster-like hyperplasia of tumor cells floating in extracellular mucous fluid, comprising 90% of the tumor mass. Despite their generally favorable prognosis, optimal management, particularly regarding antiestrogen therapy, in small (less than or equal to 3 cm), node-negative breast cancers, remains uncertain.
Methods: We conducted a retrospective review of 77 women treated between 2010 to 2018 and diagnosed with node-negative, pure mucinous breast tumors less than or equal to 3 cm, with a follow-up period of at least 5 years. Factors including age, type of surgery, margin status, adjuvant therapies, and treatment outcomes were analyzed. Patients with non-pure mucinous histology were excluded.

Results: Of the 163 patients identified, 86 were excluded due to non-pure mucinous histology, nodal involvement, tumors >3 cm, or less than 5 years of follow-up. The median age at diagnosis was 70 years (range 40-88), with a median follow-up of 93 months (range 60-158). Average tumor size was 1.42 cm (range 0.10-3.0 cm). Surgical management included lumpectomy alone in 4 patients (5.2%), lumpectomy with lymph node evaluation in 53 patients (68.8%), and mastectomy with or without lymph node evaluation in 20 patients (26.0%). Adjuvant treatments included radiation in 38 patients (49.4%) and endocrine therapy with aromatase inhibitor or tamoxifen in 45 patients (58.5%). Of these, 2 patients received chemotherapy followed by endocrine therapy (2.6%). 36 patients (46.8%) completed at least 5 years of endocrine therapy. Only 3 (3.9%) local recurrences were seen and no distance recurrences. Of the patients with a local recurrence, none of them completed 5 years of antiestrogen therapy following breast conservation and 2 received radiation. The average time to recurrence was 69 months (range 30-120). Overall survival was 88.3%, and breast cancer-specific survival was 100%.
Conclusions: Our findings call attention to reconsidering the necessity of antiestrogen therapy for pure mucinous breast tumors less than or equal to 3 cm with node-negative status, given the very low local recurrence rate observed overall. Despite fewer than half of patients completing a 5-year course of antiestrogen therapy, no distant recurrences or breast cancer related deaths were observed with a median follow-up exceeding 7 years, suggesting a limited benefit with antiestrogen therapy. These results underscore the favorable prognosis of mucinous carcinomas, supporting a more personalized approach for adjuvant endocrine therapy recommendations, such as considering patient comorbidities and life expectancy. Further research with larger cohorts and extended follow-up would be beneficial to further refine treatment guidelines for early stage mucinous breast carcinomas.
Keywords: Mucinous breast cancer, antiestrogen therapy, node-negative breast cancer, adjuvant treatment, early-stage breast cancer

P5-12-22: Semi-Quantitative Stratification of Breast Cancer Biomarkers using the APIS Breast Cancer Subtyping Kit

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Background: Precise evaluation of breast cancer biomarkers is crucial for determining appropriate patient management strategies. Histopathologists typically use semi-quantitative scoring systems to translate subjective immunohistochemistry (IHC) observations into objective quantitative data, enhancing the categorization of marker status. The APIS Breast Cancer Subtyping Kit, an RT-qPCR based diagnostic tool, measures the relative expression of mRNA target genes (ESR1, PGR, ERBB2, and MKI67) from invasive breast cancer tissue, providing both positive/negative results for each biomarker and a molecular classification. This study introduces additional RNA expression cut-off values to demonstrate that a semi-quantitative approach can further stratify target expression using

the APIS kit.

Methods: Formalin-fixed paraffin-embedded (FFPE) breast cancer samples (N=368), obtained via core needle biopsy or resection, were analyzed. IHC scores were correlated with copy numbers, determined by a validated digital PCR (dPCR) assay, to establish cut-off values corresponding to IHC classifications. The dynamic ranges of the normalized RNA copy numbers for each target were also evaluated. These copy number cut-offs were then used to assess the corresponding ΔCt values (RNA expression results) from the APIS kit. This allowed for the expression of each target to be classified into 3-4 semi-quantitative ranges.

Results: The semi-quantitative approach successfully classified the expression levels of all targets. A broad dynamic range of normalized RNA copy numbers was observed for ESR1, PGR, and ERBB2 (0-767, 0-307, 0-422 RNA copies, respectively), whereas MKI67 showed a narrower range (0-1.8 RNA copies). Central IHC categories exhibited overlapping ΔCt values for all targets, likely due to tumor heterogeneity and the different measurement techniques of IHC and RT-qPCR. Despite these variances, the established cut-off values defined a semi-quantitative scale for each target, facilitating refined classification of biomarker expression into high positive, moderate, low positive, and negative categories. For HER2/ERBB2 expression, the categories were high positive, low positive (HER2-low) and negative.

Conclusions: This study established a ΔCt semi-quantitative scale for evaluating targets with the APIS Breast Cancer Subtyping Kit, by leveraging the dynamic range of RNA expression. This is particularly significant for classifying HER-low, which has emerged as crucial for identifying patients who may benefit from novel anti-HER2 therapies.

P5-12-23: Effectiveness of Online Education in Improving Clinicians' Knowledge and Confidence in Managing Adverse Events Associated with Novel Antibody-Drug Conjugate Therapy for Breast Cancer

Zhizhi Fiske, Stephen Dunn, Eloise Ballard, Jamie Habib, Rebecca Dent, Giuseppe Curigliano

Background: Antibody-drug conjugates (ADCs) have significantly improved treatment outcomes for breast cancer, and optimal adverse event management is key to maintaining a good quality of life for patients. The objective of this study was to assess the effect of an online continuing medical education (CME) activity on clinicians' knowledge in the different safety profiles of ADCs used for the treatment of breast cancer and their confidence in implement appropriate strategies in clinical practice to monitor and manage adverse events associated with novel ADCs.
Methods: This CME activity consisted of a 15-minute video discussion between 2 expert faculty with synchronised slides. Educational effect was assessed using a repeated-pair

design with pre-/post-assessment. 3 multiple choice questions assessed knowledge, and 1 question rated on a Likert-type scale assessed confidence, with each individual serving as their own control. A McNemar's test assessed significance of improvement in the percentage of correct responses to knowledge questions from pre- to post-assessment. P values < .05 are statistically significant. The activity launched on 27th of June, 2023, with data collected through 5th September, 2023 being reported in the current study.
Results: 64 oncologists and 33 obstetricians/gynaecologists who answered all the assessment questions were included in this analysis. Analysis of pre- vs post-intervention responses demonstrated a significant improvement in overall knowledge of both physician learner groups, with 56% more oncologists answering all questions correctly after education (64% post- vs 41% pre-CME) and 100% more obstetricians/gynaecologists answering all questions correctly after education (36% post- vs 18% pre-CME). Specifically, the knowledge regarding the different safety profiles of ADCs used for the treatment of breast cancer increased from 76% pre- to 85% post-CME for oncologists (P<.001) and from 55% pre- to 69% post-CME (P<.01) for obstetricians/gynaecologists, respectively. Additionally, 27% of oncologists and 27% of obstetricians/gynaecologists reported increased confidence in effectively managing adverse events associated with novel ADC therapy for breast cancer, and that increase was, on average, 57% and 100% among the two physician groups, respectively.

Conclusions: This analysis demonstrates the positive educational impact of an online CME activity on the essential aspects of adverse event management of novel ADC therapy for breast cancer among key physician learner groups, including in areas where clinicians' baseline knowledge levels are already relatively high. As ADCs become more widely used in clinical practice, it will be important to educate clinicians on appropriate approaches to monitoring and managing treatment-related adverse events for individual patients and provide guidance to help facilitate best practices of interdisciplinary working.

P5-12-24: Outcomes of Metastatic Breast Cancer Patients Initiated on Inpatient Chemotherapy Due to Visceral Crisis

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Background: Historically, oncological patients received chemotherapy while hospitalized to closely monitor treatment-related adverse events. Currently, outpatient administration is standard for metastatic breast cancer (mBC) patients, with hospitalization typically reserved for acute complications. While inpatient chemotherapy/biotherapy (IC) remains a selectively used treatment option for admitted patient with visceral crisis, guidelines for selecting appropriate patients for this therapy are lacking. This study aims to analyze the characteristics and outcomes of mBC patients who were admitted with visceral crisis and received IC. The results will be used to inform early guideline development.

Methods: In an observational, single-institution, retrospective descriptive study using data from the prospectively collected MD Anderson Breast Cancer Management database, we identified patients with mBC who received Cycle 1 Day 1 (C1D1) of a systemic therapy regimen while inpatient between 2016-2023. The primary objective was to assess the relationship between variables including breast cancer subtypes, age, systemic therapy regimen, and overall survival (OS) defined as the time to death or to hospice enrollment. Tumor characteristics included histology type and receptor status (estrogen receptor (ER), progesterone receptor (PR), and HER2). The median overall survival was calculated by checking the median time between the initiation of C1D1 to death or to enrollment into hospice care. For the secondary objective, the Cox regression models were utilized to examine the association between these variables and OS.
Results: We identified 76 female mBC patients, with a median age of 52 years, and a relatively equal distribution among the three subtypes. The median OS was 3.28 months for all patients. Patients with HER2+ subtypes had a lower risk of death or hospice enrollment compared to other subtypes. HER2+ mBC patients had better outcomes and longer median OS (18.99 months) compared to TNBC (2.52 months) and HR+ (2.73 months). Multivariate analysis showed that a higher baseline INR was independently associated with worse OS, while age at the time of IC administration, breast cancer subtype, systemic therapy regimen, and number of prior lines of systemic therapy did not influence OS.
Conclusion: Our study highlighted the overall poor outcomes of mBC patients who receive IC. However, the data suggest that IC might be most appropriate for selected patients with HER2+ mBC. These results should be validated in prospective and/or large multi-center retrospective data analyses.

P5-12-25: Single-center retrospective cohort study evaluating neutropenia and growth factor use with sacituzumab govitecan in patients with HR+/HER2- and triple negative metastatic breast cancer

Samantha Fisch, Joshua Chin, Laura Quintal, Melanie Majure, Michelle Melisko, Jo Chien, Hope S. Rugo, Laura A. Huppert

Introduction: Sacituzumab govitecan (SG) is FDA-approved for the treatment of both metastatic triple negative breast cancer (mTNBC) and hormone receptor positive (HR+)/HER2- metastatic breast cancer (MBC). In phase III clinical trials, SG caused grade 3 neutropenia in ~50% of patients. However, the real-world incidence of SG-induced neutropenia and the practice patterns regarding the use of adjuvant growth factor use are not well characterized.

Methods: In this single retrospective cohort study, we identified patients with HR+/HER2- or TNBC MBC who received SG between 2020-2024 per standard of care. We used manual review of electronic health records to identify key clinical characteristics, treatment history, safety parameters, and documented use of growth factor support via granulocyte colony stimulating factor (GCSF, either filgrastim or pegfilgrastim) while on treatment with SG.

Results: We identified 74 patients with MBC who were treated with SG, including 45 patients with mTNBC (60.8%), 27 patients with HR+/HER2- MBC (36.5%), and 2 patients with heterogenous expression who were categorized as HR+/HER2+ MBC (2.7%). Median age was 56.5 years (range 28.4 - 81.1 years). Patients with mTNBC received a median of 2 prior lines of chemotherapy (range 0-5) and patients with HR+/HER2- disease received a median of 8 lines of prior therapy including 4 prior lines of chemotherapy (range 2-14 total lines, 0-8 lines of chemotherapy). Median time on SG was 4.4 months (range 0.26-39.8 months) for patients with mTNBC and 1.9 months (range 0.26-15.6 months) for patients with HR+/HER2- MBC. Most patients experienced any grade neutropenia while on SG (n=60, 81.1%), including most patients with mTNBC (n=37, 82.2%) and HR+/HER2- MBC (n=21, 77.8%). Grade 3 neutropenia was common during SG (n=39, 52.7%), with similar rates among patients with mTNBC (n=25, 55.6%) and HR+/HER2- MBC (n=12, 44.4%). Rates of neutropenic fever were low: in total 5/74 (6.8%), of whom 4/45 (8.9%) were mTNBC. A total of 8/74 (10.8%) patients were hospitalized for SG-related neutropenia with median length of stay 3.5 (range 1-10) days. Dose delays for any reason occurred in patients with mTNBC (n=18, 40.0%) and HR+/HER2- MBC (n=8, 29.6%), about half of which were due to neutropenia (for mTNBC n=7, 15.6%, HR+/HER2- MBC n=4, 14.8%). Dose reductions for any reason were also common among patients with mTNBC (n=21, 46.7%) and HR+/HER2- MBC (n=18, 66.7%), including some due to neutropenia (for mTNBC n=7, 15.6%, for HR+/HER2- n=4, 14.8%). Most patients discontinued SG due to disease progression for both mTNBC (n=43, 95.6%) and HR+/HER2- MBC (n=24, 88.9%). Two patients with HR+/HER2- MBC (7.4%) discontinued therapy due to toxicity. The remaining patients are still on therapy or discontinued for unrelated reasons. Most patients received GCSF during treatment with SG (n=64, 86.5%), most of whom received filgrastim (n=62, 83.8%) and a minority of whom received peg-filgrastim (n=7, 9.5%). In total 34/74 (45.9%) patients received primary GCSF prophylaxis and 24/74 (32.4%) patients received secondary GCSF prophylaxis. Rates of primary and secondary prophylaxis varied by subtype for mTNBC and HR+/HER2- respectively (primary: 37.8% and 55.6%; secondary: 40.0% and 22.2% respectively).

Conclusion: In this single center retrospective study, >80% patients with mTNBC and HR+/HER2- MBC on SG experienced any grade neutropenia. Similar to published trials, nearly half of patients in both subgroups experienced grade 3 neutropenia but rates of hospitalizations and neutropenic fever were <10% in this heavily pre-treated, real-world cohort. Rates of SG dose reduction occurred in ~50% of patients, but rates of discontinuation due to toxicity were low. GCSF prophylaxis, either primary or secondary, was used in ~80% of patients. Additional studies are needed to clarify which patients may be at highest risk for SG-induced neutropenia and the optimal growth factor regimens to improve outcomes.\

P5-12-26: The Reuse of Cyproheptadine, a First-Generation Antihistamine, as an N-WASP Inhibitor in Breast Cancer Metastasis

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Background: Cyproheptadine is a first-generation antihistamine primarily used to alleviate allergy symptoms, with a long clinical use history and a well-established safety profile. Recently, the concept of drug repurposing has gained traction, and Cyproheptadine has shown potential applications in cancer treatment. Breast cancer metastasis continues to be a major clinical hurdle, making the exploration of new therapeutic strategies imperative for enhancing the survival rates of breast cancer patients. N-WASP is a protein crucial for cytoskeletal reorganization, facilitating cell motility and invasion, both of which are essential processes in cancer metastasis. We hypothesis that it may be possible to improve patient outcomes by blocking the pathways through which cancer cells metastasise by inhibiting N-WASP.
Methods: To investigate the effect of Cyproheptadine on cell proliferation, adhesion, motility, and its specific impact on N-WASP protein expression at both mRNA and protein levels as well as downstream events, two breast cancer cell lines, MDA-MB-231 and MCF-7, and HECV, an endothelial cell line, were subjected to comprehensive in vitro biological assays (including cytotoxicity tests, cell growth assay, Electric Cell-substrate Impedance Sensing (ECIS), wound scratch assay, immunofluorescence, qPCR and western blotting). Molecular docking was carried out by computer-assisted modelling to model the interaction between N-WASP and Cyproheptadine at the atomic level.
Results: Cyproheptadine demonstrated a significant inhibitory effect on cell proliferation in all three cell lines tested. In MDA-MB-231 cells treated with the drug, proliferation decreased by 15.72% ($P=0.0007$), while in MCF-7 cells, it decreased by 43.18% ($P<0.0001$), and in HECV cells, it reduced by 33.51% ($P<0.0001$). Furthermore, Cyproheptadine markedly impaired cell adhesion and migration in the ECIS assay. Wound healing assay revealed a 16.68% reduction in migration rate in MDA-MB-231 cells ($P=0.0002$), 35.89% reduction in MCF-7 cells ($P <0.0001$) and 27.32% reduction in HECV cell lines ($P= 0.0070$) indicating diminished motility.
Cyproheptadine treatment led to a significant downregulation of N-WASP protein expression confirmed using immunofluorescence assays and Western blot analysis. Additionally, qPCR results demonstrated that Cyproheptadine was not able to regulate N-WASP mRNA expression, suggesting that the mechanism of action likely occurs at the post-transcriptional, translational or posttranslational level. Molecular docking revealed that Cyproheptadine had a binding profile with multiple amino acids of key domains of N-WASP protein. These results indicate that Cyproheptadine effectively impairs key cellular functions associated with cancer metastasis, including proliferation, adhesion, and migration, through the downregulation of N-WASP protein expression, but not at the transcription level.
Conclusion: These results propose a new therapeutic application for Cyproheptadine, beyond its conventional use as an antihistamine. Cyproheptadine shows significant promise in inhibiting N-WASP protein expression, thereby disrupting critical processes necessary for

breast cancer metastasis. This could potentially offer a dual-purpose treatment strategy for managing metastatic breast cancer.

P5-12-27: HER2 Status and Clinical Outcomes in Breast Cancer: A Retrospective Analysis

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Background: HER2 status is a prognostic marker in breast cancer (BC). Studies have demonstrated that patients with high HER2 expression have poor prognosis compared to HER2 negative (HER2-neg) disease.(1,2) Currently, BC is classified into categories based on HER2 expression and hormone receptor status (estrogen receptor (ER) and progesterone receptor (PR)). Previously, HER2 status was defined as HER2-neg or HER2- high; however, in 2022, the FDA approved fam-trastuzumab deruxtecan-nxki in the management of metastatic HER2-low BC.3 Though publications from large international academic institutions have studied HER2-low BC, there is a need to understand the biology and characteristics of HER2-low BC patients in the United States in a community cancer setting.(4,5,6) 85% of cancer patients in the US receive oncologic care at a community cancer center.7

Methods: In this retrospective study of metastatic BC patients, the prevalence, histology, and clinical outcomes were analyzed based on HER2 status in a community cancer center in the United States. 2,637 patients were diagnosed with BC between 2010-2020, and 2,470 were excluded due to their early stage diagnoses, male sex, or indeterminate HER2 status. Patients with stage IV BC as defined by the American Joint Committee on Cancer criteria were included. Patients were classified as HER2-high, low, or negative based on IHC scores and FISH. HER2-high is defined as IHC Of 3+ or 2+ with FISH amplification, HER2-low is IHC 1+ or 2+ and FISH negative whereas HER2-neg is IHC 0. Demographic, histologic, and outcome data was abstracted. Z-test of proportions were performed to compare the groups.

Results: 167 patients were studied, 21.6% being HER2-high, 40.7% HER2-low, and 37.7% HER2-neg. The median age at diagnosis of stage IV BC for HER2-high was 65, HER-low was 66.5, and HER2-neg was 70. No statistically significant difference was observed between ductal or lobular histology. Hormone receptor positive (HR+) disease was more prevalent among all HER2 groups than hormone receptor negative (HR-). 86% of the HER2-neg patients were HR+ compared to 58% HER2-high ($P<0.01$). Additionally, 84% of HER2-low patients were HR+ compared to 58% HER2-high ($P<0.01$). HR- disease was more prevalent in HER2-high patients at 41.6% compared to 16.1% in HER2-low and 14.2% in HER2-neg patients ($P<0.01$). 89.4% of HER2-high patients had a high Ki-67 proliferation rate (above 20%) compared to 64.1% of HER2-low ($P<0.05$) and 67.6% of HER2-neg ($P=0.08$). Brain metastatic disease was more common in HER2-high (11.1%) compared to HER2-low (8.8%) and HER2-neg (6.3%). Bone metastatic disease was more commonly seen in HER2-low

(57.4%) and HER2-neg (57.1%) compared to HER2 high disease (47.2%). The median time from initial BC diagnosis to development of stage IV was 5.9 months in HER2-neg, 14 months in HER2-low, and 13.3 months in HER2-high patients. Of the HER2-neg patients, those with triple negative BC defined as HR- and HER2-neg had a median time of 2.1 months from diagnosis to developing stage IV disease. Majority of patients were treated with chemotherapy, and HER2 targeted treatment was only given to HER2-high patients. 68 patients were lost to long term follow-up; however, of the patients evaluable, the median overall survival (mOS) in HER2-high patients was 24 months, HER2-low was 36.4 months, HER2-neg was 33 months, and the triple negative subgroup was 20.2 months.

Conclusion: Stage IV BC accounts for 5% of all BC. HER2-high patients presented with high Ki-67 rates and had the lowest mOS. HER2-low patients had the highest mOS and the longest median time between initial BC diagnosis and development of stage IV disease. Future studies with larger cohorts of ethnically diverse, metastatic BC patients treated in community cancer centers should be pursued evaluating the clinical and cost-effectiveness of HER2 targeted therapy in HER2-low BC patients.

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P5-12-28: Single Institutional Experience with Metaplastic Breast Cancer Comparing Outcomes based on Timing of Chemotherapy, Chemotherapy Regime and Subtypes of Metaplastic Breast Cancer.

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Background: Metaplastic breast cancer (BC) is a rare, aggressive form of breast cancer that generally does not respond well to standard chemotherapy. Optimal treatment for patients presenting with metaplastic breast cancer is yet to be defined. Here we review our institutional experience and compare outcomes based on timing of chemotherapy, chemotherapy regimen used and subtypes of metaplastic BC.
Methods: We conducted a retrospective analysis of patients diagnosed with metaplastic BC at our institution between 2002 and 2023. Patients were analyzed based on receipt of chemotherapy and timing of chemotherapy administration. For patients receiving neoadjuvant chemotherapy (NAC), pathologic complete response rates (pCR) based on chemotherapy regimen were determined. Additionally, we compared outcomes based on subtype of metaplastic BC. 3-year overall survival (OS) and 3-year progression free survival (PFS) were estimated using Kaplan Meier method.
Results: Of the 31,292 patients treated with breast cancer at our institution, there were 60 patients with metaplastic BC. Patients were excluded if they presented with metastatic disease or if they did not undergo active treatment, which left 52 patients for this analysis. The median age at diagnosis was 64.9 (range=32-93.1). All patients were female. 63.5% self-identified as White, 32.7% as Black, and 3.8% as other. Poorly differentiated tumors comprised 84.6% of the total. TNBC phenotype made up 80.7%, while 7.7% of patients had HER2+ disease and 11.5% were ER+. One patient had both ER+ and HER2+ disease. The median pathologic tumor size was 2.8 cm (range=0-15.5) and 11.5% were clinically node positive at presentation. Metaplastic subtypes included 19 squamous (37%), 15 matrix-producing (29%), 9 spindle cell (17%), 6 mixed (12%) and 3 were not further classified (6%). Chemotherapy choice was at the providers discretion.
Overall, 45 patients (86.5%) received chemotherapy, with 21 (40.3%) receiving adjuvant chemotherapy and 24 (46.1%) receiving NAC. Of those receiving NAC, 3 (12.5%) achieved a pCR. 6 (25%) patients received a neoadjuvant regimen containing pembrolizumab, one of which achieved pCR. There was no difference in pCR rates among patients receiving a pembrolizumab containing regimen vs those who did not, 16.7% vs 11.1% (p=1), though the numbers were small. NAC receipt was associated with higher clinical stage; 41.6% of patients receiving NAC had clinically Stage IIA and 20.8% were clinically Stage IIB. For those

receiving NAC the 3-year OS was 66% and the 3-year PFS was 51%. This was significantly worse than those that received adjuvant chemotherapy (3-year OS was 84%, 3-year PFS was 77%) or no chemotherapy at all (3-yr OS 83%, 3 year-PFS 67%), though these patients had earlier stage disease (the majority were clinical stage IA in both groups). 93% of matrix producing tumors were TNBC. 83% of spindle cell subtype were TNBC and had the best 3-year OS (86%) and PFS (88%). Squamous histology tumors were the most diverse with 63% TNBC, 26% ER+, and 11% HER2+. The 3-year PFS for mixed histology (33%) was significantly worse than for spindle cell subtype (88%) (p=0.02).
Conclusions: This study provides valuable insights into the treatment outcomes and subtype-specific characteristics of metaplastic BC. Despite the small sample size, notable correlations between intrinsic breast cancer phenotypes and metaplastic subtypes emerged, with the spindle cell subtype exhibiting the highest 3-year OS and PFS while the mixed phenotype had the lowest PFS and OS. This stands in contrast to other similar series previously published, with many reporting spindle cell as having worse overall outcomes. Although neoadjuvant chemotherapy, including regimens with pembrolizumab, showed limited pCR rates, these findings underscore the need for more extensive research to define optimal therapeutic strategies for metaplastic BC.

P5-12-29: Treatment outcomes of patients with de novo oligometastatic breast cancer treated with “curative intent” at a single institution

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Background: De novo oligometastatic breast cancer (OMBC) is often defined as limited disease with up to five distant lesions in two or fewer organs at initial presentation. Studies have suggested overall favorable outcomes for OMBC patients, making curative intent treatment appealing. Trials have attempted to address primary surgery in all de novo MBC (not OMBC) and, separately, ablative metastasis-directed therapy in OMBC (not all de novo) without survival benefits. However, no trial has addressed the cumulative benefits of removing the primary tumor, ablating all detectable distant metastatic lesions, and optimal systemic therapy with molecular subtype-specific multidrug systemic therapy in de novo OMBC. Management of de novo OMBC remains controversial. We analyzed outcomes of patients with de novo OMBC treated with curative intent.
Methods: We analyzed data for patients with de novo OMBC who were treated with physician-expressed curative intent at a single institution between 1/2000 – 12/2020. We identified patients by performing a simple keyword search within institutional electronic medical records that included physician-entered terms “oligometastases” AND “breast cancer” AND “curative intent” or “curative hope” and had at least one visit in clinic. Term variants such as “oligometastatic” for “oligometastases” were also included. All resulting charts were then manually reviewed for inclusion criteria. We more strictly defined OMBC as one organ (including bone) involved with metastatic lesions and 4 or fewer lesions per

metastasized organ. All patients were required to have had definitive breast surgery for inclusion into the analytic cohort. Recurrence-free survival (RFS) and overall survival (OS), beginning at time of surgery, were evaluated using Kaplan-Meier methods.
Results: A total of 39 patients were identified: 12 hormone-receptor (HR) positive (+)/HER2+, 2 HR negative (-)/HER2-, 21 HR+/HER2-, 4 HR-/HER2+. Most patients (33, 84.6%) had 1 oligometastatic lesion at diagnosis, 3 (7.7%) had 2 lesions, 2 (5.1%) had 3 lesions, and 1 (2.6%) had 4 lesions. 100% of patients underwent breast surgery; 15 (38.5%) underwent lumpectomy and 24 (61.5%) underwent mastectomy. Median age at time of surgery was 47 (range 28-69). Most patients (29, 74.4%) also underwent radiation therapy (RT) to the breast. Two-thirds of patients (26, 66.7%) underwent metastasis-directed therapy (MDT). The most common MDT type was RT (22/29, 75.9%). With median follow-up of 5.8 years (y), 27/39 (69%) patients had not recurred. Of 12 recurrences, 100% were new distant metastases. Median RFS was 7.1y (95% CI 4.87-not reached). Five-year RFS was 62% (95% CI 46-84%). Median RFS was 7.1y (95% CI 2.01-not reached) among HER2+ patients and 6.98y (95% CI 4.62-not reached) among HR+/HER2- patients. Landmark RFS was not impacted by whether a patient received MDT. Median OS was not reached (95% CI 7.42-not reached) and five-year OS was 77% (95% CI 61-95%).
Conclusions: The use of curative intent treatment in OMBC at a single institution was most common in patients with a single metastasis in one organ. Survival outcomes were favorable among this highly select cohort of patients, with nearly two-thirds of patients free of relapse at 5y and similar RFS among the HER2+ and HR+/HER2- cohorts. Validation of these findings in prospective series and further studies evaluating optimal treatment strategies in this setting are needed. The benefits of primary surgery and MDT in this population remain unknown.

P5-12-30: Characterizing Metastatic Breast Cancer (MBC) Exceptional Responders

Ahmed Mohamed, Hadil Zureigat, Beatriz Sorato, Jame Abraham, Shimoli Barot

Background: While the 5-year survival for MBC remains dismal at 29%, certain patients with a significant and/or long-lasting treatment response are known as exceptional responders. They are characterized by either having a partial or complete response (PR/CR) to treatment that is otherwise effective in <10% of similar-stage patients orexhibiting an unusually sustained response, at least 3x the median response.

Aim: We identified clinical, pathological, genetic, and genomic characteristics of MBC exceptional responders.

Methods: We included individuals ≥18 years old diagnosed with MBC by pathology and treated at Cleveland Clinic Taussig Cancer Institute from 2000-2023. The 3-year mark was used to define an exceptional treatment response. We obtained demographics, medical history, and details of BC diagnosis and treatment using electronic medical records. Genetic

results were obtained using the common hereditary cancer gene panel while comprehensive genomic profiling provided the genomic data.

Results: 27 exceptional responders were identified, all female with a median age at diagnosis of 53 years (range 46-61). 23/27 (85.2%) were White, 3 were Black, and 1 was Hispanic. 20/27 (74.1%) had never smoked, while the remainder were former smokers. 40.7% (11/27) of the exceptional responders had a previous history of early-stage BC. Most exceptional responders (18/27, 66.7%) had ER-positive/HER2-negative disease while 6/27 (22.2%) had HER2-positive and 3/27 (11.1%) had triple-negative MBC. Metastatic sites included bone (16/27, 59.3%), distant lymph nodes (16/27, 59.3%), lung (13/27, 48.1%), liver (10/27, 37%), pleura (5/27, 18.5%), and brain (3/27, 11.1%). Throughout their treatment course, 32 instances of exceptional response to various treatments were observed, including: aromatase inhibitors (AI, 11/32, 34.3%), CDK4/6 inhibitor+AI (5/32, 15%), capecitabine (3/32, 9%), T-DM1 (2/32, 6%), CDK4/6 inhibitor+fulvestrant (2/32, 6%), fulvestrant (2/32, 6%), trastuzumab+AI (2/32, 6%), trastuzumab (2/32, 6%), trastuzumab+pertuzumab (1/32, 3%), capecitabine+tucatinib (1/32, 3%), and capecitabine+neratinib (1/32, 3%). The best response to treatment among exceptional response instances was CR in 3/32 cases (9.3%), and PR in 29/32 cases (90.6%). The average duration of exceptional response was 4 years (range 3-6). 2/27 patients (7.4%) had a germline pathogenic BRCA2 mutation, and 1 had a BRCA1 mutation. Additionally, 11/27 (40.7%) had other germline genetic mutations, including CDKN2A p.16INK4a, MSH3 c.2254-19T>G, MSH6 c.3740C>G p.Thr1247Ser, and MSH2 c.874A>T. Genomic abnormalities were identified in 9/27 (33.3%) patients, including MAGI2 N477fs* 1+, MAP2K4 loss exons1-7, ESR1 Y537S, ERBB2 p.L755_E757delinsS, GATA3 P409fs, MSH6 c.3740C>G p. Thr1247Ser, MTOR C1483Y, ROS1 amplification, NF1 S1470N, FGFR1 14981, GNAS R201C, MYC amplification, PIK3CA E545K, PTCH1 c.3025G>A p.G1009R, BRCA2 D2084Y, PARK2 duplication exon 2-4, PTEN Y27N, FBXW7 S203L, RICTOR amplification, TP53 L111M0, NTRK1 V588V, MTOR D371H, DDR2 L623F, FBXW7 S203L, ATM G558A, PRDM1, WISP3, TP53 E51*, TP53 loss S367fs* 1+, MTOR L943V, ATM L3026H, and NTRK1 A457S.

Conclusions: MBC exceptional responders were mostly White females with a median age of 53 years, never smokers and had a history of early-stage disease prior to metastases. Endocrine therapy, CDK4/6 inhibitors, HER2 targeted treatments and capecitabine combinations predominantly led to exceptional responses. A significant portion of patients had germline mutations and genomic abnormalities. Our findings can provide insight on potential shared factors influencing exceptional responses in MBC, guiding future research and personalized treatments.

PS4-09: Investigating the Impact of a Culturally Tailored Education Intervention Cancer Clinical Trial Participation

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Background: Cancer clinical trials (CCT) advance knowledge in cancer care. Access to CCT may be facilitated by patient knowledge. We evaluated the impact of a culturally tailored CCT educational video intervention via a pre- and post-test survey on CCT interest and knowledge dissemination.

Methods: A CCT education intervention was developed with community engagement from persons who identified as Black, Hispanic, or Chinese, the predominant background of persons in our immediate catchment area. The video educational intervention was available in Spanish and Chinese. Community input was to make the survey available prior to the diagnosis of cancer; therefore, participants included a general population (GP), persons who did not have a cancer diagnosis. Pre and post knowledge assessments were utilized to evaluate knowledge (11 questions) and intention-to-share information/participate in a CCT (6 questions) using a Fisher-exact test. Data were analyzed after stratifying by race.

Results: The sample size included: 148 (70%) men, 58 (28%) women, 2 (1%) transgender, 2 (1%) non-binary; white, non-Latinx 125 (60%), Black 60 (29%), Asian 2 (1%), Latinx 52 (25%). Significant findings included, for Black participants, the score for the question "Once I sign a consent form, I must stay in the clinical trial until my doctor tells me I'm done" improved significantly between pre- and post-intervention ($p=0.0104$). For white, non-Latinx participants, CCT knowledge improved for the following questions "Once I sign a consent form, I must stay in the clinical trial until my doctor tells me I'm done." ($p = 0.0181$), "Clinical trials of new medicines or treatments are carefully reviewed for safety by a group of research experts and community members." ($p = 0.0314$) and "You can only join a clinical trial after you have tried all other options." ($p = 0.0316$). The intention to gain more information about CT from their physicians and discussing CT with family ($p<0.05$) improved significantly in all groups.

Conclusion: A tailored educational intervention significantly knowledge based questions and impacted the intention to learn more about CCT and to share the CCT information learned.

PS10-01: Racial differences in the prevalence of biomarker alterations, treatment patterns, and clinical outcomes in hormone receptor–positive, human epidermal growth factor receptor 2–negative metastatic breast cancer: A national cohort study

Pegah Farrokhi

Background: Despite advancements in targeted therapies for hormone receptor–positive (HR+), human epidermal growth factor receptor 2–negative (HER2−) metastatic breast cancer (mBC), data assessing racial differences in biomarker prevalence and outcomes are lacking. This study assessed the prevalence of biomarker alterations, treatment patterns, and clinical outcomes in Black and White patients with HR+, HER2− mBC in the United

States (US) who received next-generation sequencing (NGS)-based testing.
Methods: This observational cohort study utilized the nationwide, electronic health record–derived, de-identified Flatiron Health–Foundation Medicine Clinico-Genomic Database. Adult patients with mBC diagnosed from January 2017 to March 2022 were included and followed until September 2022. Patients who lacked race data, participated in a clinical trial, or had <6 mo of follow-up were excluded. This subgroup analysis included only patients with HR+, HER2− mBC, and assessed PIK3CA, AKT1, PTEN, ESR1, and BRCA1/2 alterations. All patients had NGS-based tumor tissue testing and/or liquid biopsy. Pathogenic variants, or those likely to be pathogenic, were used to identify alteration status. BRCA1/2 results were available with no distinction between somatic or germline mutations. A logistic regression analysis was used to assess the likelihood of receiving treatment. The Kaplan–Meier method and a Cox regression analysis were used to assess overall survival (OS) from diagnosis of mBC.
Results: Of 2,384 patients with HR+, HER2− mBC, 303 (13%) were Black and 2,081 (87%) were White. Median time from mBC diagnosis to first NGS testing was similar in both groups (4.6 vs 4.8 mo; p=0.12). Compared with White patients, Black patients had a lower prevalence of PIK3CA mutations (34% vs 42%; p=0.03), but there was no significant difference in the prevalence of PTEN (13% vs 12%; p=0.70), ESR1 (16% vs 17%; p=0.60), AKT1 (4% vs 7%; p=0.06), or BRCA1/2 (12% vs 10%; p=0.20) alterations. A total of 2,191 patients received first-line (1L) treatment: 90% of Black patients and 92% of White patients. In the 1L, CDK4/6 inhibitors (CDK4/6i) were used less frequently in Black vs White patients (53% vs 66%; p<0.01), whereas chemotherapy (CT) was used more frequently (27% vs 18%; p<0.01). After adjusting for baseline characteristics, the odds of receiving 1L CDK4/6i was 38% lower in Black vs White patients (OR 0.62; 95% CI: 0.48–0.81; p<0.001). In the second line (2L; n=1,527), where standard of care varies depending on clinical characteristics and biomarkers, CDK4/6i (37% vs 38%; p=0.88) or CT (32% vs 30%; p=0.49) use was similar between Black and White patients. In patients receiving 2L therapy having PIK3CA mutations, there was no significant difference in 2L PI3Kα-specific inhibitor use between Black and White patients (34% [14/41] vs 26% [74/285]; p=0.40). Median OS was significantly shorter in Black vs White patients (34.1 vs 42.1 mo; p=0.004). After adjusting for baseline characteristics, biomarkers, and treatments, Black patients had a shorter OS (HR 1.23; 95% CI: 1.01–1.50; p=0.04). There was a nonsignificant trend toward a shorter OS in Black vs White patients treated with 1L CDK4/6i after adjusting for baseline characteristics and biomarkers (HR 1.24; 95% CI: 0.94–1.64; p=0.13).
Conclusions: In this US study, Black patients with HR+, HER2− mBC who received NGS testing were less likely to have PIK3CA mutations, more likely to receive 1L CT, and less likely to receive 1L CDK4/6i vs White patients, with worse outcomes. This survival difference highlights unmet medical needs in Black patients. Further research is warranted to identify and mitigate racial disparities with HR+, HER2− mBC.

PS10-02: Pre-diagnosis Physical Activity and Racial Disparities in Breast Cancer Survival Outcomes: a Multiethnic Cohort Study

Yijia Sun

Background and objectives: Physical activity (PA) is widely recognized for its health benefits, but its association with breast cancer (BC) survival remains inconclusive. Racial differences in this context have not been well-studied. In this study, we aimed to assess whether racial disparities exist in pre-diagnosis (3 years before diagnosis) PA, adolescent PA, and lifetime PA among BC patients. We also examined the association between PA and BC survival outcomes and whether PA could explain racial differences in these survival outcomes.
Methods: We included BC patients from the Chicago Multiethnic Epidemiologic Breast Cancer Cohort who completed a baseline questionnaire on demographic and risk factors. PA was measured using the metabolic equivalent of task (METs) - hours/week based on patients' self-reported PA duration and intensity. A neighborhood disadvantage index was calculated after geocoding of patients' residential addresses. Ordered logistic regression models were used to assess adjusted odds ratios (aOR) comparing African American (AA) to European American (EA) patients across different PA categories and levels. Cox proportional hazards models were fit to estimate adjusted hazard ratios (aHR) for 3 survival outcomes: overall survival, BC-specific survival, and recurrence-free survival (RFS). Further, we conducted mediation analysis to examine whether racial differences in PA and neighborhood disadvantage influenced BC patients' survival outcomes.
Results: A total of 2,020 patients were incuded in the study, with a median follow-up of 5.4 years. The mean age was 54.7 years, and the mean pre-diagnosis PA was 20.3 METs hours/week. Compared to EA patients (n=1,361 [67%]), AA patients (n=659 [33%]) were significantly less likely to participate in higher levels of total (aOR=0.62, 95% CI: 0.49-0.77), vigorous (aOR=0.61, 95% CI: 0.49-0.76), and moderate (aOR=0.65, 95% CI: 0.52-0.81) pre-diagnosis PA. Similar disparities were observed in lifetime PA, but no substantial differences were found in adolescent PA between AA and EA patients. AA patients had a significantly higher risk of all-cause mortality (aHR=1.53, 95% CI: 1.15-2.04) and a greater risk of recurrence or death (aHR=1.56, 95% CI: 1.18-2.04) than EA patients; the higher risk was for BC-specific mortality, though was not statistically significant (aHR=1.39, 95% CI: 0.84-2.29). We observed a "J-shaped" relationship between survival outcomes and pre-diagnosis total PA. Compared to physically inactive participants (<4 METs hours/week) before diagnosis, those with total PA level of 14-30 METs hours/week had a 43% lower all-cause mortality risk (aHR=0.57, 95% CI: 0.36-0.89) and a 36% reduced risk of recurrence or death (aHR=0.64, 95% CI: 0.42-0.98). However, excessive pre-diagnosis total PA (>30 METs hours/week) was not found to be beneficial for survival outcomes. Mediation analysis revealed there was not a mediation effect of PA on racial differences in survival outcomes. Interestingly, we observed that neighborhood disadvantage could explain 53% of the overall survival difference and 50% of RFS difference between AA and EA patients with stage I-III BC.
Conclusion: Moderate-to-vigorous PA before diagnosis significantly reduced mortality and

recurrence risk in this multiethnic BC patient cohort. AA patients showed lower engagement in pre-diagnosis and lifetime PA compared with EA patients, potentially contributing to racial disparities in BC survival outcomes. While PA did not mediate the racial disparities in survival, promoting moderate levels of PA could improve health outcomes among breast cancer patients, particularly among those living in disadvantaged neighborhoods.

PS10-03: Socioeconomic disparities in long-term heart failure risk of trastuzumab with or without anthracyclines in early-stage breast cancer: A SEER-Medicare Database Analysis

Karissa Britten

Background: Our group recently presented data from the SEER-Medicare database describing long-term cardiotoxicity associated with trastuzumab (T) and/or anthracyclines (A) in early-stage breast cancer (EBC) patients (SABCS 2022, P3-03-13). Here, we present the disparities analysis stratified by available socioeconomic variables (SEVs) from individual zip code and census data.
Methods: We performed an analysis of the SEER-Medicare database from 2005 to 2016, including patients with stage I-III EBC at diagnosis with no prior history of congestive heart failure (CHF). The primary outcome was development of CHF based on ICD coding. Patients were connected to most recently available census data via state and zip codes. SEVs included race/ethnicity, per capita income (PCI), level of education, percent (%) living in poverty, and % not speaking English at home. Descriptive statistics compared SEVs to clinicopathologic characteristics. Multivariate cox proportional hazards models were built stepwise down with an alpha of 0.05, first accounting for all planned SEVs, then including cardiac comorbidities, and finally the primary exposure variables (T and/or A).
Results: Of the initial 214,014 patients with EBC identified in the primary analysis, census data was available for 206,605 patients, with 8,302 receiving T without A, 1,977 receiving T and A, and 13,176 receiving A without T (the remainder received neither T nor A). Patients of Black, Hispanic, and American Indian/Alaskan Native (AIAN) race/ethnicity had increased incidence of large, high-grade tumors and nodal involvement as compared to White and Asian American/Pacific Islander (AAPI) patients. Lower PCI, higher % living in poverty, and lower level of education were also linearly associated with increased tumor size, grade, and N stage. Only the highest quartile of patients not speaking English at home was associated with these higher risk clinical features. Univariate cox proportional hazards model showed that all SEVs were associated with significant differences in the risk of developing CHF; however, multivariate analysis revealed % not speaking English at home and % living in poverty were no longer significant so were removed from subsequent models. Adjusting for significant cardiac covariates and the exposure variables, the multivariate socioeconomic model revealed that Black patients had a 23% higher risk of CHF (Hazard Ratio [HR] 1.23, 95% CI 1.19-1.27, $p<0.001$) and AAPI patients had a 22% lower risk of CHF (HR 0.88, 95% CI 0.83-0.93, $p<0.001$) compared to White patients.

Hispanic and AIAN patients did not have a significantly different risk of CHF than White patients (HR 1.00, 95% CI 0.92-1.08, p=0.99 and HR 1.13, 95% CI 0.96-1.35, p=0.149; respectively). Adjusting for race/ethnicity and cardiac comorbidities, patients living in a zip code in the lowest quartile of PCI had an 18% higher risk of CHF (HR 1.18, 95% CI 1.15-1.22, p<0.001) as compared to those in the highest quartile. Risk of CHF associated with the exposure variables showed similar trends as those reported in the primary analysis: patients receiving both T and A remained at highest risk of developing CHF (HR 1.23, 95% CI 1.13-1.34, p<0.001) compared to the baseline population (who had received neither T nor A), followed by A without T (HR 1.20, 95% CI 1.16-1.24, p<0.001) and T without A (HR 1.15, 95% CI 1.09-1.21, p<0.001).
Conclusions: Among patients with EBC, after adjusting for cardiac comorbidities, Black patients and those living in an area with low PCI had higher risk of developing CHF. The addition of socioeconomic variables to a multivariate model did not impact the primary analysis trend showing increased risk of CHF with T and A, followed by T without A, as compared to the baseline population. These findings illustrate the importance of developing strategies that address both risk factor modification and social determinants of health to mitigate disparities in cardiac outcomes in oncology.

PS10-04: Identifying Risk Factors for High Allostatic Load in a Racially/Ethnically Diverse Cohort of Breast Cancer Patients

Anna Vaynrub

Introduction: Allostatic load (AL), an indicator of cumulative physiological stress and neuroendocrine dysregulation in response to chronic environmental challenge, has been associated with breast cancer (BC) incidence and mortality. Prior studies have found a 1.5-1.8-fold relative increase in all-cause mortality among BC patients with higher AL. Our objective was to determine whether demographic, clinical, and lifestyle factors are associated with AL in a diverse cohort of BC patients.

Methods: Patients diagnosed with stage 0-IV breast cancer between 2007-2023 at Columbia University Irving Medical Center (CUIMC) in New York, NY were consented to complete a survey on demographic, clinical and lifestyle factors and allow access to their electronic health record (EHR) for research purposes. To calculate AL score, we extracted EHR data on systolic/diastolic blood pressure, heart rate, and laboratory results (albumin, alkaline phosphatase, BUN, creatinine, glucose, white blood cells). AL was calculated by assigning one point for each of 9 diagnostic biometrics exceeding the reference range. Patients were dichotomized into “high” and “low” AL groups based on the median score. Descriptive statistics, univariate and multivariate logistic regression analyses were conducted to estimate odds ratios (OR) and 95% confidence intervals (CI) for the associations between demographic, clinical and lifestyle factors with the main outcome of high AL.

Results: Among 1730 evaluable patients, median age at diagnosis was 57 years (IQR: 47, 67) and 37.8% self-identified as non-Hispanic White (NHW), 12.8% non-Hispanic Black (NHB), 35.8% Hispanic, and 8.1% Asian/Pacific Islander/Other. Based upon survey responses, 29.1% had a high school education or less, 42.8% were married, 44.2% were employed, and 29.9% reported an annual household income <$15,000. Median body mass index (BMI) was 26.63 kg/m2 (IQR: 23.02, 30.90). In terms of lifestyle factors, 32.8% of patients were ever smokers, 56.8% self-reported regular alcohol use, 60% followed a restricted diet of some variety (e.g., low fat, low carbohydrate, vegetarian, vegan), 46.7% did not exercise regularly, 65.4% endorsed taking vitamins regularly, and 31.8% reported taking herbal supplements. The median AL score was 3.0 (IQR: 2.0, 4.0) (approximating the mean score and binarized score distribution of a BC cohort based in the U.K. Biobank). Pulse, systolic blood pressure, and blood glucose were the most common biomarkers to contribute to high AL (elevated in 74.3%, 67.1%, and 65.3% of high AL patients, respectively). BC patients with high vs. low AL were older, more likely to self-identify as NHB or Hispanic, have lower education and income, report other chronic illnesses, have a higher BMI, report less regular alcohol use, have lower physical activity levels, and lower vitamin/supplement use. In multivariable analysis, factors associated with high AL included older age (OR=1.01, 95% CI=1.00-1.01), NHB vs. NHW race (OR=1.14, 95% CI=1.04-1.24), and being unemployed vs. employed (OR=1.12, 95% CI=1.02-1.22). Diagnosis of stage III or IV BC compared to stage I disease was also significantly associated with high AL (OR=1.23, 95% CI=1.02-1.50 and OR=1.20, 95% CI=1.02-1.41, respectively).

Discussion: In our diverse study population of BC patients, we found a significant correlation between sociodemographic and clinical factors and a biomarker of physiological stress, which has been associated with BC mortality. AL may be a useful biomarker to assess the effects of modifiable lifestyle factors and social determinants of health to improve BC clinical outcomes and reduce health disparities.

PS10-05: Door-to-door Breast Cancer Screening in 22,278 populaces from Feb. 2020-April 2024: Breast Cancer Hub's Trendsetting Grassroots Sustainable Solutions, overcoming the Disparity, & Challenges in the Rural Remote Villages in Poverty, of Assam, India

Lopamudra Das Roy

Background: There is a huge gap in Breast Cancer survival globally with the death rate being significantly higher in developing countries due to taboo, ignorance, lack of awareness, sociocultural, economic, and environmental barriers, leading to inaccessibility to healthcare facilities, proper guidance, and treatment management. Healthcare providers in rural India regularly see women coming in an advanced stage and in Assam, Northeast (NE) India, the situation is grimmer.
This study is based on an analysis of the database of door-to-door Breast cancer screening

of the rural villages in Assam from February 2020 to April 2024, reporting the Breast Cancer Screening status, disparities leading to late detection and death, and the success of Breast Cancer Hub's Grassroots approach, providing sustainable solutions.
Methods: In this study, we report the data from February 2020 to April 2024 (N=22,278), of in-person Door-to-Door Breast Cancer Screening by Breast Cancer Hub (BCH), a nonprofit organization in the remote 18 villages in Assam, India. BCH-generated questionnaire on health and lifestyle, family history, demography, socio-economic condition and other determining conditions. The data was incorporated from notebook used by BCH teams in the field, into Excel sheets. A complete descriptive statistics is used for understanding the data. For goodness of fit test, chi square is used. All the analysis have been compiled with the help of SPSS version 21.
Results: This is the pioneering study, as BCH is the first group to conduct door-to-door Breast Cancer Screening in N=22,278 villagers (8085 families), in the underprivileged rural Seventeen villages and One Tea Garden Estate in Assam.
From our evaluation, we confirm that the awareness of Breast Cancer Symptoms was <1% among the villagers. The Breast Self-Exam was performed by 0% of the populace. No participant (0%) underwent Clinical Breast Exam. Women 40 years and above, never performed screening mammograms (0%) or ultrasounds (0%) but only visited the hospital when the symptoms were extremely severe eventually getting diagnosed at a late stage leading to death. We investigated the socio-economic condition and huge barrier to care due to financial constraints, villagers living on a daily wage and health always took the last priority as they fall below the poverty line. In addition, the lifestyle is influenced by the tremendous intake of tobacco in all forms, especially adding tobacco, betel nut, slaked lime, and other ingredients to the betel leaves and chewing in the form of Paan. We encountered 76 suspicious cases, that needed further evaluation and screening. But, during screening, the team faced challenges, as the villagers could not financially afford to travel to cancer diagnosis-equipped hospitals which are extremely far away, leading them to succumb to faith healers and eventually death. The family members avoided taking patients to hospitals as it would be a full-day affair and they couldn't miss their daily wage, as food is their priority. Also, they were not aware of government healthcare cards for the Below Poverty Line population. Therefore, BCH created a sustainable framework, by teaching every member Breast Self-Examination (BSE), providing BSE card in local language, accompanying suspicious cases to hospitals, providing transport, generating villagers' income certificates and health cards, helping with hospital registration, patient communication with healthcare professionals, providing Aid for diagnosis and treatment not covered under the government schemes, dropping patients home with follow-ups, counseling, support, taking the same patients back to the hospital on the next appointments, assisting with the comprehensive treatment process, case by case.
Discussion: BCH is the trendsetter to execute this framework in the untapped villages in India, revolutionizing the Breast cancer scenario, and driving impactful changes in Breast Cancer survival.

PS10-06: Access to Innovative Medicines for Advanced Breast Cancer as a Catalyst for Health Systems Strengthening in Low- and Middle-Income Countries

Fatima Cardoso

Background: Breast cancer (BC) is the leading cause of cancer-related deaths among women worldwide, with a disproportionate number of these deaths occurring in low- and middle-income countries (LMICs) due to limited access to effective therapies. In response, The Max Foundation (Max) and its Humanitarian PACT for Advanced Breast Cancer (ABC) partners, including the ABC Global Alliance, the American Society for Clinical Pathology, Cepheid, Novartis, and Lilly, launched a multi-stakeholder program to provide treatment access and address broader healthcare barriers in select LMICs. A country's ability to achieve its health goals largely depends on the capacity of its healthcare workforce. The introduction of innovative medicines for ABC has created the catalyst for the Humanitarian PACT to support both individual patients and broader health systems strengthening in LMICs through healthcare professional (HCP) training.
Methods: The Humanitarian PACT for ABC provides access to CDK4/6 inhibitors and aromatase inhibitors for women living with HR+/HER2- ABC in select LMICs free-of-charge, while also supporting the strengthening of healthcare infrastructure and knowledge through clinical and diagnostics training for HCPs in the country. Training sessions, both on-site and virtual, were led by Experts Consultants covering diagnosis, pathology, clinical decision-making, medication management, and disease monitoring as part of the program's implementation. Medical oncology training sessions focused on situational analysis, patient selection, side effects management, and patients monitoring. Pathology training sessions focused on diagnosis and biomarker assessment.
Results: From October 2023 to June 2024, 96 post-menopausal HR+/HER2- ABC patients from 8 countries; the Bahamas, Benin, Cambodia, Jamaica, Mozambique, Nepal, Saint Lucia, and Seychelles were enrolled in the program and received access to treatment. On-site and virtual training was provided to 29 HCPs, including oncologists, pathologists, and pharmacists from these countries. Patient retention, adherence, and side effects management will be analyzed in each country with main challenges and barriers to be further identified.
Conclusions: We believe that by providing access to high-impact oncology medicines in LMICs, we are equipping HCPs with the most important tools they need to treat patients effectively. Access to medicines serves as the most critical catalyst for strengthening health systems, ensuring that other investments in infrastructure and clinical care improvement occurs and have lasting impact for patients. HCP training plays a crucial role in supporting health systems strengthening by building the capacity of HCPs to deliver quality care, improve patient outcomes, and address health system challenges. This program underscores the transformative impact of leveraging novel medicines as a pathway to strengthening health systems in LMICs, paving the way for sustainable advancements in global oncology, and most importantly providing patients with access to care and medicines resulting in improved outcomes.

PS10-07: The Molecular Subtypes of Breast Cancer: A Single Institution Experience after a Decade of the Syrian War

Maher Saifo

Background: Breast cancer is the most commonly diagnosed type of cancer and the leading cause of cancer-related death among women. Breast cancer mortality rates in Syria rank among the highest in the world. There are few studies about breast cancer epidemiology or patient characteristics in Syria. This study aimed to evaluate estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression in Syrian patients with breast cancer.
Methods: This retrospective study included all new patients with a confirmed diagnosis of breast cancer via biopsy in 2023. The data were retrieved from the Albairouni University Hospital (ABUH) cancer registry.
Results: 1460 patients were included in the study. BC is the most commonly diagnosed cancer in ABUH (21.6%). The patients were distributed among the majority of hormonal receptors (HR)+/HER2- (711, 48.7%), followed by HR+/HER2+ (327, 22.4%), followed by HR-/HER2+ (271, 18.6%) and HR-/HER2- (151, 10.3%).
Conclusion: More than 50% of breast cancer cases in Syria are HER2+ and triple-negative, which may indicate a poor prognosis. Syrian healthcare practices, including cancer registry practices, were negatively impacted by a decade-long war. Data collection regarding other pathological findings, patients' characteristics, and clinical outcomes is still in progress.

PS10-08: Appalachian Mobile Mammogram Program Achieves Unprecedented Outcomes by Repeatedly Reaching Underserved Women

John L. Bell

Introduction: Breast cancer remains the number one incident female cancer in the United States (US). In 2024, it is estimated that 310,720 new breast cancers will be diagnosed in US women and approximately 42,250 women will die of the disease.1 Despite ongoing controversies and conflicting data, screening mammography remains the gold standard examination for early detection and improved survival related to breast cancer. Mobile mammography units (MMU) are especially crucial for patient populations that lack access to recommended routine screening. Detecting breast cancer at earlier stages leads to a reduction in disease progression and mortality.2,3,4Through multiple return visits by the MMU to the same sites and outreach efforts in underserved areas, The University of Tennessee Medical Center's (UTMC) MMU now presents one of the largest series ever published or reported in the US. This series includes 48,385 mammograms conducted between 2008 and 2023, serving 20,254 unique women. The underserved population in this area faces barriers such as receiving fewer healthcare services, socioeconomic disparities, and lack of transportation to fixedmammography sites. Many of these women are without permanent mammography equipment in their home counties so the MMU allows accessible mammographyscreening. Disadvantaged women are more heavily impacted by breast

cancer compared to less vulnerable populations.4 UTMC's MMU service area covers 23 counties (five of which have no "fixed/permanent" mammogram services) in East Tennessee consisting of both urban and primarily rural Appalachian populations.METHODS: A retrospective data analysis was performed on a prospectively maintained database for our MMU Breast Health Outreach Program (BHOP). Data points included demographic information, date of mammogram, sequence of imaging (first, second, third, etc. study), BIRADS assignment (initial and final), biopsy results (if obtained), and status (alive/dead with/without disease). Frequency and percentage statistics were performed.RESULTS: Of the 20,254 women screened on the MMU, 11,037 (54.5%) women were screened once. However, 9,217 (45.5%) women had two or more screeningsperformed on the MMU (range - 2 to 16 mammograms). Due to this high follow-up rate supported by the MMU program administrative infrastructure and our stated intention to return annually to identical locations, 238 cancers were identified in women for an incidence proportion of 0.5% (238/48,385). Of the cancers, 190 were either Stage 0 (n=54) or Stage I (n=136) representing 80% of the diagnosed cancers. In addition, only two (0.8%) patients have expired due to breast cancer and only one patient has experienced a distant failure (bone only) for a distant failure proportion of 0.4%.DISCUSSION: This is one of the largest analyses ever undertaken regarding mobile screening mammography in the US. The results reveal the expected incidence proportion of 0.5% in this asymptomatic, primarily rural population. More importantly, of the cancers identified, 80% were early stage which led to a disease-specific mortality of 0.8%. Focused follow-up of this vulnerable population leads to early-stage diagnosis and improved outcomes, not otherwise seen in underserved, rural screening populations.

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PS10-09: Occupational Pesticide Exposure and Poor Prognosis Breast Cancer in Brazilian Women: Epidemiological Insights and Molecular Mechanisms.

Carolina Panis

The participation of women in the agricultural sector and its associated health consequences are inadequately addressed. In countries such as Brazil, where agriculture is a primary economic driver, female rural workers are at an increased risk of developing chronic diseases, including cancer, due to frequent exposure to harmful substances such as pesticides. This study aimed to characterize pesticide contamination among women during occupational activities, assess its impact on breast cancer (BC) aggressiveness, and explore the underlying molecular mechanisms. Conducted in the Southwest region of Paraná state in Brazil, an area characterized by extensive pesticide use and family-based farming, the study found that this region has higher rates of BC diagnoses and mortality compared to the national average, with pesticide usage reaching six times the national average. This case-control study prospectively included 758 individuals. We identified that the primary routes of contamination for these women are during equipment decontamination and clothes washing. Urine tests from most BC-affected women revealed the presence of glyphosate, atrazine, or 2,4-D. Epidemiological analysis demonstrated that this population has a 41% higher diagnosis rate and a 14% higher mortality rate than unexposed women. The crude association indicated a significantly higher risk of BC among women exposed to pesticides (OR: 1.58, 95% CI 1.18-2.13). Adjusted analyses showed a lower, non-statistically significant association (OR: 1.30, 95% CI 0.87-1.95). Stratification based on disease profile revealed a significantly higher risk of lymph node metastasis (adjusted OR: 2.19, 95% CI 1.31-3.72) in women exposed to pesticides. Analysis of non-tumoral mammary tissue from women without BC but exposed to pesticides showed elevated levels of oncogenic mediators such as PPAR-γ and TNF-α compared to unexposed women. Additionally, in a subset of BC patients with a poor prognosis, pesticide exposure was linked to impaired systemic immune responses (with reduced levels of interleukins 1ß, 12, and 17A), oxidative stress imbalance, and increased expression of tumor-promoting molecules (TGF-β1 and CTLA-4). These findings indicate that occupational pesticide exposure significantly increases the risk of developing aggressive BC, which is associated with a poor prognosis signature due to immune response impairment. In response to the severity of these findings, we have initiated an educational program to raise awareness among rural women about the risks related to pesticide exposure. Over 1500 women have participated in this project, receiving training on safe pesticide handling and the use of protective equipment during clothes washing. Additionally, free personal protective equipment and periodic medical screenings are being provided.

PS1-01: Tumor Infiltrating Lymphocytes (TILs) as a Predictive Marker of Pathological Complete Response (pCR) in a Diverse Patient Population with Early Triple Negative Breast Cancer (TNBC) Treated with the Neoadjuvant KEYNOTE-522 Regimen.

Riya Albert, Joshua Thomas, Navid Sadeghi, Sangeetha Reddy, Glenda Delgado, Heather McArthur, Samira Syed, Deborah Farr, Nisha Unni

Background: Triple negative breast cancer (TNBC) is a highly heterogeneous and aggressive subtype of breast cancer that accounts for approximately 15% of all breast cancer diagnoses and is associated with a poor prognosis. Based on the KEYNOTE-522 (K522) trial, the FDA approved the use of neoadjuvant immunotherapy, Pembrolizumab along with chemotherapy for high-risk early stage TNBC in July 2021. Specifically, the K522 study demonstrated an increased pathological complete response (pCR) rate of 64.8% vs 51.2% and 36-month event-free survival (EFS) of 84.5% vs 76.8% for Pembrolizumab with chemotherapy vs. chemotherapy alone, respectively. The presence of tumor infiltrating lymphocytes (TILs) is a predictive biomarker of pCR and improved survival in patients with TNBC. In this retrospective cohort study, we examine a clinically diverse patient population who received the K522 regimen to determine whether TILs confer a predictive value in response to neoadjuvant pembrolizumab treatment compared to other tumor and clinical factors.

Methods: We reviewed electronic medical records of 271 patients with early TNBC who received neoadjuvant chemo immunotherapy with the K522 regimen at a tertiary care university hospital, UT Southwestern (UTSW), and affiliated safety-net hospital, Parkland Memorial (PM) between August 2021 and May 2024. 184 patients completed the neoadjuvant portion of treatment followed by surgery and fit the criteria for our analysis (UTSW: 120, PM: 64). Baseline patient and tumor characteristics were described. Two proportion Z-tests and Chi-squared tests for independence were used to compare pCR rate between patient ethnicities and univariate logistic regression was used to evaluate the association between binary presence of TILs, grade, and residual cancer burden.

Results In total, 57% achieved a pCR; TILs were reported in 52.8% of pathology reports. The presence of TILs was associated with a significantly improved pCR rate of 70% compared to 48% in individuals without TILs (p=0.0027). The median age of our patient population was 51 years. Of which, 34.8% self-identified as Caucasian, 31.0% Hispanic, 25.5% Black, and 8.7% other. There was no statistically significant difference in pCR rate or presence of reported TILs across different ethnicities. Of note, Hispanic patients with TILs had an increased rate of pCR (80.0%) than Hispanic patients without TILs (54.3%) (p=0.0323). No other ethnic group with TILs showed any statistical association with pCR when compared to their non-TIL presenting counterparts. Controlling for tumor grade, patients with TILs were 2.442 times more likely to have a pCR (CI: 1.310—4.553; p=0.0050). Patients with grade 3 TNBC were 2.89 times more likely to have a pCR (CI: 1.275—11.822; p=0.0170). Node positivity in conjunction with TILs also showed statistically significant pCR rates versus all other subgroups (p=0.0051).

Conclusions The K522 trial did not collect racial data as a baseline demographic characteristic, while our study had a significant percentage of Hispanic and Black populations treated with this regimen. This may account for our pCR rate of 57%, which is lower than what was reported on the K522 trial as studies have shown a lower pCR rate in Black population compared to other ethnicities. Our data suggests that TILs can be considered a predictive marker of pCR rate and treatment response in patients with early-stage TNBC. This is particularly relevant in Hispanic patients with TILs achieving a higher rate of pCR than the rest of our population. This may indicate that TILs have potential to guide treatment decisions for certain subgroups. Current College of American Pathologists guidelines do not mandate standardized or uniform reporting of TILs in pathology reports. While TILs could be considered a potential predictive marker in early stage TNBC, more emphasis needs to be placed in standardized reporting of TILs and larger studies are needed for further validation.

PS1-02: Impact of racial differences in circulating blood components and stromal tumor-infiltrating lymphocytes (sTILs) on outcomes in triple negative breast cancer (TNBC)

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Introduction: TNBC is overrepresented in Black women, and Black patients (pts) with TNBC have worse clinical outcomes compared to non-Black pts. Neoadjuvant chemotherapy (NACT) +/- immunotherapy is current standard of care for early-stage TNBC. Immune response parameters including sTILs are important predictors of response to NACT and long-term outcomes in TNBC. Circulating blood cell counts may be indicators of the systemic immune environment and thus play a role in response to NACT. Racial differences in sTILs and peripheral white blood cell components and their combined impact on outcomes are not well studied in TNBC.
Methods: 469 pts with stage I-III TNBC enrolled in a prospective registry between 2011-2023 who received NACT (n=321) or NACT + pembrolizumab (P) (n=148) were included in analysis. Absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and absolute monocyte count (AMC) were extracted from complete blood count (CBC) obtained prior to starting NACT. sTILs were centrally quantified as previously described. Impact of sTILs and CBC components (ANC, ALC, AMC, NLR (neutrophil to lymphocyte ratio), LMR (lymphocyte to monocyte ratio), and platelet to lymphocyte ratio (PLR)) on pathologic complete response (pCR) and recurrence free survival (RFS) was evaluated.
Results: Among 469 pts, 33% had node positive disease; 81% were White, 14% Black, and 2% Asian. Pts who received NACT+P were more likely to have higher T stage (p=0.005) and TNM stage (p=0.004) and shorter follow up compared to those who received NACT alone. Black pts had lower ANC (median 3.6 vs 4.6 k/uL, p<0.001) and NLR (median 1.6 vs 2.5, p<0.001), and higher ALC (median 2.2 vs 1.8 k/uL, p=0.003) and LMR (median 4.4 vs 3.8,

p<0.001) compared to non-Black pts. AMC was numerically lower in Black pts, and PLR was similar between Black and non-Black pts. sTILs were available for 55% (n=257) of pts; Black pts had higher sTILs compared to non-Black pts (median 40% vs 10%, p=0.019). There was no association of sTILs with ANC, ALC, NLR, LMR, or PLR. There was a weak inverse association of sTILs with AMC (p=0.013). pCR rate was similar in Black and non-Black pts (52% vs 55%). sTILs were associated with pCR both as a continuous variable (odds ratio, OR 1.12 for every 10% increase, p=0.009) and at 30% cut point. None of the CBC components were associated with pCR. Increasing AMC and LMR were associated with lower RFS (AMC: hazard ratio, HR 1.11 for every 0.4 k/uL increase, p=0.019, LMR: HR 1.28 for every 1.0 increase, p=0.002). None of the other CBC components, sTILs, nor race were associated with RFS. On multivariable analysis that included T stage and nodal status, AMC and LMR were each independently associated with RFS (AMC: HR 1.10, p=0.054; LMR: HR 1.07, p=0.016).
Conclusion: Among all circulating blood cell components, only the ones that included monocytes (AMC and LMR) independently impacted RFS, with higher monocytes being associated with worse outcomes in TNBC. Positive correlation between circulating monocytes and tumor-associated macrophages (TAMs) has been previously reported. It is also possible that the chemokine axis may recruit inflammatory monocytes into tumor sites, which may in turn differentiate into TAMs. We noted weak inverse association of AMC with sTILs, which also suggests some relationship between monocytes and the tumor immune microenvironment (TIM). Compared to non-Black pts, Black pts were more likely to have immune-enriched tumors (higher sTILs) but had more unfavorable peripheral white blood cell immune environment (higher LMR). These findings may partly explain why high sTILs do not translate into improved pCR rate and RFS in Black pts. Further investigation is warranted into the interaction between TIM, circulating monocytes, and race and how these interactions impact response to therapy.

PS1-03: Prediction of Prognosis and Efficacy of Neoadjuvant Therapy in HER2-Positive Breast Cancer Patients Based on Tertiary Lymphoid Structures

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Background: Tertiary lymphoid structures (TLS) are lymphoid tissues that form in pathological conditions such as chronic inflammation, infection, or tumors. TLS play a crucial role in antigen-specific immune responses, participating in anti-tumor immune reactions and other immune-related processes. Studies have shown that TLS expression is associated with the efficacy of immunotherapy in triple-negative breast cancer (TNBC). However, the significance of TLS in HER2-positive breast cancer remains unclear. Trastuzumab plays a vital role in blocking the HER2 signaling pathway and, through antibody-dependent cellular cytotoxicity (ADCC), enhances the killing effect of immune cells on tumors. The relationship between TLS expression and the efficacy of targeted therapy is not well-defined. This study utilizes public databases (METABRIC) to confirm the significant

association between TLS and patient prognosis. Moreover, the correlation between TLS expression and the efficacy of neoadjuvant therapy in HER2-positive breast cancer is validated using the I-SPY2 data. Additionally, pre-treatment biopsy samples from HER2-positive breast cancer patients were collected, and multiplex immunofluorescence staining was employed to verify the close relationship between TLS expression and therapeutic efficacy.

Methods: We first explored the prognostic value of TLS expression levels in HER2-positive breast cancer patients from the METABRIC database. A total of 224 HER2-positive breast cancer patients were selected from the METABRIC dataset. Using the R software's survminer package res.cut function, we determined the optimal cutoff value for TLS expression at 0.32, categorizing patients into high TLS group (n=53) and low TLS group (n=171). Furthermore, the CIBERSORT algorithm was used to illustrate the differences in the immune microenvironment between the high and low TLS expression groups in HER2-positive breast cancer patients. Signal pathway enrichment analysis was performed on HER2-positive breast cancer sequencing data from the I-SPY2 publicly available dataset (n=245), and samples were grouped based on TLS expression (high TLS group n=68, low TLS group n=177). By analyzing the I-SPY2 data, we evaluated the role of TLS expression in predicting the pathological complete response rate (pCR) in HER2-positive breast cancer patients receiving dual-targeted therapy, as well as the impact of adding pertuzumab. Additionally, pre-treatment biopsy samples from HER2-positive patients undergoing neoadjuvant therapy were collected, and multiplex immunofluorescence staining was employed to verify the close relationship between TLS expression and therapeutic efficacy.Results: In HER2-positive breast cancer patients from the METABRIC database, Kaplan-Meier survival curves showed that the survival probability of the high TLS group was significantly higher than that of the low TLS group. At a median follow-up of approximately 60 months, the survival probability of the high TLS group was about 75%, compared to less than 60% in the low TLS group. The survival difference between the two groups was statistically significant (HR 1.644, 95% CI 1.082 to 2.500, p=0.041). CIBERSORT algorithm analysis revealed that in HER2-positive breast cancer tumors, those with high TLS expression had significantly increased infiltration of CD8+ T cells, activated memory CD4+ T cells, follicular helper T cells, and M1 macrophages. In contrast, tumors with low TLS expression had higher levels of M0 macrophages and resting dendritic cells. Subsequently, GSEA analysis was conducted on two cohorts, using NES > 1, p-value < 0.05, and p.adjust < 0.25 as criteria for significant enrichment pathways. Significant differences were found in tumor signaling pathway activation between patients with different TLS expression levels in HER2-positive breast cancer samples. High TLS expression patients showed upregulation of immune and inflammation-related pathways, indicating a stronger immune response. In contrast, low TLS expression patients showed upregulation of cell proliferation and signaling pathways, indicating increased tumor cell proliferation activity. In I-SPY2 trial, the high TLS group showed enrichment in immune-related pathways such as B cells, dendritic cells, and mast cells, with more active inflammatory features (e.g., STAT1 signaling and chemokine 12). Conversely, the low TLS group was more enriched in proliferation-related pathways (e.g., Module11_Prolif) and Basal index, suggesting

significant biological differences in immune and proliferative activities between high and low TLS tumors. Data from the publicly available I-SPY2 dataset suggested that in the control group (paclitaxel + trastuzumab), the pCR rate for "TLS high" patients was 45%, whereas the pCR rate for "TLS low" patients was 17%. In the pertuzumab group (control group plus pertuzumab), the pCR rate for "TLS high" patients was 63%, compared to 58% for "TLS low" patients. The addition of pertuzumab significantly improved the pCR rate in the TLS low expression group. Univariate interaction analysis of TLS expression with the addition of pertuzumab showed significance (P = 0.019). In "TLS low" group, the addition of pertuzumab significantly increased the pCR rate, more than tripling it (58% vs. 17%, P = 0.0027), while there was no statistically significant difference in "TLS high" group (63% vs. 45%, P = 0.6372). Biopsies from the primary tumors of 20 newly diagnosed HER2-positive patients who received neoadjuvant therapy were collected, and multiplex immunofluorescence staining was performed using markers DAPI, CD20, CD4, and CD8. Preliminary results indicated that patients with a Miller-Payne (MP) score of G5 had tumors with abundant TLS structures, whereas patients with MP scores of G1-G2 had significantly fewer TLS in their tumor sections.
Conclusion: Our study demonstrates that TLS expression is a significant prognostic marker in HER2-positive breast cancer. High TLS expression is associated with improved survival and a more active immune microenvironment, as evidenced by increased infiltration of immune cells such as CD8+ T cells and activated memory CD4+ T cells. Conversely, low TLS expression correlates with increased tumor cell proliferation and poorer prognosis. Furthermore, our analysis of the I-SPY2 dataset reveals that TLS expression can predict the efficacy of neoadjuvant therapy, with the addition of pertuzumab notably enhancing the pathological complete response rates in TLS low-expressing tumors. Multiplex immunofluorescence staining of pre-treatment biopsies corroborates these findings, showing a close relationship between TLS abundance and therapeutic efficacy. These results suggest that TLS could serve as a valuable biomarker for stratifying patients and tailoring treatment strategies in HER2-positive breast cancer, potentially leading to improved clinical outcomes.

PS1-04: Tumor-infiltrating lymphocytes (TILs) and response to neoadjuvant chemotherapy in young patients with breast cancer

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Background: Increased TILs are associated with higher pathologic complete response (pCR) rates after NACT for breast cancer, but scant data exist in patients ≤ 40 years old, in whom age-related differences in host and tumor immune microenvironment may exist. We assessed the extent and composition of immune infiltration in breast tumors of young women undergoing NACT and correlated with clinicopathologic features and treatment response.

Methods: Patients with stage I-III breast cancer who had NACT and available pre-treatment tumor tissue were identified from a prospective cohort study of women with breast cancer diagnosed at age ≤ 40 years. Multiplexed immunofluorescence was used to quantify cytotoxic T (CD8+), T helper (CD3+CD8-), T regulatory (Treg, FOXP3+CD3+), exhausted T (PD1+CD8+), and PDL1+ cells in stroma and tumor as a percentage value of positive cells. Univariate analyses tested associations of TIL levels (high vs. low, divided based on median) with clinicopathologic variables and compared TIL levels (continuous) between pCR and non-pCR cases. Logistic regression tested associations between TIL subtypes (continuous, per 10% increase) and pCR.
Results: Among 194 patients, median age was 36 years (range 22-40 years), 14% were positive for BRCA1/2 mutations, and most had grade 3 (71%), > 2 cm (87%), node-positive (79%) tumors. Forty-one percent of tumors were hormone receptor (HR)-positive/HER2-negative, 33% HER2-positive/HR-positive or -negative, and 27% triple-negative. Sixty-one patients (31%) experienced pCR after NACT. Patients with recent pregnancies (≤ 5 years prior, n=80) had higher stromal infiltration of T helper (P=.020) and cytotoxic T (P=.002) cells as well as higher intratumoral infiltration of cytotoxic T (P=.001), Treg (P=.018) and exhausted T (P=.049) cells compared to those who were nulliparous (n=52), pregnant at diagnosis (n=7) or pregnant > 5 years prior (n=24). BRCA1 mutations (n=19) were associated with high levels of exhausted T cells in stroma (P=.021) and tumor (P=.048). Grade 3 tumors (n=137) had higher levels of T helper cells in stroma (P=.030) and tumor (P=.010). Triple-negative (n=52) and HER2-positive (n=63) tumors had higher stromal Treg infiltration (P=.046). Triple-negative and stage III tumors (n=66) were associated with high levels of PDL1+ cells in stroma (P=.033) and tumor (P=.005), respectively. No differences in TILs were seen according to age, race, ethnicity or histological subtype. Patients with pCR had greater stromal Treg (P=.0189), intratumoral T helper (P=.0043) and intratumoral PDL1+ cell infiltration (P=.0372). Increased stromal Tregs was predictive of pCR, controlling for breast cancer stage, grade and subtype (OR 1.63, 95% CI 1.12-2.28, P=.011). Increased levels of all intratumoral TIL subtypes was predictive of pCR, with odds ratios ranging from 1.14 (95% CI 1.02-1.29, P=.026) for PDL1+ cells to 2.02 (95% CI 1.31-3.12, P=.002) for Tregs for each 10% increment, controlling for breast cancer stage, grade and subtype.
Conclusions: The distribution of TIL subtypes in young patients' breast tumors pre-treatment varied according to recency of pregnancy, BRCA1/2 mutation status and tumor characteristics. High levels of most TIL subtypes was associated with improved response to NACT, independent of breast cancer subtype. The relationship between TILs and pCR in young women appears similar to that in older women, suggesting that tumors of younger and older patients may not be fundamentally different when analyzed according to clinically relevant biologic features. Characterization of immune subpopulations could help refine the predictive value of TILs in young patients with breast cancer, who may benefit from individualized escalated and de-escalated treatment strategies.

PS1-05: Genomic characteristics related to histology-based immune features in breast cancer

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Background: The immune cell component of the tumor microenvironment is an important modulator of tumor progression and suppression. In patients with breast cancer, tumor-infiltrating lymphocytes (TILs) represent a large part of antitumor immunity along with tertiary lymphoid structure (TLS), as they predict favorable prognosis and good treatment response. In this study, we scored for immune-related histology features using whole slide H&E images of the TCGA-BRCA dataset and analyzed these distinct features relative to gene expression patterns and molecular intrinsic subtypes.
Method: A total of 1035 cases from TCGA-BRCA dataset were evaluated for TILs, plasma cells, high-endothelial venule associated lymphoid aggregate (HALA) and TLS. For HALA and TLS, location relative to tumor (non-tumor, peritumor, and intratumoral area) and number was determined. For each pathologically defined immune feature (i.e., no TLS vs TLS+), we then searched for associated gene expression features and used the top 100 significantly upregulated genes to make signatures for tumor group-based analyses. Upregulated immune modules in high-TIL tumors using a 10% TIL level cutoff were compared based on PAM50 molecular subtype (luminal A/B, HER2-enriched, basal-like). The clinical significance of histology-based gene signatures and high-TIL related immune modules was validated using bulk RNAseq and survival data from the SCAN-B cohort (N=6329).
Results: HER2-enriched (HER2E) and basal-like breast tumors exhibited the highest mean TILs, with plasma cells more frequently observed in these subtypes. HALAs were present in 36.4% of cases and TLS were found in 6.5% of cases, also predominantly in HER2E and basal-like tumors. Gene signatures related to intratumoral HALA and intratumoral TLS correlated with better survival outcomes, while peritumoral HALA correlated with worse outcomes on the overall TCGA data set. In addition to TILs- and plasma cell-associated gene signatures, intratumoral HALA and intratumoral TLS correlated with HER2E and basal like tumors. High-TIL tumors revealed 164 enriched immune modules, with unique upregulated modules in each molecular subtype. For example, in luminal tumors, immune modules had little relation to favorable prognosis. High-TIL HER2E and basal-like tumors had distinct immune gene signatures linked to improved survival, with HER2E being more associated with B-cell prognostic features and basal-like subtype more associated with T-cell prognostic features.
Conclusion: Different subtypes of breast cancer exhibit distinct tumor immune microenvironments, both histologically and molecularly. These differences in immune properties and biological characteristics should be considered when developing precise treatment strategies to achieve the best therapeutic efficacy for patients.

PS1-06: Intratumor tumor-infiltrating lymphocytes (iTILs) and spatial distribution pattern of stromal TILs (sTILs) evaluated by HALO AI are prognostic indicators of triple-negative breast cancer

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Introduction: A high density of tumor-infiltrating lymphocytes (TILs) has been recognized as an indicator of favorable prognosis of triple-negative breast cancers (TNBCs), and measurement of TILs infiltrating in the stroma of peripheral zone of cancer tissue are considered to be of the optimal clinical significance. However, some studies on other cancer types have indicated that TIL densities in both stromata of peripheral and central zones and in tumor cell nests were associated with prognosis. In this study, we evaluated the density and spatial distribution pattern of TILs manually and with an artificial intelligence (AI) modality and investigated their prognostic implication.
Materials and methods: We evaluated 70 surgically resected TNBC specimens from patients who did not receive neoadjuvant chemotherapy. CD3+ TILs and CD8+ TILs were detected by immunohistochemistry. The manual evaluation was performed by counting the total number of CD3+- and CD8+- TILs in peripheral stroma (psTILs), in central stroma (csTILs), and in tumor cell nests (iTILs) in five high-power fields (HPFs) on photomicrographic images. The evaluation with AI was performed by calculating densities of these TILs in 10 tiles (one tile: 500 x 500 μm square) after deep-learning of annotated areas of tumor cell nests, stroma, and other regions on digital slide glasses with HALO AI (Indica Labs, NM, U.S.A.). With AI, the peripheral zone was defined as the region centered on the boundary separating the host tissue from the cancer tissue with a width of 1 mm, and the central zone as the tissue inside the peripheral zone. psTILs, csTILs, and both peripheral and central iTILs (piTILs and ciTILs) were measured. With reference to receiver operating characteristic curves, the optimal cut-off values between high and low TILs were determined to discriminate clinical outcome of the patients. Spatial distribution patterns of TILs were also defined: infiltrated (high psTILs/high csTILs) and non-infiltrated patterns. Recurrence-free survival and overall survival analyses were performed with Cox proportional hazards model analyses.
Results: Median follow-up duration was 9.4 years, and 14 patients suffered recurrences and 13 patients died. In manual measurements, cut-off values in totally five HPFs were 1000, 300, 700, 300, 10, and 5 in CD3+ psTILs, CD8+ psTILs, CD3+ csTILs, CD8+ csTILs, CD3+ iTILs, and CD8+ iTILs, respectively. In AI measurements, cut-off values in totally 10 tiles were 1,500, 1,000, 500, 250, 30, and 15 in CD3+ psTILs, CD8+ psTILs, CD3+ csTILs, CD8+ csTILs, CD3+ ciTILs, and CD8+ ciTILs, respectively. By Cox univariate analyses, low CD3+ iTILs and low CD8+ iTILs with AI measurements showed higher risks [recurrence: hazard ratio(HR) 3.11, 95% CI 1.01-9.59, P = 0.047 for CD3+ ciTILs; HR 4.30, 95% CI 1.18-15.7, P = 0.027 for CD8+ ciTILs; HR 5.88, 95% CI 1.30-26.6, P = 0.021 for CD3+ csTILs; death: HR 3.57, 95% CI 1.16-11.0, P = 0.026 for CD3+ ciTILs; HR 4.30, 95% CI 1.18-15.7, P = 0.027; HR 2.23, 95% CI 0.99-10.5, P = 0.052; HR 3.98, 95% CI 1.09-14.5, P = 0.036 for CD3+ csTILs], but other psTILs and csTILs did not show prognostic significance. With regard to spatial

distribution of CD3+ TILs, non-infiltrated patterns showed significantly worse prognoses than infiltrated pattern (recurrence: HR 8.52, 95% CI 1.11-65.7, P = 0.039; death: HR 8.98, 95% CI 1.16-69.3, P = 0.035). Similar results were shown by manual evaluations. The CD3+ ciTILs, CD8+ ciTILs, CD3+ csTILs, and spatial distribution pattern with AI measurements were significantly or nearly significant prognostic values independently of patient age, pT, pN, stage, and adjuvant chemotherapy.
Conclusions: Evaluation of TILs not only in peripheral stroma but also in central stroma and tumor cell nests appeared important in TNBCs. iTILs and spatial distribution pattern of TILs might be applicable to discern patients who are eligible for dose de-escalation from those who require standard perioperative systemic therapy to TNBC.

PS1-07: AI-Derived Tumor-Infiltrating Lymphocytes Enhance the Model with Baseline Stromal Tumor-Infiltrating Lymphocytes and Ki-67 in Predicting Pathologic Complete Response in an Early-Stage Triple-Negative Breast Cancer Prospective Trial

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Background: The tumor immune microenvironment is crucial in shaping the response to neoadjuvant chemotherapy in triple-negative breast cancer (TNBC). Stromal tumor-infiltrating lymphocytes (sTILs) and Ki-67 are important biomarkers to predict treatment response in TNBC (Abuhadra et al., 2023). However, manual assessment of sTILs faces challenges due to variability in interpretation across different observers (Van Bockstal et al. 2021). To obtain a consistent predictive model for treatment response with chemotherapy in TNBC, we utilized artificial intelligence (AI) methods for enhancement.

Material & methods: We collected 408 hematoxylin and eosin (H&E)-stained images from ARTEMIS (NCT02276443) arm 1 pretreatment core biopsies, divided into discovery and validation cohorts as per a previous work (Abuhadra et al., 2023). We also used the same criteria to evaluate pathologic complete response (pCR), Ki-67, and sTILs. H&E images exhibiting metaplasia and giant cells were excluded, resulting in 201 slides for the discovery cohort and 193 slides for the validation cohort, with one slide per case.
To calculate the TILs score, we applied AI pipelines on H&E images to identify epithelial, lymphoid, stromal, and other cells (AbdulJabbar et al., 2020), and to segment tissue into tumor, stroma, parenchyma, necrosis/hemorrhage, and adipose tissue (unpublished). To ensure the accuracy of cell classification, we combined the two pipelines to refine cell recognition. Specifically, cell types identified outside the tumor area but misclassified as epithelial cells underwent a secondary prediction. AI-derived TILs (AI-TILs) was calculated as the proportion of identified lymphoid cells within the tumor and stromal tissues, excluding other cell types.

Results: To evaluate the AI pipelines, 20 images from discovery cohort were manually

annotated by three pathologists, resulting in 8010 cells. We achieved an average balanced accuracy of 91.2% across the identified cell types. In the discovery cohort (n=201; pCR rate 42%, 85/201), AI-TILs was notably associated with manual sTILs (Spearman's rho=0.49, P<0.001). In a multivariable model consisting of AI-TILs and Ki-67 to predict pCR, with AI-TILs cutoff (0.173, range: 0.03-0.73) established through recursive partitioning analysis, we found that higher AI-TILs levels were significantly associated with pCR (Odds ratio, 4.57, 95% CI=1.63-12.80, P=0.004). Manual sTILs showed a similar pattern (Odds ratio, 3.71, 95% CI=1.97-6.99, P<0.001) in a multivariable model with Ki-67. When combining Ki-67, sTILs and AI-TILs, the significant association between AI-TILs and pCR was retained (Odds ratio: 3.01, 95% CI=1.04-8.72, P=0.042). Using the same model, we achieved an AUC of 0.74 and a precision of 0.68, marginally improving the model consisting of Ki-67 and sTILs (AUC 0.71, precision 0.68). In the validation cohort (n=193; pCR rate 42%, 82/193), the AI-TILs score performed consistently, maintaining correlation with sTILs (rho=0.39, P<0.001). By employing the model constructed in the discovery dataset with Ki-67, sTILs, and AI-TILs to predict pCR, we achieved an AUC of 0.69 and a precision of 0.67, comparable to the model with Ki-67 and sTILs alone (AUC 0.68, precision 0.66). Calibration plots and Hosmer-Lemeshow test indicated that the model including AI-TILs aligned better with the actual data compared to the model without AI-TILs.

Conclusion: This study demonstrates that AI-TILs from baseline biopsies can be a promising biomarker to enhance the prediction for neoadjuvant chemotherapy response in TNBC, alongside existing predictors such as manual sTILs, underscoring the potential clinical value of AI-TILs due to its reproducibility and objectivity.

PS1-08: Deciphering the Tumor-Immune Landscape and Mechanisms of Response and Resistance to Neoadjuvant Therapy in Early-Stage Breast Cancer Using Single-Cell RNA Sequencing

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Background: For most early-stage aggressive breast cancers (BC), neoadjuvant therapy (NAT) with chemotherapy (CT), with/without HER2 blockade or immune checkpoint blockade (ICB), is the current standard of care. However, around half of them will not respond to NAT and have a higher risk of recurrence. This study aims to provide insights into the cellular and molecular heterogeneity, potential resistance mechanisms, and predictive biomarkers for NAT response by analyzing the tumor and immune landscape using single-cell RNA sequencing (scRNA-seq).

Methods: Tissue samples were prospectively collected from pre-treatment biopsies, post-treatment tumor beds, and normal adjacent tissue of patients treated with NAT for luminal B-like (LumB), HER2-positive (HER2+), and triple-negative breast cancer (TNBC). Cells

were processed using the 10x Genomics Chromium Next GEM Single Cell 5' platform and analyzed for gene expression and T-cell and B-cell repertoire profiling. Following data pre-processing, cell clusters were annotated using unsupervised clustering and canonical marker genes. Comprehensive analyses involving copy number variation (CNV), pathway analysis, pseudotime trajectory, and ligand-receptor (LR) interactions were conducted. Differences in gene expression and cell proportions were assessed using t-tests and Wilcoxon rank-sum tests.

Results: We obtained high-quality scRNA-seq data from 30 patients across various time points, totaling 286,346 cells. NAT responders were 1/4 in LumB, 5/5 in HER2+, 7/14 in TNBC with CT alone, and 5/7 in TNBC treated with CT-ICB. After annotation, tumor tissue was enriched in immune cells and specific cancer-associated fibroblasts (CAFs) compared to normal adjacent tissue. Across BC subtypes, we observed differences in tumor cell origins, tumor microenvironment (TME) composition, and its dynamics under NAT. TNBC tumor cells indicated a basal/luminal progenitor origin, while LumB and HER2+ tumor cells displayed a luminal hormone-responsive origin. Also, LumB had lower levels of immune cells compared to TNBC and HER2+, especially tumor-reactive CXCL13+ and effector T cells. NAT influenced cell subtype dynamics, decreasing T and B cells and increasing CAFs in all BC subtypes. Post-NAT, there was a reduction in naïve CD4 and CD8 T cells and Tregs, but an increase in resident memory and T helper cells. Of interest, responders showed a significant increase in the CD8 effector/Treg ratio post-NAT compared to non-responders ($p<0.001$), likely due to the higher cytotoxic effect of CT on Tregs, which contributes to its antitumor efficacy. Response to NAT also correlated with higher proportions of proliferating T cells in the G2/M cell cycle phase ($p<0.01$), while higher levels of T cells in the G1 phase were associated with non-responders.
Of interest, a granular analysis of the T cell compartment revealed clonotypic variations and CXCL13+ CD8+ T cell expansion in pre-treatment samples linked to NAT response. Cell-cell interaction analyses further highlighted LR interactions specific to responders between tumor cells and CXCL13+ CD4+/CD8+ T cells, NK cells, proliferative T cells, and B cells. On the other hand, LR interactions specific to response to CT-ICB versus CT alone in TNBC were primarily observed among immune cell subsets, particularly between CXCL13+ CD4/CD8+ T cells and T regs and B cells (via CXCL13/CXCR5, TNFSF4/TNFRSF4, CCL5/CR5, CCL3/CCR5 or TNF/ICOS LR pairs), suggesting the necessity of immune cell communication in lymphoid aggregates for an effective immune response.
Finally, tumor cell sub-clonal analysis by inferred CNV revealed distinct patterns of genomic and transcriptomic heterogeneity among epithelial cells and TMEs.

Conclusions: Our findings underscore tumor-immune landscape complexity and offer insights into cellular dynamics, potentially unveiling predictive biomarkers of response to NAT in BC.

PS11-01: [18F]Fluoroestradiol (FES)-PET as a predictive measure for endocrine therapy in patients with newly diagnosed metastatic breast cancer: Results from EAI142

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Background: Multiple single institution studies have shown that FES-PET predicts clinical benefit of endocrine based therapy for patients with metastatic breast cancer (MBC). ECOG-ACRIN trial EAI142 is a multicenter imaging biomarker study of FES-PET to assess baseline FES uptake qualitatively and quantitatively prior to initiation of first-line endocrine therapy with or without CDK4/6 inhibitors in MBC. The primary objective is to determine the negative-predictive value (NPV) of FES uptake for predicting clinical benefit in patients with estrogen-receptor positive (ER+) MBC at 6 months after treatment with endocrine based therapy. In this analysis, we present the primary analysis: the imaging results and 6-month follow-up data in the fully enrolled cohort.

Methods: From 2016-2022, 14 centers enrolled 105 female patients; 98 completed study procedures and were treated with endocrine based therapy. [18F]Fluorodeoxyglucose (FDG)-PET at baseline was encouraged, but not required, and was obtained in 66 patients. SUVmax of up to 5 of the most prominent lesions were recorded and qualitatively assessed for FES uptake in all patients. A sub-set analysis was done using FDG to guide lesion selection in the 66 patients who had both FES and FDG scans with the goal to better identify FES-negative lesions and heterogeneity in FES uptake. Patients were followed for progression-free survival (PFS) at 6 months (primary endpoint) and at 2 years (secondary endpoint). To determine the reproducibility of quantitative assessment of tumor FES uptake, 10 patients underwent a second FES-PET scan within 12 days of the first scan and prior to initiation of treatment in a test-retest sub-study.

Results: Of the 450 lesions identified at baseline, the majority were in bone or soft tissue (262 bone, 124 lymph node, 40 visceral). Most patients had 5 or more evaluable lesions (83/98, 85%). The majority of patients were postmenopausal (80/98, 82%) and treated with an aromatase inhibitor (69/98, 70%) and CDK4/6 inhibitor (73/98, 74%). We identified a wide range in uptake (SUVmax of 1.2-44.7), but only 19/98 (19%) patients had any lesions with qualitatively mild, minimal or absent FES uptake. No patient had an average SUVmax for the most prominent lesions less than the pre-specified cut-off of 1.5, therefore an NPV was unattainable. A total of 16/98 (16%) patients progressed at 6 months. Of 4 patients with heterogenous uptake, 1 (25%) progressed at 6 months. In the sub-set analysis, FDG -guided lesion location did not identify any additional FES-negative lesions. We observed excellent reproducibility in 79 test-retest lesions (SUVmax 1.5-20.2)

with a lesion level intra-class correlation coefficient of 0.94. Two-year PFS will be updated at the time of presentation.

Conclusions: In patients undergoing first-line therapy for ER+ MBC, we evaluated ER expression by both qualitative and quantitative FES-PET. NPV could not be assessed due to a lower than expected proportion of FES-negative scans and rate of progressive disease at 6 months needed to meet the assumptions used for power calculations. Test-retest studies confirmed quantitative overall and lesional reproducibility of FES-PET.

PS11-02: [18F]fluoroestradiol (FES) PET/CT to guide 2nd line treatment decision in patients with ER-positive HER2-negative advanced breast cancer (ABC) progressing on 1st line aromatase inhibitor and CDK4/6 inhibitor: early results of the ESTROTIMP trial.

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Introduction: Post CDK4/6i second line endocrine therapy-based treatment options have recently expanded for patients with ER-positive/HER2-negative ABC, with new endocrine therapies (ngSERDs) and combination therapies (PI3KCA/AKTi, CDKi) being available or under investigation. Additionally, antibody-drug conjugates such as T-DXd (Destiny-Breast 06, ASCO 2024) have also demonstrated prolonged efficacy in the post-CDK4/6i setting and could become an attractive treatment option. Interestingly, while ER loss/downregulation is a universal mechanism of resistance to endocrine therapy, [18F]fluoroestradiol (FES) PET/CT enables non-invasive assessment of ER expression and heterogeneity in ABC lesions. This information may guide therapy selection in the post-CDK4/6i setting, potentially reducing the use of ineffective endocrine therapies in these patients.

Methods: ESTROTIMP is an ongoing multicenter trial in France, prospectively enrolling 152 patients with progression of ER-positive/HER2-negative ABC on first-line endocrine therapy in combination with a CDK4/6 inhibitor. After standard of care [18F]fluorodeoxyglucose (FDG) PET/CT, all patients undergo an FES PET/CT scan. Treating physicians complete therapeutic management questionnaires before and after the FES PET/CT to evaluate the impact on therapeutic management. Nuclear medicine physicians complete reading forms for both FDG and FES PET/CT scans. Patients score pain and apprehension for both FES PET/CT and biopsy on a Visual Analogue Scale (VAS). Quality of life and second-line progression free survival (compared to a matched retrospective cohort) will be assessed after 12 months. An interim analysis of the first 30 assessable prospective

patients has been conducted.

Results: FES uptake in ABC lesions was classified as all lesions ER-positive (when compared with FDG PET/CT) in 19 of 30 patients (63.3%), mixed ER-positive/negative lesions in 7 (23.3%), and all lesions ER-negative in 4 patients (13.3%). A therapeutic management change based on incorporation of FES PET/CT results was implemented in 11 of 30 patients (36.7%; 99% confidence interval 16.0 - 61.6). Therapy selection was changed in 9 patients and diagnostic testing in 5, including 3 patients with changes in both. FES PET/CT helped confirm the initial management plan in 17 of 19 patients without changes in the management decision. Patients reported significantly lower pain and apprehension VAS scores for FES PET/CT compared to biopsy (Pain: Mean [SD], 1.00 [2.304] vs. 2.95 [3.197]; p=0.0051. Apprehension: Mean [SD], 2.47 [3.126] vs. 4.81 [3.636]; p=0.0111).

Conclusions: FES PET/CT is a helpful tool to inform therapeutic management decisions in patients with progression of ER-positive/HER2-negative ABC on first-line therapy and procedurally showed lower pain and apprehension scores compared to biopsy.

PS11-03: Comparative analysis of [18F]FES- and [18F]FDG-PET in patients with metastatic ER+ lobular breast cancer

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BACKGROUND: Detection of invasive lobular carcinoma (ILC) with standard imaging modalities can be challenging. [18F]fluorodeoxyglucose (FDG) PET is commonly used in the work up of first presentation of metastatic breast cancer (MBC), but may be suboptimal for ILC due to lower metabolic activity compared to invasive ductal cancer. Imaging with [18F]fluoro-17b-estradiol (FES) PET measures whole-body estrogen receptor (ER) expression, which is generally high in ILC. Whether FES-PET outperforms FDG-PET in detecting metastatic ILC (mILC) lesions -and should therefore be the first-step imaging modality for mILC work up- remains unclear. Therefore, we compared the sensitivity of FDG- and FES-PET to detect ILC metastases, in patients who were enrolled in the prospective IMPACT-MBC study.
METHODS: In IMPACT-MBC (NCT01957332), 200 patients with newly diagnosed MBC underwent baseline FDG-PET, FES-PET, computed tomography (CT) scan and bone scintigraphy in addition to a metastasis biopsy. In this subanalysis, patients with ER+ mILC were included. Metastases were visually identified on FDG- and FES-PET and expressed as the total number and percentage of all metastases detected on all imaging modalities together. Results were analyzed on patient and lesion level.
RESULTS: Twenty-six patients with ER+ ILC were included, with a median age of 57 years.

Mean number of detected metastases per patient was 34 (range 2-77) with FDG-PET and 33 (range 0-88) with FES-PET. In 12 patients (46%), FES-PET detected more metastases than FDG-PET, whereas FDG-PET detected more metastases than FES-PET in another 12 patients (46%), and in two patients (8%) the same number of metastases was detected with both scans. In total, 1101 metastases were detected with all imaging modalities. A total of 853 metastases were detected with FDG-PET and 859 metastases were detected with FES-PET. This results in a sensitivity of 77.5% (95% CI 75 – 80) for FDG-PET and 78% (95% CI 76 – 80) for FES-PET.
CONCLUSIONS: This is the largest prospective cohort comparing FDG-PET with FES-PET for baseline work up in mILC. FES-PET does not outperform FDG-PET in detecting ILC metastases, and the two techniques have similar sensitivity on a group level. For clinical practice, in light of wider availability of FDG-PET and detection of equal or more ILC metastases in the majority of patients, FDG-PET remains the first-step imaging modality for mILC. However, in a considerable part of patients with mILC, FES-PET detects more ILC metastases than FDG-PET. Therefore, in case of persistent clinical dilemmas or doubts, an additional FES-PET should certainly be considered in mILC.

PS11-05: Novel 4D radiomics applied to dynamic FES PET images to improve prediction of ER-positive breast cancer outcomes for ER-targeted therapy

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Introduction: Identification of noninvasive biomarkers to predict tumor biology and provide prognostic indicators in ER positive breast cancer are of essence. [18F]fluoroestradiol (FES) is an FDA-approved tracer that measures functional ER expression and predicts response to ER-targeted therapy. The goal of this study was to test novel 4D analysis of dynamic volumetric FES PET patient imaging data to predict outcomes in patients with metastatic ER positive breast cancer undergoing endocrine therapy.
Methods: We utilized the Rad-Fit method, previously tested in 4D analysis of dynamic [18F]fluorodexosyglucose (FDG) breast cancer patient data sets, to identify and characterize intratumor subregions through unsupervised clustering of tumor voxels according to each voxel's time-activity information, summarized by functional principal components (Chitalia, PMID: 3367764). The analysis was performed on a set of dynamic FES PET data taken over a single body section with one or more tumor sites from a previously studied cohort of 38 patients with locally advanced or metastatic breast cancer taken prior to endocrine therapy (Linden, PMID: 1668272). The FES imaging data were analyzed using the Rad-fit method and radiomics measures were compared to patient outcome data (PFS and OS) collected in the original study. Tumors were clustered from two up to twelve clusters and the optimal clustering of each tumor was chosen by utilizing a scaled silhouette score. The mean, standard deviation, minimum, and maximum values of the 4D features of subregion sum of squared error (SSE), distance between sub regions, and

the total number of intratumor subregions were used to build prognostic models of overall survival (OS) and progression free survival (PSF). We employed Kaplan-Meyer plots to determine if our prognostic models could significantly separate our population into high and low risk groups.
Results: 4D features significantly separated this cohort into a high or low risk group for OS in a single tumor per subject (C=0.73, p=0.0018) or averaging all features per subject (C=0.65, p<0.00025) model. Mean and maximum distance are the most significant radiomic features in the Cox-PH model with all features. Combining the significant radiomic predictors of maximum and mean distance with tumor site SUV Max resulted in improved separation of high and low risk groups for the single tumor (C=0.71, p=0.0014) and average tumor (C=0.61, p=0.00018) models.
Conclusions: In this exploratory study, we show that 4D radiomic features extracted from dynamic FES PET images using the established Rad-Fit method can add significantly to prediction of OS in advanced ER positive breast cancer. Metrics of tumor subregion distance, reflective of the overall tumor heterogeneity in FES PET images, appear to perform as the best radiomic predictors for risk stratification of OS.
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PS11-06: Leveraging AI to Predict Recurrence-Free Survival in Breast Cancer Patients through Image-Based assessment of Tumor Characteristics

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Background: Early-stage hormone receptor positive (HR+) human epidermal growth factor receptor 2 negative (HER2-) breast cancer is typically treated with endocrine therapy with or without chemotherapy. While several commercially available genomic assays exist to assess risk of recurrence and guide adjuvant systemic therapy selection, these platforms are time consuming, costly, and may not fully capture disease heterogeneity when performed on a single tissue block. Radiographic imaging allows for a global assessment of tumor heterogeneity, but new approaches are needed to develop effective prognostic imaging tools. Therefore, we developed and validated an AI-based model incorporating pre-treatment DCE-MRI features with clinicopathologic features to predict recurrence risk in HR+/HER2- breast cancer.

Methods: The model was trained on 522 women (29.5% neoadjuvant chemotherapy), pooled from a multi-institutional clinical trial and one independent institution, and validated on 496 women (31.2% neoadjuvant chemotherapy), pooled from three independent health systems. All patients had HR+/HER2- breast cancer and pre-treatment DCE-MRI. The model inputs included clinicopathological data (age, race/ethnicity, T stage, N stage, grade) and DCE-MRI features that capture the spatial characteristics of the tumor and

surrounding tissues. These image-based features are computed using a validated AI-based segmentation model developed for early-stage breast cancer. Model outputs are thresholded to optimize patient stratification in the training set, resulting in categorical outputs of high or low risk. Finally, we assessed the added prognostic value of our model trained with both clinicopathologic and imaging features against the model trained with clinicopathologic features alone.

Results: In the validation cohort, patients had a median follow-up time of 4.6 years, median age of 53 years, and 16% were African American. The majority (90.3%) of women had T1-T2 disease, and 34.9% had 1-3 involved lymph nodes. The 5-year recurrence free survival (RFS) for women predicted as low risk by the model was 93.1% (95% CI: 89.0, 95.6%) compared to 78.0% (95% CI: 67.3, 85.6%) for women predicted as high risk. The unadjusted hazard ratio (HR) for the predicted high vs low recurrence risk groups was 3.8 (95% CI: 2.0, 7.2) at 5 years. After adding age, race/ethnicity, T stage, N stage, and grade into a Cox model, the adjusted HR for high vs low risk groups was 3.2 (95% CI: 1.6, 6.6). The prognostic signature was added to the clinical-only risk score both as a continuous variable and as risk groups, with significant improvement in RFS prediction ($p<0.01$) over the clinical-only model, as assessed using a likelihood ratio test.
Subgroup analyses indicated the model was prognostic regardless of age or nodal status. In women less than 50 years, the 5-year RFS for the low risk group was 89.1% versus 75.9% in the high risk group (HR: 2.8, 95% CI: 1.1, 7.1). In women greater than 50 years, the 5-year RFS was 94.6% in the low risk group vs 80.3% in the high risk group (HR: 4.4, 95% CI: 1.8, 10.6). Effective risk stratification into low risk and high risk was accomplished in both the lymph node negative (HR: 3.8, 95% CI: 1.6, 9.1) and lymph node positive patients (HR: 4.0, 95% CI: 1.5, 11.1).

Conclusions: Our AI-based prognostic tool incorporating pre-treatment DCE-MRI allows personalized treatment planning in real time in women with early-stage HR+/HER2- breast cancer. The prognostic benefit exceeded that of clinical features alone and was observed regardless of age and lymph node involvement. Further studies are ongoing to assess the ability of the model to identify patients most suitable for therapy escalation (e.g., chemotherapy, CDK 4/6 inhibitors) or de-escalation (endocrine monotherapy).

PS11-07: Predicting the response of locally advanced breast cancer to neoadjuvant therapy using MRI-based mathematical modeling of the I-SPY 2 dataset

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Introduction: Neoadjuvant therapy (NAT) is the standard of care for patients with locally advanced breast cancer; unfortunately, 30-65% of patients have residual disease after completion of NAT.1-2 Accurate and early prediction of individual patient response to NAT

is essential for clinicians to make changes to improve patient outcomes. Unlike current population-based methods, mechanism-based mathematical models make patient-specific predictions that capture tumor heterogeneity and longitudinal changes.3
We previously developed a model3 that achieved a concordance correlation coefficient (CCC) of 0.95 for total tumor cellularity (TTC) and a CCC of 0.94 for tumor volume (TV) between the observed and predicted changes in a dataset of 56 triple negative (TN) breast cancer patients.4 Here, we aim to show the generalizability of this approach by applying it to the multi-site, multi-subtype I-SPY 2 trial dataset of patients imaged with standard-of-care magnetic resonance imaging (MRI).5

Methods: I-SPY 2 is a clinical trial for locally advanced breast cancer that acquired dynamic contrast-enhanced (DCE) and diffusion-weighted (DW) MRI scans before (V1), three weeks into (V2), and after completion of (V3) the first course of NAT.5 Our subset of 93 patients includes 42 hormone (estrogen and/or progesterone) receptor-positive/human epidermal growth factor receptor 2-negative (HR+/HER2-), 22 HER2+, and 29 TN breast cancer patients.
Our mathematical model is a partial differential equation characterizing the rate of change in voxel-wise tumor cellularity, NTC($\bar{x}$,t), as a function of cell movement constrained by tissue mechanical properties, logistic proliferation, and death due to NAT.3-4 We calculated NTC($\bar{x}$,t) at each visit from the apparent diffusion coefficient maps obtained from DW-MRI scans.3 The breast and tumor tissue was segmented by clustering and defined the modeling geometry. Drug concentration was initialized as proportional to contrast agent accumulation obtained from DCE-MRI. We calibrated global drug efficacy and spatially-resolved proliferation rates to the V1 and V2 NTC($\bar{x}$,t) data and ran the calibrated model forward to make patient-specific predictions of tumor status at V3.3

Results: At the time of submission, we have completed our analysis on 77 patients, achieving CCC values of 0.94 and 0.91 between the observed and predicted TTC and TV (from V1 to V3), respectively. Fisher's z-tests between these CCC values and those achieved in the previous single site study4 indicated our model's predictive accuracy was statistically equivalent in the two data sets. In general, model predictions for TTC at V3 were highly accurate, but the predictions for TV and local cellularity at V3 were less accurate. The model was limited in capturing tumor tissue compression, which often led to overestimated TV and underestimated local cellularity at V3. However, our model was successful in predicting which voxels will have no observable tumor cells at V3. As a result, the median absolute percent difference across all voxels between the observed and predicted change in NTC($\bar{x}$,t) had a cohort mean of 11.3%.

Discussion: Our results indicate our mechanism-based mathematical model informed by early changes to NAT can accurately predict tumor status after a course of NAT, supporting the feasibility of personalized treatment using only clinically-available MRI data. For

instance, after a patient receives three NAT cycles and imaging, we can calibrate a model and make predictions for different available treatment schedules, and a clinician can use the optimal predicted schedule(s) to guide treatment.

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PS11-08: MRI improves multi-modal AI system for breast cancer diagnosis and prognosis

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Background: MRI is the most sensitive imaging modality for breast cancer detection and is not affected by breast density. Screening MRI has higher specificity than mammography in high-risk populations, including women with a family history of breast cancer, BRCA1/2 mutations, and a personal history of breast cancer. The ACS screening guidelines recommend MRI supplemented with mammography for women at high risk (≥ 20%-25% lifetime risk). MRI is also used for diagnosing breast cancer when mammography and ultrasound are inconclusive. We investigate how MRI can improve cancer detection and risk prediction with a multi-modal AI system. Current standard-of-care risk models, such as the TC model, rely solely on clinical variables and do not account for the rich information in imaging data. Other existing AI systems typically analyze single imaging modality, usually mammography. Our multi-modal transformer (MMT) learns from longitudinal imaging data of multiple modalities, FFDM, DBT, US and MRI.

Methods: We utilized the NYU Multimodal Breast Cancer Dataset, comprising 1,372,455 exams from 298,670 patients (age 30-108, mean 56.55 years, SD 12.00 years) between 2010 and 2022, for MMT training and evaluation. Our objective is to predict whether a patient currently has cancer and, if not, assess the risk of developing cancer in the future, incorporating data from all available, present and prior, breast imaging. Our method involves three steps: (1) training modality-specific feature extractors separately to generate image-level and patch-level feature embeddings; (2) combining image embeddings with additional variables including age, modality, study date and view; (3) feeding the combined embeddings into a transformer for cancer prediction. The model outputs two predictions, the patient's probability of having cancer and the patient’s risk of getting cancer within 5 years.

Results We evaluated our model on a subgroup of patients who had at least one MRI in their records. The MMT model achieved an AUROC of 0.943 (95% CI: 0.935, 0.950) for cancer detection and 0.796 (95% CI: 0.765, 0.826) for 5-year risk prediction across all modalities. We separately compared our model’s AUROC on non-MRI exams and MRI exams with the corresponding baselines. For non-MRI exams, the MMT model with MRI data, achieved an AUROC of 0.939 (95% CI: 0.929, 0.948) for cancer detection and 0.778 (95% CI: 0.742,

0.810) for 5-year risk prediction, which improved the baseline MMT model without MRI by 0.024 and 0.044 (two-sided DeLong's test, P < 0.01 for both) respectively. These results demonstrate that incorporating MRI improves both cancer detection and risk prediction for non-MRI exams. For MRI exams, the MMT model achieved an AUROC of 0.947 (95% CI: 0.934, 0.958) for cancer detection, improving by 0.029 (two-sided Delong's test, P < 0.01) compared to an MRI-only baseline. This indicates that including prior imaging enhances the effectiveness of MRI in detecting cancer. However, for risk prediction on MRI exams, there was no significant improvement (ΔAUROC 0.004: two-sided DeLong's test, P = 0.94). Additionally, MMT's risk prediction AUROC on MRI exams was lower than other modalities (0.719, 95% CI: 0.615, 0.813), suggesting that MRI alone has less predictive power for future risk.

PS11-09: Improving risk estimation for women with dense breasts

Shu (Joy) Jiang, Debbie Bennett, Graham Colditz

Purpose: Women with dense breasts on screening mammography present a clinical challenge as the USPSTF guidance is lacking on recommendations for supplemental screening. Therefore, we aimed to apply a dynamic risk prediction model that incorporates current and prior screening mammogram images to better refine risk among women with dense breasts.
Methods We analyzed a prospective WashU clinic-based cohort of 10,099 cancer-free women at cohort entry (recruited from women attending routine screening between Nov 3, 2008 and February 2012). Follow-up through October 31, 2020 identified 478 pathology-confirmed breast cancer cases. The cohort included 27% non-Hispanic Black women. An external validation cohort included 18,360 women screened at Emory from January 1, 2013 and followed through December 31, 2020. This included 42% non-Hispanic Black women and 332 pathology-confirmed breast cancer cases.
We trained a dynamic model using repeated screening mammograms at WashU to predict 5-year risk. We then applied the model to the external validation data to evaluate discrimination performance measured by AUC and calibrated to US incidence from SEER.
Results In the overall external Emory validation data set, 48.6% of women who remained free from breast cancer were classified as dense (BI-RADS C or D) and 49.1% of women who developed breast cancer (cases) were classified as dense. Time to diagnosis of breast cancer was evenly distributed from first available mammogram to 8 years of repeated screening. Using 3 years of prior mammogram images available at the current screening visit, we obtained a 5-year AUC of 0.80 (95% CI, 0.78, 0.83) for prediction of subsequent breast cancer risk when controlling for age and BI-RADS density. This represents a significant improvement over only using the current visit mammogram AUC 0.74 (95% CI, 0.71, 0.77) for risk estimation (p<0.01). We repeated analysis in women with dense breasts (n cases = 163) and the 5 year AUC was 0.79 (95%CI 0.75 to 0.84) for risk prediction. When calibrated to SEER incidence rates, a risk ratio of 15.7 was observed comparing high (>0.04%) to very low (<0.003%) 5-year risk. The dynamic model classified 14.8% of the women with dense breasts as high risk among whom 53% of all cases were diagnosed within 5 years. The

dynamic model effectively identifies a subgroup of women with dense breasts who are at high risk and exceeding lifetime risk thresholds in current guidelines for risk reduction. This subgroup of women with dense breasts would be the most likely to benefit from additional imaging. Identification of very low-risk groups of women with dense breasts would also allow for appropriate reassurance and standard mammography screening protocols without need for supplemental screening recommendations.
Conclusion Among women with dense breasts, incorporating information from previous screening mammogram images improves 5-year breast cancer risk prediction beyond static models used in clinics today. This model can identify those women with dense breasts who are at high risk of developing breast cancer, who might most clearly benefit from supplemental screening or risk reduction strategies.

PS12-01: Olaparib-induced early dynamics of tumor immune microenvironment in triple-negative or ER-low breast cancer: insights from a window of opportunity MEDIOLA trial of olaparib and durvalumab with serial biopsies

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Background: Increasing evidence indicates that PARP inhibitors can modulate the tumor microenvironment (TME), making them promising candidates for combination therapy with immune checkpoint inhibitors (ICIs). To demonstrate the clinical efficacy and biological impact of PARP inhibitor treatment followed by ICIs, we conducted a window of opportunity MEDIOLA trial with olaparib and durvalumab before standard neoadjuvant chemotherapy (NAC) in stage II/III triple negative breast cancer (TNBC) or ER-low breast cancer (NCT03594396).

Methods: Olaparib (300mg bid) was given continuously for 4 weeks, with a single 1500 mg dose of durvalumab on day 15, followed by standard NAC (4 cycles of doxorubicin /cyclophosphamide, then 4 cycles of docetaxel). FDG-PET/MRI scans were performed at baseline, 2 weeks, and 4 weeks; CT scans at baseline and 4 weeks. Serial tumor biopsies were collected at baseline, after 2 weeks of olaparib (before durvalumab) (on treatment 1; OT1), and 2 weeks post-durvalumab after 4 weeks of olaparib (OT2). Whole exome sequencing (WES) and functional RAD51 foci assays were conducted on fresh frozen baseline biopsies. Immune cell dynamics were analyzed using multiplex IHC (mIHC) on baseline, OT1, and OT2 samples.

Results: Fifty-four female patients (median age 40, range 24-68) were enrolled. Pathologic complete response (pCR) was achieved in 38 patients (70.4%) (Im SA et al. SABCS 2021). Pathogenic germline BRCA1/2 (gBRCA1/2) mutations were present in 17/54 (31.5%), and functional RAD51 foci assays showed homologous recombination deficiency (fHRD) in

27/54 (50.0%). fHRD was significantly associated with early metabolic response to olaparib by FDG-PET at 2 weeks (63.0% vs 25.9%; p = 0.006), and borderline association after 4 weeks of olaparib and durvalumab (85.2% vs 63.0%; p = 0.062). fHRD was linked to RECIST response by CT at 4 weeks (63.0% vs 33.3%; p = 0.029), and pCR rate was significantly associated with metabolic and RECIST responses at 4 weeks (p < 0.001 and 0.027, respectively). WES revealed mutations including TP53 (79.6%), PIK3CA (11.1%), PTEN (7.4%), RB1 (5.6%), and PALB2 (1.9%). There was significantly more infiltration of CD3+, CD4+, and CD8+ T-cells at OT1 and OT2 compared to baseline (all adjusted p < 0.05). Surprisingly, patients with metabolic response at either 2 or 4 weeks and those who achieved pCR showed unique temporal changes in the TME. The boosted infiltration of exhausted CD8+PD-1+ and CD8+TIM3+ T-cells, along with activated CD8+CD25+ T-cells, was prominent at OT1 timepoint in metabolic and pathologic responders (all adjusted p < 0.001). This finding suggests that olaparib can induce significant immune modulation in CD8+ T-cell subsets as early as 2 weeks after treatment initiation. Moreover, both CD8+ and CD4+CXCL13+ T-cells expanded in responsive tumors at OT1 (all adjusted p < 0.01), underscoring the crucial role of CD4+ helper T-cells during olaparib-mediated immune modulation. Most of these changes observed at OT1 persisted through OT2 biopsies. However, immune cell densities at baseline by themselves showed no correlation with pCR or metabolic/radiological responses. From a tumor biology perspective, baseline CD4+CXCL13+ and CD20+CXCR5+ cells were higher in fHRD tumors (all adjusted p < 0.05). Temporal expansions of CD8+PD1+ and CD8+TIM3+ T-cells at OT1 were more pronounced in those with fHRD or gBRCA1/2 mutations, suggesting a significant influence of fHRD at baseline on the TME.

Conclusions: The unprecedentedly high rate of pCR in this study highlights the enhanced anti-tumor effects mediated by olaparib-induced immune modulation followed by ICI. Through mIHC, we demonstrated that temporal changes in the TME were reflective of early response and pCR and in part dependent on HRD status. More in-depth analyses of TME dynamics linked with genomic and transcriptomic information are underway, which may provide further insights into tumor cell characteristics and their temporal interactions with TME.

PS12-02: CXCL11 in the tumor immune microenvironment modulates resistance to endocrine therapy in hormone receptor-positive breast cancer

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Despite advances in the treatment of hormone receptor-positive (HR+) breast cancer (BC), this disease subtype continues to be a major cause of mortality. The tumor immune microenvironment (TIME) plays a crucial yet not fully understood role in the natural history of HR+ BC. This study aims to clarify the impact of the TIME on HR+/HER2-negative BC response to antiestrogen treatment.

We collected samples from postmenopausal patients with stage I-III HR+ BC, treated for 2-3 weeks with estrogen deprivation (ED) induced by letrozole. Treatment response was categorized based on the Ki67 scores at the time of the surgery (on treatment tumors, onTx): ED-sensitive (ED-S) if natural log (ln) of the Ki67 score ≤1.0 (i.e. ≤2.7% Ki67+ cells) vs. ED-resistant (ED-R) if ln ≥2.0 (i.e. ≥7.4% Ki67+ cells).

Analysis of H&E tumor sections revealed that stromal tumor-infiltrating lymphocytes (sTILs) were significantly higher in ED-R compared to ED-S tumors ($p=0.0001$). Next, using both cyclic immunofluorescence (cycIF) and spatial transcriptomics, we found that the TIME of ED-R breast tumors was primarily infiltrated by CD8+ T cells, with their abundance being significantly higher in ED-R vs. ED-S tumors both prior to and after letrozole treatment ($p<0.001$ in preTx and $p<0.001$ in onTx tumors). In ED-R tumors specifically, CD8+ T cells were further enriched upon estrogen suppression ($p<0.001$), while Tregs decreased upon treatment ($p<0.01$).

We next utilized a co-culture system to determine whether CD8+T cells can stimulate the proliferation of HR+BC cells. MCF7 and T47D cells were seeded in estrogen-free medium (EFM) in multi-well plates, with transwell inserts containing CD8+ T cells separated by a membrane with 3.0-μm pores. In this system, the cancer and T cells share the conditioned medium, thus allowing for paracrine cell-cell communication without direct contact between T cells and cancer cells. MCF7 and T47D exhibited 1.5- to 2.0-fold higher estrogen-independent proliferation when co-cultured with CD8+T cells ($p=0.0115$ and $p=0.0003$, respectively).

Immune cell recruitment to cancer sites occurs through chemotactic cytokines. Thus, we investigated chemokine expression in RNA-seq data of ED-R vs. ED-S tumors. CXCL9, CXCL10, and CXCL11 chemokines were significantly enriched in ED-R compared to ED-S tumors ($p<0.0001$ for all genes; both preTx and onTx). Likewise, their receptor CXCR3 was similarly enriched in ED-R vs. ED-S tumors (preTx $p=0.0106$, onTx $p<0.001$). Publicly available datasets of patients with HR+BC treated with antiestrogen therapy showed that higher expression of CXCL9 (HR 1.36; $p=0.016$), CXCL10 (HR 1.71; $p<0.0001$), and CXCL11 (HR 1.5; $p=0.0016$) is associated with shorter relapse-free survival.

We next investigated whether these chemokines play a causal role in resistance to ED using hormone-dependent MCF7 and T47D cells. Treatment with recombinant CXCL11 markedly upregulated phospho-AKT and phospho-ERK1/2 and stimulated estrogen-independent proliferation of both cell lines ($p<0.0001$). In addition, CXCL11 levels measured by ELISA increased 1.5- to 2.0-fold in media conditioned by breast cancer cells for 6 days when co-cultured with CD8+T cells compared to breast cancer cells alone.

CXCR3 and CXCR7 are key receptors that interact with CXCL11. To assess their role in CXCL11-induced signaling, MCF7 and T47D cells were treated with a CXCR3 inhibitor (AMG487) and a CXCR7 antagonist, alone or in combination, in EFM with CXCL11. In

particular, AMG487 strongly inhibited CXCL11-induced p-AKT/p-ERK activation in MCF7, while the effect was only partial in T47D cells.
Taken together, our results suggest that CXCL11, a CD8+T cell-associated chemokine expressed in the breast TIME, promotes resistance to estrogen deprivation in early HR+BC, possibly through CXCR3. Further studies will investigate the use of inhibitors of CXCL11 receptors as a therapeutic strategy to reverse the TIME-mediated antiestrogen resistance.

PS12-03: Molecular characterization of the NeoPalAna Endocrine Resistant (ET-R) Cohort: implications for CDK4/6 inhibitor (CDK4/6i) and ET resistance mechanisms in primary estrogen receptor positive (ER+) and HER2 negative (HER2-) breast cancer (BC)

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Background: Resistance to ET is a major contributor to BC mortality. CDK4/6i overcomes ET-R. However, some ER+/HER2- BC are intrinsically resistant to ET/CDK4/6i. Here we report the primary endpoint of complete cell cycle arrest (CCCA) and correlative studies of the NeoPalAna (NCT01723774) ET-R Cohort to examine resistance biomarkers for palbociclib (PAL) + anastrozole (ANA).

Methods: NeoPalAna is a multi-cohort single arm phase 2 neoadjuvant endocrine (NET) trial of PAL/ANA in clinical stage II/III ER+/HER2- BC. The ET-R Cohort enrolled patients (pts) with tumor Ki67 >10% after at least 4 weeks of standard (SOC) NET to receive PAL/ANA for 4 28-day cycles before surgery if Ki67 was ≤10% on C1D15 biopsy (Ki67C1D15). Pts switched to chemotherapy or underwent surgery if Ki67C1D15 >10%. The primary endpoint was C1D15 CCCA (Ki67C1D15 ≤2.7%). Simon's optimal 2-stage design (stage 1 n=12, stage 2 n=25) was used to detect a CCCA rate ≥20% vs ≤5% (power 0.9, alpha 0.1).
Correlative endpoints included genomic and proteomic analyses of tumor tissues in relation to response by Ki67C1D15 ≤10% (PAL-Sensitive or PAL-S) or >10% (PAL-Resistant or Pal-R). Tumor biopsies prior to SOC ET (Baseline or BL), before PAL/ANA (C1D1), and at C1D15 were analyzed for whole exome sequencing (WES) for 27 pts at the earliest timepoint (13 BL, 5 at C1D1, and 9 at C1D15), RNAseq for 33 pts at all possible timepoints (BL n=20, C1D1 n=15, C1D15 n=28) and global proteomic analysis on 16 C1D15 frozen cores (11 PAL-S and 5 PAL-R). Differentially expressed genes (DEGs) and Hallmark gene set enrichment analyses (GSEA) were performed controlling false discovery rate (FDR).

Results: Between Aug 2016 and Mar 2021, 34 pts were enrolled, with a median age 53 (30.4~77.6) years and a median 6 weeks of prior NET. This ET-R cohort was enriched for high grade tumors (3% G1, 47% G2, 47% G3) and mutations implicated in ET resistance. The top 6 mutated genes included TP53 (41%), PIK3CA (33%), NCOR1 (22%), NF1 (22%), RUNX1 (19%) and CDH1 (19%), similar between PAL-S vs -R BCs. CCCA rate was 57.6% (19/33; 95% CI: 39.2-74.5%). The trial met its primary endpoint (exact binomial test $p<0.0001$ against null CCCA of 5%).
At BL, PAM50 subtype was determined for 20 pts with RNAseq performed, which revealed 8 LumA (all PAL-S), 8 LumB (5 PAL-S, 3 PAL-R) and 4 non-Lum BCs (3 Basal and 1 HER2 E, all PAL-R). Principal component analysis (PCA) of 630 DEGs (FDR adj. $p\leq0.5$) at BL demonstrated tight clustering of PAL-S BCs, with PAL-R BCs appeared to scatter away in a gradient. Top positively enriched DEGs in PAL-R BCs included CCNE1, IDO1, IRF4, BRCA2, EZH2, and several immune cell markers. Top upregulated signaling pathways in PAL-R BCs included interferon (IFN) γ signaling, E2F targets, allograft rejection, MYC targets, G2M checkpoint, mTORC1 and TNF signaling, inflammatory response, IL6-JAK-STAT, and IFN α signaling. Estrogen response pathways were significantly downregulated in PAL-R BCs. Comparing C1 and BL, ET upregulated multiple pathways including allograft rejection, EMT, IFN γ response, etc. Adding PAL led to significant reductions in G2M checkpoints, E2F and MYC targets, IFN, mTOR pathways by both RNA and proteomic analyses. However, these pathways remain highly upregulated in PAL-R vs PAL-S at C1D15. CIBERSORT analysis which deconvoluted bulk RNAseq to estimate and compare cell type fractions at C1D15 and BL indicated a significant increase in several immune cell types including CD8 T cells, Plasma cells, M1 and M2 macrophages, and monocytes in PAL-S but not PAL-R BCs.

Conclusion: Neoadjuvant PAL/ANA led to 58% CCCA at C1D15 in ET-R BC. Specific oncogenic pathways and immune tolerance markers, such as IDO1, were enriched in PAL-R. Our data also suggest immune modulatory effects of CDK4/6i/ET in association with ET/PAL response.

PS12-04: HDACi combined with anthracycline elicits interactions between MHC-II+ triple-negative breast cancer and CD69+CD4+Trm orchestrating synergistic immunotherapy

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Background: Early TNBC (eTNBC) lacks biomarkers for predicting the benefits of immune checkpoint blockade (ICB). Therefore, identifying indicators for the high-benefit patients and finding synergistic target for low-benefit populations are crucial. Tumor-associated MHC-II (tsMHC-II) are linked with the benefits of ICB in eTNBC, though the mechanisms remain to be explored. This study aimed to research the interaction mechanisms and translational targets between tsMHC-II and CD4+ T cell subgroups by NeoTennis clinical trial cohorts.

Method: RNA-seq, mIHC, and spatial analysis were used to quantify MHC expression in the tumor and spatial relations within the TME. In vivo murine cohorts combined with CyTOF were utilized to elucidate the mechanisms of tsMHC-II and CD4+ Trm cells in chemo-ICB. Co-culture of cell line, organoid, and T cell were used to investigate the regulatory factors of tsMHC-II. TMA and sc-RNA seq were used to explore the clinical significance, molecular characteristics of MHC-II+ tumor.
Results: The NeoTennis trial included eTNBC patients in a two-phase clinical trial involving anthracycline induction followed by sequential nab-paclitaxel and PD-1 inhibitor (Toripalimab). Analysis of baseline and anthracycline-induced biopsy samples using mIHC (Pan-CK, HLA-A, HLA-DR, CD4, CD8, CD69, Foxp3, T-bet) allowed for the clustering of patients based on MHC-I and MHC-II expression in the tumor epithelium, resulting in four subtypes. The ROC predictive model based on the outcomes and MHC subtypes was constructed to find that the MHC-I+ MHC-II+ subtype and tsMHC-II had the highest AUC scores (0.811 and 0.848, respectively), whereas the CPS was only 0.419. Meanwhile, tsMHC-II expression in the pCR group was significantly higher than in the Non-pCR group at baseline while MHC-I showed no difference. Spatial analysis revealed that CD69+CD4+ resident-memory T cells (Trm) had the strongest spatial association with MHC-II+ tumors, with this relationship being most prevalent in the pCR group. Dynamic analysis of changes before and after anthracycline showed that the proportion of MHC-II+ tumors and CD4+ Trm cells increased in the pCR group after treatment. In vivo models combined with CyTOF revealed that there was a significant increase in CD4+ Trm cells, Th1 cells, and iNOS+ macrophages in the tumor after anthracycline. After knocking out the MHC-II molecules in tumor cells, the anthracycline-induced increase in CD4+ Trm cells and Th1 cells was reduced, and the tumors grew faster than the control group. Conversely, when CD4+ T cells were depleted following doxorubicin-treated, the efficacy of sequential anti-PD1 was diminished. The above findings showed that anthracyclines could upregulate tsMHC-II, leading to the production of CD4+ Trm. Analysis of TMA from 300 cases in FDSCC and sc-RNA seq of TNBC confirmed that MHC-II+ TNBC was associated with an extremely good prognosis (HR=0.28, 95%CI=0.12-0.64; p=0.003), while 22.4% of TNBC patients had high tsMHC-II. Moreover, the tsMHC-II+ tumor cells exhibited upregulated IFN-gamma response. RNA-seq showed that patients resistant to anthracycline-induced MHC-II upregulation had highly active HDAC. In vitro, HDAC inhibitors (Entinostat) could upregulate MHC-II in TNBC cell line and PDOs, thereby promoting increased contact with CD4+ T cells. In vivo, combining anthracyclines with Entinostat increased the infiltration of CD4+ Trm cells, Th1 cells, and CD8+ T cells, and inhibited tumor proliferation, after which, sequential anti-PD1 therapy further inhibited tumor growth for a longer time.
Conclusion eTNBC patients with high tsMHC-II expression benefited from neoadjuvant anthracycline with sequential anti-PD1 ICB. Anthracyclines could promote the upregulation of tsMHC-II in tumor cells and induce the production of CD4+ Trm. For anthracyclines-resistant patients, combining neoadjuvant anthracycline with HDACi could significantly enhance the effectiveness of ICB.

PS12-05: PARP7 inhibition combined with radiotherapy overcomes ICI resistance in breast cancer

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Background: Radiotherapy (RT) has shown promise in overcoming immune checkpoint inhibitor (ICI) resistance in breast cancer preclinical models and early-stage clinical trials. However, RT/ICI synergy is not observed in all tumors and the induction of systemic anti-tumor (i.e., "abscopal") responses is relatively infrequent. The goal of this study was to identify targetable pathways that may guide patient selection and augment RT/ICI synergy by screening a panel of clinically representative breast cancer immune-competent mouse models. Our studies identify the mono-ADP-ribosyltransferase PARP7 as a biomarker of RT/ICI responsiveness that, when inhibited, results in significantly greater induction of interferon-stimulated genes (ISGs) and systemic anti-tumor immune responses.
Methods: We profiled four Trp53-/- breast cancer syngeneic allograft tumor models (2250L, 2208L, 2336R, 2225L) in vivo for RT/ICI abscopal effects. Female BALB/c mice were bilaterally injected in the fourth mammary fat pad with tumor cells. Treatment began once the tumor reached 6mm, with mice being randomly divided into 4 groups: non-treated (NT), RT (3 x 8Gy), ICI (anti-PD-1, anti-CTLA4, both at 10mg/kg, 2x/week), and RT+ICI. RT was administered only to the left tumor and abscopal effects were based on measurement of the contralateral tumor relative to NT animals on day 10 after treatment initiation. Additional treatment arms were added for 2250L tumor: PARP7i (RBN-2397, 30mg/kg, daily for 10 days), RT+PARP7i, ICI+PARP7i, and RT+ICI+PARP7i. mRNA-sequencing was performed on the four syngeneic NT tumor models or 48h after treatment with RT (8Gy) +/- PARP1/2i (Olaparib, 1uM), using Illumina TruSeq stranded mRNA library prep on a NextSeq2000. DESeq2 was used for differential gene expression analysis of normalized read counts. A panel of three human (MDA-MB-231, MDA-MB-436, and MCF7) and six murine breast cancer models (2250L, 2336R, 2208L, 2225L, EO771, and mWnt) were profiled in vitro for ISG expression 48hr following treatment with RT (8Gy) +/- PARP7i (RBN-2397, 50nM) using RT-qPCR. Cells were transfected with synthetic nucleic acids using lipofectamine 3000 and RNA was isolated 6h post treatment for RT-qPCR. Statistical comparisons used two-tailed t-tests with a $p<0.05$ significance threshold.
Results: All four syngeneic breast cancer models were ICI resistant, and only two demonstrated abscopal response when treated with RT/ICI (2250L, 2208L). RNA-sequencing of NT and RT samples identified genes in the PARP family as being significantly upregulated between abscopal responding models (2250L, 2208L) and non-abscopal models (2336R, 2225L), with PARP7 being uniformly higher expressed among the abscopal competent lines ($p <0.000001$). RT+PARP7i induced significantly greater IFNB1 levels compared to RT+PARP1/2i in 2250L cells (85-fold vs 6-fold, $p<0.0001$). Synergistic induction of ISGs by RT+PARP7i was observed across the panel of human and murine models (response additivity scores of 1.38-12.94). PARP7i enhanced IFNB1 induction in response to both ISD90 and poly(I:C) suggesting that PARP7 suppresses both RNA- and DNA- sensing pathways. In vivo, RT+ICI+PARP7i can overcome ICI resistance and induce a

systemic immune response in the 2250L model, resulting in significantly smaller contralateral tumors compared to RT+ICI alone (p=.0483).
Conclusion: Breast cancers that express PARP7 may be targetable with PARP7i in combination with RT/ICI to enhance ISG expression in the tumor microenvironment and overcome ICI resistance. Future studies will characterize the mechanism of this effect and its potential clinical translation.

PS12-06: An In Situ Tumor Vaccine Against Triple Negative Breast Cancer

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Objective: Immunotherapies offer limited clinical benefit as a monotherapy in triple negative breast cancer (TNBC) and require the addition of cytotoxic chemotherapy to improve responses in patients. Resulting from the Keynote-522 clinical trial, the current standard-of-care chemo-immunotherapy regimen for localized TNBC patients causes Grade 3 or higher toxicity in 76% of patients with chemotherapy contributing to over 90% of the observed toxicity. Therefore, there is an urgent need to develop novel chemotherapy-free immunotherapy regimens with superior tumor control and minimal toxicity. Although radiation therapy (RT) has traditionally been used as an adjuvant local treatment, in rare cases it can serve as an in situ tumor vaccine by eliciting a systemic anti-tumor immune response through the “abscopal” effect. The goal of this study is to identify novel RT and targeted therapy combinations that serve as a potent anti-tumor vaccine in TNBC patients with minimal toxicity. Methods: A 4T1 syngeneic, orthotopic mouse model of TNBC was used to screen a library of 19 epigenetic drugs in combination with tumor-directed RT in vivo. The efficacy of OTX015, the top hit from the screen, in combination with RT was validated and compared to each treatment component alone and a chemo-immunotherapy regimen of doxorubicin + paclitaxel + PD1 inhibition. Tumor-free mice following treatment were administered a tumor re-challenge in the contralateral mammary fat pad to assess the development of tumor-specific immune memory. Cytotoxic T cell depletion studies were performed to establish the role of CD8+ T cells in mediating the effects of the RT + OTX015. Results: We identified OTX015, a bromodomain and extraterminal domain (BET) inhibitor, as the top hit in our in vivo drug screen. RT (8 Gy x 2 on days 2 and 4) + OTX015 (100 mg/kg by oral gavage on days 1-4) promoted significant tumor control relative to vehicle ($p < 0.0001$), OTX015 alone ($p < 0.0001$), RT alone ($p = 0.0015$) and the chemo-immunotherapy regimen ($p = 0.0129$). 2/8 mice treated with RT + OTX015 not only showed complete tumor regression but also resisted tumor re-challenge. Mice treated with RT + OTX015 also exhibited significant reduction in metastatic burden. CD8+ T cell depletion abrogated both the local and systemic tumor control afforded by RT + OTX015. Conclusions: Using an in vivo drug screen, we identified that a short course of tumor-directed RT in combination with pharmacological BET inhibition serves as an in situ tumor vaccine. Our results support that RT + OTX015 offers superior tumor control relative to the current standard-of-care chemo-immunotherapy regimen and elicits immunological memory in a cytotoxic T cell dependent fashion in a pre-clinical model of TNBC.

PS12-07: DNA methyltransferase 3A (DNMT3A) protein expression in triple-negative breast cancer (TNBC): Impact on clinical outcomes, gene expression, and tumor microenvironment

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Background: Given the heterogeneity of TNBC, identification of biologically and clinically distinct TNBC subtypes with unique therapeutic vulnerabilities is critically needed. Preclinically, DNMT3A protein expression is predictive of sensitivity to DNMT inhibitors (DNMTi). Additionally, treatment with DNMTi enhances antitumor immune responses. Ongoing (NCT05673200) and completed (NCT02957968) trials are evaluating the addition of DNMTi to chemoimmunotherapy for TNBC. However, it is unknown whether DNMT3A expression is associated with distinct TNBC biology or clinical outcomes.
Methods: We measured DNMT3A protein using immunohistochemistry in early-stage TNBC tumors and evaluated its association with clinicopathologic characteristics, gene expression (RNASeq), spatial tumor immune microenvironment features (52 proteins using Nanostring GeoMx® Digital Spatial Profiler [DSP]), and recurrence-free survival (RFS). DNMT3A expression was categorized as negative (no staining) or positive (high: moderate/strong staining in >25% of nuclei; low/intermediate: weak staining in any % nuclei or moderate/strong staining in ≤25% of nuclei).
Results: Among 345 patients, the median age was 54 years; most tumors were > 2 cm (53%) and N0 (62%). 281 (81%) were DNMT3A+ (high: 127 [37%], low/intermediate: 154 [45%]). DNMT3A+ TNBC were more often grade 3 (94% vs 86%, p = 0.037), with Ki-67 >15% (85% vs 67%, p = 0.002), and N+ (40% vs 26%, p= 0.043). There were no differences in age, menopausal status, tumor size, PD-L1 or stromal tumor-infiltrating lymphocytes (sTILs) between DNMT3A+ or DNMT3A- TNBC. To assess the impact of DNMT3A on the natural history of early TNBC, we focused our survival analyses on a subset of 103 (30%) patients who did not receive systemic therapy. DNMT3A expression was associated with worse RFS on a multivariable model adjusting for tumor size, nodal status, and sTILs (aHR 4.6, 95% CI 1.05-19.96, p=0.043; univariable results: HR 3.3; 95% CI: 0.79, 13.99; p = 0.102). The 5-year RFS (95% CI) of DNMT3A+ TNBC without chemotherapy was 66% (53-76), compared with 93% (59-99) for DNMT3A- TNBC. While TNBC with sTILs ≥ 30% had favorable 5-year RFS regardless of DNMT3A expression (86% DNMT3A+ vs 100% DNMT3A-), TNBC with sTILs <30% had particularly poor outcomes when also expressing DNMT3A (5y RFS 54% [38-67] for sTIL low and DNMT3A+ TNBC vs 90% [47-99] for sTIL low and DNMT3A- TNBC). RNA-Seq data (available for 154 [63%] of 345 tumors, regardless of receipt of chemotherapy) revealed differential expression of 234 genes between DNMT3A+ (n=126) and DNMT3A- (n=28) TNBC (e.g. MUC19, CEACAM5, and ZNF606, among others). GeoMx® DSP data (available for 289 [84%] tumors) showed that DNMT3A+ TNBC exhibited lower expression of CD3, CD4, CD8 (T cells) and granzyme B in the tumor compartment; lower expression of HLA-DR (antigen presentation), CD56 (NK cell), FAP

alpha, SMA (fibroblasts) and CTLA4 (immune checkpoint) in the stromal compartment; and lower expression of CD127 (memory T cell), CD11c, CD14, CD68 (myeloid/macrophages), CD34 (stem cell), EpCAM and PanCK (epithelium), fibronectin (extracellular matrix) and TIM3 (immune checkpoint) in both the tumor and stromal compartments. Expression of Ki-67 was higher in both the tumor and stromal compartments of DNMT3A+ TNBC.
Conclusions: DNMT3A expression was associated with worse clinical outcomes in TNBC (particularly in sTIL-low tumors), higher tumor proliferation, and diverging gene expression and tumor immune microenvironment profiles. While no differences in sTILs were noted by H&E quantification according to DNMT3A status, GeoMX DSP analyses revealed an immunosuppressed tumor and stromal microenvironment in DNMT3A+ tumors, characterized by decreased T, NK, myeloid, and antigen presentation markers. These data support targeting DNMT along with immunotherapy in DNMT3A+ TNBC as is currently being evaluated in NCT05673200.

PS12-08: Biological sex-linked immune modulation but not cross-sex estrogen therapy reduces the efficacy of PARP inhibition in the treatment of Brca1 breast tumors.

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Background: Breast cancer (BC) treatment for trans feminine individuals is complicated by their use of gender-affirming estrogen therapy (ET). There is no clinical guideline to manage ET during BC treatment in these medically underserved patients. It is unknown whether continuing ET during BC treatment affects a successful outcome. We used a preclinical model to study the response of Brca1mut breast tumor to a PARP-inhibitor, in the presence of ET.
Method: Tumor survival study using immune competent FVB/NJ mice was conducted by implanting fragments of K14-Cre Brca1f/fp53f/f tumors into female (n=45), male (n=28), and male mice receiving 60 µg of weekly subcutaneous ET (n=44). When the tumor reached 5 mm, male mice receiving ET were first randomized to stop or continue ET, then mice in each arm were randomized to be treated with a PARP inhibitor (olaparib) or were left untreated until study endpoint where the tumor reached 20 mm. A 10-day study in FVB/NJ mice was set up with the same eight arms as above (n=5/arm) whereby tumors were harvested after 10 days of olaparib treatment for RNASeq. Finally, to test the hypothesis that the male immune system modulated PARP inhibition, we conducted a six-arm tumor survival study in immune deficient NSG mice (n=15/arm): female+olaparib, female, male+olaparib, male, male+continued ET+olaparib, male+ continued ET. Log-rank test was used to compare tumor-specific survival. Differential gene expression was analyzed using limma. Gene set enrichment was conducted using mouse Hallmark gene sets and Camera.
Results: Only arms treated with olaparib were reported here. In the tumor survival study using FVB/NJ mice, females had the longest median survival time of 103 days. Males had

significantly a shorter survival than females (75 days, p=0.02). Males that stopped ET had significantly shorter survival (38.5 days) than females (p=0.003) and males (p=0.004). Males that continued ET also had shorter survival (50 days) than females (p=0.001) and males (p=0.16). There was no difference between males that stopped and continued ET (p=0.56). There was no survival difference between untreated study arms (median 14 to 19 days; p>0.05).
No gene was differentially expressed between FVB/NJ male and female mice at FDR<0.05. Interferon alpha and gamma response gene sets were significantly upregulated in males compared to females while six gene sets (xenobiotic metabolism, bile acid metabolism, heme metabolism, reactive oxygen species, peroxisomes, and adipogenesis) were significantly downregulated (FDR<0.05). Tumors from males that continued ET were significantly upregulated for proliferative gene sets—G2M checkpoint, E2F targets, MYC targets, and mitotic spindle—compared to males, males that stopped ET, and females (FDR<0.05). Tumors from males that stopped ET showed decreased proliferation and increased inflammatory responses when compared to males, males that continued ET, and females (19 to 23 significantly enriched gene sets; FDR<0.05).
The differences in tumor-specific survival previously observed in FVB/NJ mice were ablated in NSG mice. There was no difference between NSG females, males, and males that continued ET (median 69 days, all p>0.05).
Conclusion: Male mice have poorer outcomes with PARP-inhibition than females, irrespective of cross-sex hormone treatment. Our survival study and transcriptomic data in immune competent mice suggested that although ET had profound molecular effects in the tumors, ET-modulated effects were not as strong as the effect of biological sex on olaparib efficacy. Our study in immune deficient mice confirmed that the male innate immune system played a major role in conferring poorer response to PARP-inhibition in males.

PS12-09: Neoadjuvant pembrolizumab or placebo plus chemotherapy followed by adjuvant pembrolizumab or placebo for high-risk, early-stage triple-negative breast cancer: Overall survival and subgroup results from the phase 3 KEYNOTE-522 study

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Background: In the phase 3 KEYNOTE-522 study (NCT03036488), neoadjuvant pembrolizumab + chemotherapy followed by adjuvant pembrolizumab showed statistically significant and clinically meaningful improvements in the dual primary endpoints of pathological complete response and event-free survival versus neoadjuvant placebo + chemotherapy followed by adjuvant placebo in patients with high-risk, early-stage triple-negative breast cancer (TNBC). At the prespecified analysis of the key secondary endpoint of overall survival (OS) (data cutoff, 22 Mar 2024), the hazard ratio (HR) for OS favored pembrolizumab (0.66 [95% CI], 0.50-0.87; P=0.0015). KEYNOTE-522 met the success

criterion for OS, with a P value that crossed the prespecified significance boundary of 0.00503. Here, we present OS results in key prespecified subgroups, including patients by residual cancer burden (RCB) categories. Post-hoc analyses of OS in patients with T2N0 disease at baseline are also provided.
Methods: Eligible patients with previously untreated, nonmetastatic, centrally confirmed TNBC (stage T1c N1-2 or T2-4 N0-2 per American Joint Committee on Cancer) were randomized 2:1 to neoadjuvant pembrolizumab 200 mg Q3W or placebo, both given with 4 cycles of paclitaxel + carboplatin, followed by 4 cycles of doxorubicin or epirubicin + cyclophosphamide. After definitive surgery, patients received adjuvant pembrolizumab or placebo for 9 cycles or until recurrence or unacceptable toxicity. The Kaplan-Meier method was used to estimate the subgroups for OS. HRs (95% CIs) for OS subgroups were based on the Cox regression model with Efron's method of tie handling with treatment as a covariate. RCB was assessed by the local pathologist at the time of definitive surgery.
Results: 1174 patients were randomized to pembrolizumab (n=784) or placebo (n=390). At the prespecified data cutoff for OS (22 Mar 2024), median follow-up (range) was 75.1 (65.9-84.0) months. In patients with baseline T2N0 disease (pembrolizumab, n=315; placebo, n=159), deaths occurred in 24 (7.6%) patients in the pembrolizumab group and 23 (14.5%) patients in the placebo group (HR, 0.51 [95% CI, 0.29-0.90]). OS rates (95% CIs) at 5 years were 93.3% (89.9%-95.6%) vs 89.2% (83.2%-93.1%), respectively. RCB rates were 63.5% in the pembrolizumab group vs 56.2% in the placebo group for RCB-0, 8.8% vs 11.5% for RCB-1, 18.4% vs 20.3% for RCB-2, and 5.1% vs 6.7% for RCB-3. Deaths occurred in 26/498 (5.2%) patients in the pembrolizumab group vs 17/219 (7.8%) patients in the placebo group in the RCB-0 subgroup (HR, 0.66 [95% CI, 0.36-1.23]), 10/69 (14.5%) vs 5/45 (11.1%) patients in the RCB-1 subgroup (HR, 1.35 [95% CI, 0.46-3.96]), 35/144 (24.3%) vs 35/79 (44.3%) patients in the RCB-2 subgroup (HR, 0.50 [95% CI, 0.31-0.80]), and 27/40 (67.5%) vs 16/26 (61.5%) patients in the RCB-3 subgroup (HR, 1.26 [95% CI, 0.68-2.34]). OS in subgroups based on PD-L1 combined positive score will be included in the presentation.
Conclusions: In patients with high-risk early-stage TNBC, neoadjuvant pembrolizumab + chemotherapy followed by adjuvant pembrolizumab showed clinically meaningful improvements in OS compared with neoadjuvant chemotherapy alone, with the highest survival benefit observed in patients with the least RCB.

PS13-01: Evaluation of breast cancer stem cell gene expression signatures in single-cell RNA sequencing (scRNAseq) data from the OPPORTUNE and FELINE trials, and the association with treatment resistance

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Background: Cancer stem cells (CSCs) play a key role in tumor initiation, progression, and resistance to conventional therapies. These cells possess the ability to self-renew and differentiate, contributing to tumor heterogeneity and recurrence. Preclinical data indicate

that targeting cancer stem cell-specific pathways could lead to more effective treatments and prevent relapse, thereby improving outcomes. However, there is limited clinical data to support this, in part due to the challenges of measuring CSCs in the clinical setting. Single-cell RNA sequencing (scRNAseq) data in combination with stemness gene expression signatures were investigated as a novel approach to detect and assess changes in CSC numbers in response to treatment.
Methods: Single-cell RNAseq datasets from two clinical trials were interrogated – OPPORTUNE, a window-of-opportunity trial evaluating anastrozole vs anastrozole plus pictilisib (PI3K inhibitor) in 75 patients with ER+ HER2- early breast cancer, and FELINE, a neoadjuvant trial which compared letrozole plus placebo with letrozole plus ribociclib (CDK4/6 inhibitor) in 120 patients with ER+ HER2- early breast cancer. The primary endpoint for OPPORTUNE was the inhibition of tumor cell proliferation as measured by Ki67. The primary endpoint for FELINE was the rate of preoperative endocrine prognostic index (PEPI) score 0 after neoadjuvant endocrine therapy. Thirty-four patients from the FELINE study had available scRNAseq data for analysis; this number was 62 patients for the OPPORTUNE study. Stemness gene expression signatures with published evidence of selectivity for breast CSCs were identified from the literature and their utility in detecting CSCs compared in the datasets. Subsequently, changes in CSC fraction with treatment was assessed.
Results: Eight different stemness gene signatures were identified from the published literature. Among these, four (Kim_myc, benporath_es2, Bhattacharya_hESC, and Shats_consensus) exhibited selective expression in a minority of tumor cells, with the Shats consensus signature demonstrating the highest specificity to tumor cells over non-malignant cells. Utilizing a Gaussian mixture model, we estimated that approximately 4.5% of cells within a tumor are likely stem cells. In the OPPORTUNE study, higher stemness scores were observed in luminal B compared to luminal A tumors, but there was no association with PIK3CA mutation status. Changes to Ki67 were inversely associated with the CSC fraction – tumors with higher stemness scores were less likely to achieve a complete cell cycle arrest versus endocrine-sensitive tumors. Similarly, in the FELINE trial, non-responders in the letrozole arm showed a trend towards elevated stemness scores.
Conclusion: The use of stemness gene expression signatures in scRNAseq data is a feasible method to assess changes in putative CSC fraction with treatment in breast cancer. Increased stemness scores were associated with more aggressive subtypes and resistance to treatment.

PS13-03: Decoupling of oestrogen response and proliferation in post-menopausal ER+ HER2- primary breast cancer after 2-week aromatase inhibitor treatment in the POETIC trial

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Background: Aromatase inhibitor (AI) treatment is well established and highly effective in early ER+ HER2- breast cancer (BC), but recurrence eventually develops in ~20% of patients. The PeriOperative Endocrine Therapy for Individualizing Care (POETIC) trial demonstrated that high levels of the proliferation marker Ki67 maintained after a 2-week neoadjuvant AI treatment were associated with increased risk of recurrence. We conducted bulk and spatial transcriptomics analyses to identify the tumour features that associated with poor response to AI treatment.

Methods: We analysed ER+ HER2- tumours from the POETIC trial. Included were 177 poor responders (PRs) to AI treatment corresponding to the bottom 15% Ki67 change, 190 good responders (GRs) taken from the top 50% Ki67 changes and matched to PRs for baseline Ki67 scores, and 20 untreated controls. PRs were split into 119 ESR1-high and 58 ESR1-low patients based on baseline ESR1 expression.
Bulk RNA-seq was used to determine PAM50 intrinsic subtypes. Differential expression (DE) and gene set enrichment analysis (GSEA) was performed between paired baseline and surgery samples in the three responder groups, separately for core and excision samples at surgery. DE significance was tested against a 0.1 fold change (FC) threshold and defined as padj < 0.05. The effect size of oestrogen (PGR, TFF1, GREB1 and PDZK1) and proliferation (18 genes from PAM50) responses was quantified by their mean log2 FC.
Spatial transcriptomics analysis was performed on a subset of 12 paired samples that passed quality control. Robust cell type deconvolution with the single cell BC atlas was used to define regions of stromal and tumour-rich content. Spots were clustered using a spatially aware algorithm and copy number alteration (CNA) was inferred in tumour-rich regions against a stromal reference.

Results: Broad DE was seen in response to 2-week AI treatment in GRs (4105 genes in tumours with surgical core-cuts) and PRs, ESR1-high (534 genes), but not in PRs, ESR1-low (5 genes). E2F targets and oestrogen response early hallmark genes were significantly repressed in both GRs (padj < 2.2e-16 for both) and PRs, ESR1-high (padj < 2.2e-16 and padj = 4.3e-14) relative to PRs, ESR1-low according to GSEA. However, the effect size in GRs was similar for both oestrogen response (-2.56 mean log2 FC) and proliferation genes (-2.44), but discordant in PRs ESR1-high (oestrogen response -1.93; proliferation -0.47).
As previously shown, baseline distributions of PAM50 subtypes were similar between GRs and PRs, ESR1-high. However, the number of non-LumAs switching to LumA was significantly different between the two (p < 2.2e-16; Fisher exact). In GRs 98% (118/121) of non-LumAs switched to LumA after treatment whereas only 33% (27/82) did so in PRs ESR1-high. The majority of PRs ESR1-low exhibited Basal subtype both before (~62%, 36/58) and after (~60%, 35) treatment; only 4% (2/54) of non-LumAs switched to LumA.
Spatial gene expression analysis showed substantial evidence of heterogeneity. CNA differences were found between surgery and baseline samples in 8/12 tumours. In 2 of the 8, there were >5 spatially distinct subclones across both cores. Differences in expression of oestrogen signalling and proliferation between two tumour regions in a single core were

observed in multiple tumours both with and without corresponding CNA differences.

Discussion: In PR ESR1-high tumours, AI therapy is associated with suppressed oestrogen signalling without a corresponding antiproliferative response, suggesting a decoupling of oestrogen response and proliferation in these tumours. Spatial analysis reveals frequent gene expression and copy number heterogeneity in ER+ HER2- BC. Work aiming to quantify gene expression differences between subclones is ongoing.

PS13-04: DREAM complex assembly and stable cell cycle control underlies long-term clinical response to CDK4/6 inhibition

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Background: Combinations of endocrine therapy and CDK4/6 inhibitors (CDK4/6i) used in metastatic breast cancer (MBC) result in heterogenous clinical benefit with subsets of patients having disease control ranging anywhere from a few months to more than 5 years. Understanding the mechanisms that account for these differences can enable new pharmacologic approaches to convert more of these cancers into the long-term control subset.
Methods: To elucidate the causal factors that underlie the observed clinical variability, we have utilized multi-omic analyses of patient samples as well as experimental modeling using a diverse cohort of ER+ breast cancers. For clinicogenomic analyses, we analyzed 467 patients with ER+/ HER2- MBC treated with first-line CDK4/6i at our institution (MSK) with detailed clinical follow annotation and follow up. For genomic/transcriptomic studies, we analyzed single cell RNA sequencing data, whole genome sequencing data, and Ki67 score from the neoadjuvant CDK4/6i containing arms in the FELINE trial (NCT02712723). Patient derived xenografts, patient derived organoids, isogenic cell lines were used to define the underlying mechanisms. Live cell imaging assays were performed by FUCCI cell cycle sensor to assess cell cycle transitions.
Results: Of 467 patients from MSK cohort, 129 (27.6%) had pre-CDK4/6i loss-of-function variants in TP53, corresponding to short PFS (median PFS= 8.7 months, 95% CI: 6.6–10.3; HR = 2.12, 95% CI: 1.68 to 2.68; p = 2.64e-10). This finding was validated in two large independent clinical cohorts outside MSK. In patient derived and isogenic cell lines, p53 loss had no impact on initial drug response but promoted long-term cell outgrowth and prevented emergence of senescence phenotypes. Following cells in detailed time courses revealed the propensity of p53 mutant but not wild type cells to reenter the cell cycle after initial arrest by CDK4/6 inhibition. Gene expression analysis of RNA seq data revealed a significant positive association between TP53 mutation status and DREAM complex genes, assessed by GSEA (NES= 1.51, p = 1e-4)
In models, deletion of DREAM complex component, p130-Rbl2 was found to fully recapitulate the effects of p53 loss: promoting long-term outgrowth from CDK4/6i, blocking

therapy induced senescence, and promoting cell cycle re-entry. To validate these results, we analyzed the multi-omic and histologic characteristics of serial samples from the FELINE trial. Compared with WT cases, baseline TP53 LOF had a higher amount of proliferating cells at the end of treatment, measured by Ki67 > 10% (4.5% of WT cases vs. 60% of mutant cases, respectively, OR = 26.8, p = 0.014), the per cell proliferation score, measured by ssGSEA (log2FC: 0.2 vs. 2.8, respectively) as well as a higher pseudobulk expression of DREAM complex related genes (GSEA: NES = 2.0, q = 3.7e-4).
Finally, to identify strategies that might facilitate stable cell cycle control of p53KO tumors, we investigated which targets of p53 regulate DREAM and identified p21. Given role of p21 in regulating both CDK4/6 and CDK2 we investigated whether CDK2 inhibitors would provide additional benefit to CDK4/6i. Addition of CDK2i was able to fully restore efficacy in p53KO lines as well as a patient derived model with TP53 mutation enabling irreversible cell cycle arrest and long-term growth suppression.
Conclusion: Wild type p53 facilitates DREAM complex assembly in response to CDK4/6i in ER+ breast cancer, underlying long-term disease control. p53 loss enables cells to reenter the cell cycle and facilitates the development of CDK4/6i resistance. Combined inhibition of CDK2 with CDK4/6 represents a promising therapeutic strategy for overcoming loss of p53 and enabling long-term antitumor efficacy.

PS13-05: The UNDERSTAND trial: a multi-omic platform for investigating CDK4/6 inhibitors resistance mechanisms in HR+ advanced breast cancer

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Introduction: Hormone receptor-positive, HER2-negative metastatic breast cancer (HR+/HER2- mBC) is an almost invariably incurable disease. Endocrine therapy plus CDK4/6 inhibitors (ET+CDK4/6i) represents the standard-of-care for 1st line treatment. However, primary or acquired resistance leads to tumor progression in most patients, and there is therefore an unmet need to unravel mechanisms of resistance and actionable targets in patients progressing upon CDK4/6i. To date, genomic studies pinpointed several putative resistance biomarkers. including alterations in ESR1, PIK3CA, FGFR1, TP53, RB1, KRAS, HRAS, NRAS, AURKA, AKT1, FGFR2, and CCNE2 genes. However, genomics can only partially explain resistance to CDK4/6i in HR+/HER2- mBC patients, while a combined analysis of genomic, transcriptomic and epigenetic profiles could potentially provide a more accurate characterization of primary and acquired resistance mechanisms. In this scenario, in June 2022 we launched the UNDERSTAND trial, an observational, open-label study aimed at identifying markers of resistance and tracing cancer cell evolution by sequential multi-omic analysis, in prospectively collected tissue and plasma samples from 100 HR+/HER2-

mBC patients undergoing 1st line ET+CDK4/6i.

Methods: The trial plans to collect tumor tissue at baseline (T0, prior to ET+CDK4/6i) and at disease progression (T1) in patients with accessible metastatic sites (cohort A) to longitudinally obtain genomic (FFPE), methylomic (fresh/frozen), bulk transcriptomic (fresh/frozen), single cell RNAseq (fresh tissue) and spatial transcriptomic (FFPE) data. If tumor biopsy would not feasible (bone-only disease, refusal of the biopsy procedure, lack of accessible tumor sites), blood samples will be prospectively collected at baseline, after 15 days of treatment, 3-months, 1-year or at tumor progression, for circulating methylome and ctDNA analysis (cohort B). Blood samples will be collected in cohort A patients as well. A dedicated electronic clinical record was set up with REDCap to collect demographic, clinical and sample information.

Results: Between January 2022 and July 2024, we enrolled 56 patients with HR+/HER2- advanced breast cancer. Multiple plasma samples were longitudinally collected (cohort A + B). We were able to collect baseline tumor tissue in 20 patients, including 3 relapses (cohort A). T0 samples were genomically characterized with the Illumina TSO500 NGS panel. scRNAseq data were obtained from 14 samples (ChromiumX platform, 10X Genomics), including 7 nodal, 4 liver and 3 local relapses. After stringent quality controls, we identified and annotated 90.000 high quality (mean: 6400 cells per sample), including 43000 neoplastic cells, as well as T cells, B cells, plasma cells, mast cells, myeloid cells, endothelial cells, perycites, fibroblasts and liver cells. Intra- and inter-tumor heterogeneity was investigated, and the PAM50 classifier was used, highlighting a site-specific variability of molecular subtype proportion. Exploratory case-control analysis on patients with early (<6 months) and late (>12 months) relapses confirmed the role of PIK3CA and MAPK pathway activation in therapy resistance, identifying neoplastic cells as the primary contributors. Additionally, other pathways, such as WNT, NF-κB, and JAK/STAT, were implicated in resistance, suggesting areas for further investigation. For the upcoming SABCS, we plan to analyze and present the complete results of translational analyses on matched T0/T1 tissue samples from 30 patients, including genomic profiling, bulk and scRNA sequencing data.

Conclusions: Our highly integrated infrastructure allows us to collect and integrate unprecedented longitudinal multiomic molecular data from HR+/HER2- mBC patients. A combination of genomic, transcriptomic, and epigenetic analyses will provide a more accurate characterization of primary and acquired resistance mechanisms to CDK4/6i.

PS13-07: Enhancing T-DXd Efficacy in HER2-positive Breast Cancer Resistant to HER2 ADC by Non-biased Kinase-related Target Screening

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Background: Anti-HER2 antibody-drug conjugate (HER2-ADC) therapies, such as trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd), significantly prolong survival in patients with HER2-positive metastatic breast cancer (BC) compared to physician's choice of chemotherapy with trastuzumab (The EMILIA, TH3RESA, and DESTINY-Breast clinical trials). Intrinsic or acquired resistance to HER2-ADC therapies remains a substantial clinical challenge since there is no established standard of care following progression on T-DXd. This study aimed to elucidate novel therapeutic targets that can overcome resistance to HER2-ADC therapies using unbiased approaches with synthetic lethal kinome RNA interference screening.
Methods: We conducted targeted DNA sequencing (n=15) and whole transcriptome sequencing (n=11) to investigate genetic aberrations following anti-HER2 and/or HER2-ADC therapy (T-DM1 or T-DXd) in BC patient tissue samples. We generated HER2-positive human BC cell lines resistant to T-DM1 or T-DXd. To determine the effect of the T-DM1 and T-DXd on the ERBB2 gene and HER2 protein expression, we used fluorescence in situ hybridization, droplet digital PCR, and Western blotting assay. To discern resistance mechanisms of T-DXd and identify kinase targets whose inhibition might synergistically enhance the efficacy of T-DXd, we conducted whole-genome sequencing, cDNA microarray analysis, and synthetic lethal kinome RNA interference screening using T-DXd resistant HER2-positive BC cell lines. To determine the synergistic antitumor effect of T-DXd combined with targeted therapy, we conducted Bliss synergy analysis, colony formation, and xenograft assays by mammary fat pad injection of T-DM1- or T-DXd-resistant BC cells in nude mice.
Results: Increased amplification of DNA repair-related genes (TOP2A, RAD21, RAD52, and CDK12) was found in patient tissue samples after progression to HER2 antibodies, T-DM1, or T-DXd. Significant enrichment of DNA damage repair-related gene sets was observed in the transcriptome of post-treatment human samples. Further, ERBB2 gene or HER2 protein expression was reduced compared to levels before such treatment. In HER2-ADC-resistant HER2-positive BC cell lines, we had similar upregulation of DNA repair-related genes (PCNA, ATM, RAD52) and reduction of ERBB2 gene and HER2 protein expression. By synthetic lethal kinome RNA interference screening, this non-bias screening identified the PI3 kinase, cell cycle, and DNA repair canonical pathways as potential targets to enhance the efficacy of T-DXd therapy in HER2-ADC–resistant HER2-positive BC. We confirmed that ectopic HER2 expression does not improve the efficacy of HER2 ADC in HER2-ADC–resistant HER2-positive BC. After further screening all potential targets, we found that the drugs targeting the DNA damage repair pathway were the most effective in enhancing the efficacy of T-DXd. Indeed, ATR inhibitor elimusertib led to significant HER2-ADC–resistant BC cell death in vitro (Bliss synergy score >5.0, $P<0.01$) and in four xenografts in vivo ($P<0.01$) compared to cell death with T-DXd alone. Importantly, we further observed the synergy of elimusertib and T-DXd in parental HER2-positive BC cell lines, ensuring the observed effects are not limited to resistant cell lines only.
Conclusions: Our findings indicate that resistance to HER2-ADC therapies is associated with increased DNA repair-related genes. By non-biased screening, this study provides robust evidence that targeting DNA repair pathways can significantly enhance the efficacy of T-DXd

in HER2-ADC-resistant HER2-positive BC. The potential of combining ATR inhibitors with T-DXd to overcome resistance and improve patient outcomes is a promising avenue that warrants further exploration in a clinical trial for patients with HER2-ADC-resistant- HER2-positive BC.

PS13-08: ARID1A as a Novel Regulator of Trop2-ADC Efficacy in Trop2-Low Hormone Receptor–Positive Breast Cancer

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Background: The Phase 3 TROPiCS-02 study found that the efficacy of sacituzumab govitecan (SG), an antibody-drug conjugate (ADC) directed to tumor-associated calcium signal transducer 2 (Trop2), in breast cancer (BC) does not depend on Trop2 expression levels. SG is the only FDA-approved ADC for advanced HR+/HER2− BC and triple-negative BC; it targets Trop2 by delivering SN-38, a topoisomerase I inhibitor. AT-rich interactive domain–containing protein 1A (ARID1A) is the most prevalent mutation in the SWItch/Sucrose Non-Fermentable (SWI/SNF) complexes in metastatic BC. ARID1A interacts with transcription factors involved in gene transcription and DNA repair, acting as a tumor suppressor. It is essential for maintaining luminal characteristics and influences treatment response. Patients with tumors harboring ARID1A alterations experience significantly shorter progression-free survival on selective estrogen receptor degraders than those with tumors with wild-type ARID1A. We hypothesize that loss or suppression of ARID1A expression results in a high level of Trop2 expression in HR+/HER2− BC. Identifying the mechanism linking ARID1A and Trop2, as well as determining the presence or absence of ARID1A mutations, could help identify patient groups that may benefit from combination therapy with SG and other inhibitors, enhancing treatment efficacy even in those with ARID1A mutations.
Methods: We used transposase-accessible chromatin sequencing (ATAC-seq) and an RNA-seq database (GSE124228) from ARID1A-knockout MCF7 cells to determine whether ARID1A regulates Trop2 gene transcription. To elucidate how SG regulates the ARID1A-Trop2 axis in HR+/HER2− BC, we used specific small-molecule inhibitors, siRNA, and an expression plasmid targeting ARID1A. Target proteins in the ARID1A-Trop2 axis were detected with immunofluorescence staining, immunohistochemistry, flow cytometry, Western blotting, quantitative real-time PCR, and the TCGA database.
Results: ARID1A protein expression negatively correlated with Trop2 expression in HR+/HER2− BC cell lines. Through Ataxia-Telangiectasia Mutated (ATM), the protein kinase critical for the cellular response to DNA damage activation, SG or SN-38 reduces ARID1A protein expression in HR+/HER2− BC cells. Further, ARID1A expression is diminished in tissue samples from SG-treated MCF7 xenografts resistant to both tamoxifen and abemaciclib. Mechanistic studies showed that overexpression of ARID1A reduces Trop2 mRNA and protein expression, whereas ARID1A knockdown elevates Trop2 levels in HR+/HER2− BC cells. Immunofluorescence staining data indicated an inverse correlation between ARID1A expression and Trop2 expression. ATAC-seq data revealed increased

chromatin accessibility at the Trop2 promoter in ARID1A-knockout MCF7 cells. T47D cells, which exemplify low ARID1A and high Trop2 expression, are approximately ten times more sensitive to SG than MCF7 and HCC1428 cells, which have high ARID1A and low Trop2 expression. Thus, low ARID1A expression leads to elevated Trop2 levels, resulting in increased therapeutic activity of SG.
Conclusion: We discovered that SG induces Trop2 expression through SN-38–mediated reduction of ARID1A protein expression in HR+/HER2− BC cell lines. Moreover, lower ARID1A in HR+/HER2− BC cells was associated with increased sensitivity to SG. These results suggest a novel mechanism for Trop2 ADC efficacy in HR+/HER2− BC with low Trop2 expression. Our findings highlight the importance of knowing a patient's ARID1A status to predict the efficacy of Trop2-ADCs. Further mechanistic studies will be presented at SABCS.

PS13-09: Mechanisms of Resistance to Trastuzumab Deruxtecan in Breast Cancer Elucidated by Multi-omic Molecular Profiling

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Trastuzumab deruxtecan is widely used as a treatment for metastatic HER-2 low and HER2-positive breast cancer, and preclinical studies have suggested multiple potential mechanisms of resistance. There have, however, been few large population-based studies of resistance. Following the course of trastuzumab deruxtecan (T-DXd) through the cancer cell, we combined Next Generation Sequencing (NGS), immunohistochemistry (IHC) and claims data to evaluate clinically relevant mechanisms of resistance to this agent.
A total of 1220 T-DXd-treated breast cancer samples tested at Caris Life Sciences (Phoenix, AZ) underwent whole transcriptome sequencing (Illumina, NovaSeq). HER2 IHC was performed (4B5) and scored following ASCO/CAP guidelines. Real-world clinical data were obtained from insurance claims. Time-on-treatment (TOT) and post-treatment overall survival (OS) were determined as the interval from the T-DXd initiation to the end of treatment and the last recorded clinical activity, respectively. RNA expression of 86 genes linked to numerous reported ADC resistance mechanisms were investigated for association with clinical outcome, including antigen/dimerization partners (N=5), ABC transporters (N=38), lysosomal genes (N=16), genes involved in ADCC pathway (N=9), endocytosis, intracellular trafficking and cytoskeleton organization pathways (N=6), topoisomerase isoforms (N=4), tubulin isoforms (N=5) and well-established prognostic markers in breast cancer (N=3). Multivariate analysis was conducted using RNA transcript per million (TPM) inputs as continuous variables in a Cox-Proportional Hazard model, with significance determined at $p<0.05$.
In the multivariate analyses performed in 1144 tumors collected prior to T-DXd start, associating expression levels of the 86 genes with TOT and OS revealed ERBB2 as the strongest positive predictor of TOT ($p<0.0001$; FDR adjusted $p =0.0011$) and OS ($p<0.0001$; FDR $p =0.011$); conversely, ABCC1 expression (MRP1, multidrug-resistance associated

protein 1) emerged as the strongest negative predictor of TOT (p=0.0009, FDR p=0.030) and OS (p=0.0004, FDR p =0.011). Notably, MKI67 (p=0.0099), TUBB3 (p=0.005) were negatively associated with OS, not with TOT.
Using the top quartile of ABCC1 expression (35 TPM) as cutoff, T-DXd outcome was better in ABCC1-low vs. ABCC1-high tumors (TOT: 6.0m vs. 5.2m p=0.09; OS: 24 vs. 15m, p=0.0005).
When combined with ERBB2 RNA expression (using median of 63 TPM as a cutoff), ABCC1-high tumors showed significantly shorter TOT compared to ABCC1-low in both the ERBB2-high (TOT: 4.7m, 95% CI [3.9-5.1] vs. 9.1m, 95% CI [7.3-10.7m]) and ERBB-low group (TOT: 3m [2.3-4.3] vs. 6.3 [5.1-7], p<0.0001); similar differences were observed for OS.
When ABCC1 RNA expression was combined with ERBB2 IHC, amongst all subgroups, tumors with HER2 IHC 0+ and ABCC1-high showed the shortest TOT (2.3m, [1.6-3.5m]) and OS (10.7m, [6-30.1m]) while tumors with HER2 IHC 3+ and ABCC1-low showed the longest TOT (10.7m [7.7-13.4m]) and OS (30.8 [24.1-not reached]). Patient age was not significantly associated with TOT or OS in our analyses. As expected, ERBB2 RNA expression was strongly associated with HER2 IHC results: median ERBB2 = 31 TPM in IHC intensity of 0+, 48 TPM in IHC of 1+; 84 TPM in IHC of 2+, and 445 TPM of IHC of 3+ (p<0.0001).
Using a multi-omic approach, we thoroughly surveyed gene expressions on pathways associated with antibody-drug conjugate resistance mechanisms. By examining clinical outcome data from 1144 breast cancer patients treated with T-DXd, our data suggest that at an intracellular level TDXd outcome is a function of drug concentration influx and time to efflux.

PS14-01: Rhenium (186Re) obisbemeda (rhenium nanoliposome,186RNL) for the treatment of leptomeningeal metastases (LM): Update on Phase 1 dose escalation study

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Background: Leptomeningeal metastases (LM) is a devastating systemic cancer complication most often seen with breast, lung, and melanoma, with limited treatment options and poor survival. Rhenium (186Re) Obisbemeda (186RNL) provides the combination of effective therapeutic beta radiation, simultaneous gamma decay for imaging, and ~90-hour physical half-life for optimal radiation delivery. A summary of the Phase 1 dose escalation study, expected to be completed, will be presented.
Methods: ReSPECT-LM is a Phase 1 dose escalation study for patients with LM from any primary cancer. Adult patients are given a single dose of 186RNL via intraventricular catheter (Ommaya reservoir) in an outpatient setting to determine the maximum tolerated/feasible dose (MTD/MFD), safety, tolerability, and response. Seven administered dose cohorts in a 3+3 design, from 6.6 mCi to 109.96 mCi, were anticipated.
Results: For the first 5 cohorts (n=25), doses were well-tolerated; most AEs were Grade 1

and 2 with 1 DLT in Cohort 5 (at time of abstract submission). 12 patients (48%) had breast cancer as their primary cancer. Of those evaluable at the time of abstract submission, 4 were triple negative (ER negative/PR negative/HER2 negative); 2 were ER positive/HER2 negative; and 2 were HER2 positive. Median overall survival for all patients at time of reporting was 12 months. CSF tumor cell enumeration assays were performed with a maximum reduction over baseline seen at Day 28. Radiation absorbed doses were calculated for the ventricles, cranial subarachnoid space, and spinal fluid and showed a linear increase with administered dose, with liver and spleen remaining low. Neurological symptoms were reported to improve. We expect the Phase 1 conclusion and the RP2D to be determined for conference reporting, with an additional subset analysis of the breast cancer patients.
Conclusion: A single dose of 186RNL for patients with LM has been well-tolerated, with high absorbed doses to the treatment area, acceptable organ doses, and promising clinical improvement and reductions in tumor cell count. Phase 2 is expected to begin enrollment by Q4 2024. Additionally, based on these data, a Phase 1 multidose study is planned to be initiated this year.

PS14-02: A Prospective Phase II Trial of Hypofractionated Stereotactic Radiotherapy (FSRT) for Patients with 1-10 Brain Metastases from Breast Cancer

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Purpose: Single-fraction stereotactic radiotherapy (SRT) is widely recommended in modern breast cancer brain metastasis treatment for 1-4 lesions, while hypofractionated SRT (FSRT) is rarely reported. This study aims to evaluate the efficacy and safety of FSRT in patients with 1-10 brain metastases from breast cancer.
Methods and Materials: From March 2019 to August 2023, we conducted a prospective, single-arm, phase II trial of FSRT in BM patients from breast cancer. Patients received FSRT delivered at 8 Gy per fraction in 3-5 fractions. The main inclusion criteria were breast cancer patients with 1-10 brain metastases. The primary endpoint was the local control (LC) rate, and the secondary endpoints included intracranial distant control, intracranial progression-free survival (iPFS), overall survival (OS), treatment-related toxicities, and assessments of neurocognitive function. The study is registered at clinicaltrials.gov with the identifier NCT04061408.
Results: A total of 173 patients with 436 brain lesions underwent treatment with FSRT. The median age of patients was 51 years (IQR 43-59), of which 16.1% (28/173) had 5-10 lesions, and 30.0% (52/173) had the largest lesion over 2 cm. As of February 2024, at a median follow-up time of 15 months (IQR: 8-29 months), the local control rate at 1 year and 2 years was 88.6% (95% CI: 83.6-93.9%) and 77.1% (95% CI: 69.4-85.6%), respectively. The intracranial distant control at 1 year and 2 years was 51.8% (95% CI: 44.5-60.5%) and 26.7% (95% CI: 19.5-36.6%), respectively. The median OS for these patients was 29 months

(95% CI: 21-36 months); the estimate of OS at 1 year and 2 years was 73.8% (95% CI: 67.2-80.9%) and 55.3% (95% CI: 46.4-65.8%), respectively. We included age, KPS score, presence of neurological symptoms, presence of extracranial metastases, molecular subtypes, number of lesions, maximum tumor diameter and total volume of lesions into multivariate regression analysis, and found that KPS was the only prognostic factor associated with local control rate. Among the 137 patients with evaluable lesions, the optimal treatment response was assessed by RANO-BM criteria. Except for 10 patients who did not have efficacy evaluation data, 33 patients (24.1%) achieved CR, 72 patients (52.6%) achieved PR, 18 patients (13.1%) achieved SD, and 4 patients (3.1%) were evaluated as PD. The median iPFS time for these patients with evaluable lesions was 11 months (95% CI: 9-15 months). Among the treated lesions, 27 cases out of 436 lesions (6.2%) experienced radiation necrosis. No grade 3 or 4 adverse events related to FSRT occurred in this study. Conclusions: This study demonstrated that FSRT treatment for breast cancer brain metastasis with 1-10 lesions is safe and effective option with excellent local control and a lower risk of radation necrosis.

PS14-03: Updated analyses from a Phase I study of the brain-penetrant oral SERD- SIM0270 in patients with ER-positive,HER2-negative advanced breast cancer

Qingyuan Zhang,Huiping Li, Yongmei Yin, Jiong Wu, Jian Zhang, Min Yan, Wenfeng Li, Sanyuan Sun, Yehui Shi, Hongtao Li, Hong Wang, Xiyuan Wang, Lili Zhu, Ya Li, Chunyan He, Chen Yang

Background: This is a phase I, multi-cohort, open label study of SIM0270 in patients with ER+/HER2- advanced breast cancer and the dose escalation part of phase Ia examined SIM0270 as monotherapy has been presented previously1. Here we present data from patients in the dose expansion part (Cohort S1)of SIM0270 monotherapy at the recommended dose (60mg PO QD) of phase Ia.
Methods: In Cohort S1, SIM0270 was administered orally once daily in patients with ER+/HER2- locally advanced/metastatic breast cancer who had received ≥1 prior endocrine therapy(secondary endocrine resistant) and there was no upper limit on the number of lines of prior endocrine treatment in the advanced setting; ≤2 chemotherapy regimens; prior treatment with fulvestrant or CDK4/6 inhibitors were permitted. Pre-/peri-menopausal women received ovarian suppression treatment. The key endpoints included safety, tolerability and efficacy.
Results: As of June 17, 2024, 51 patients received SIM0270 (60mg PO QD) in Cohort S1. Of all treated patients, median age was 60y and 90.2% were post-menopausal. ESR1 mutation was detected in 23.5% patients at baseline, and 74.5% patients had visceral metastases at baseline (liver 35.3%, lung 54.9%). Patients had received a median of 1 prior chemotherapy and 1 prior endocrine therapy in the advanced setting; 45.1% had prior fulvestrant, and 39.2% had prior CDK4/6 inhibitors treatment. The median duration of treatment was 114 days with a median 6.4 months follow up. The overall safety profile was consistent with that

of the dose escalation part, the incidence of TEAE was 100% and most of them were CTCAE grade 1 or 2; the most common TEAEs (incidence ≥20%) were sinus bradycardia 45% (most were grade 1 asymptomatic), ALT increased 31%, ALT increased 27%, hypertriglyceridemia 24% and urinary tract infection 24%; the incidence of ≥G3 TEAE was 24% and ≥G3 TRAE was 12% (hypertriglyceridemia 4%, electrocardiogram QT prolonged 2%, neutrophil count decreased 2%, sinus bradycardia 2%, anemia 2%). In total, 3 patients had dose interruptions and dose reductions due to TRAEs (1 hypertriglyceridemia, 1 electrocardiogram QT prolonged and 1 sinus bradycardia) and all resolved with dose modification. No patient discontinued SIM0270 due to TRAEs and no treatment related SAEs were observed. The confirmed ORR was 8.3% in 48 response evaluable patients, and CBR was 40% in 45 CBR evaluable patients. Seventeen patients remained on treatment, and the most common reason for treatment discontinuation was disease progression. Conclusions: In the expansion cohort of patients with ER+/HER2- locally advanced or metastatic breast cancer, SIM0270 monotherapy demonstrated promising evidence of clinical activity based on ORR and CBR that compared favorable to historical controls despite extensive pre-treatment including chemotherapies, CDK4/6 inhibitors and fulvestrant, which were enhanced in the subgroup patients refractory to the last treatment of patients with ESR1 mutations. SIM0270 monotherapy was well tolerated with manageable AE profile and low number of treatment discontinuation due to related AEs observed in the dose escalation part was maintained during cohort expansion part at dose of 60mg QD. Additional analyses are ongoing. (NCT05293964)

Reference ：
1. 2023 SABCS PS 15-01, A first-in-human phase 1 study of SIM0270, a brain-penetrant oral selective estrogen receptor degrader (SERD),in patients with ER+/ HER2- locally advanced or metastatic breast cancer.

PS14-04: SIM0270, a brain-penetrant oral SERD, in combination with everolimus in patients with ER+/HER2- advanced breast cancer: the phase Ib study

Jian Zhang, Jiong Wu, Xinhong Wu, Huiping Li, Wenfeng Li, Yongmei Yin, Sanyuan Sun,Yuping Sun, Ying Wang, Hong Wang, Weimin Xie, Xiyuan Wang, Lili Zhu,Ya Li, Qi Fu, Chunyan He, Chen Yang

Background: SIM0270 is a novel, brain-penetrant orally biologically active SERD with pure antagonistic properties that result in sustained inhibition of ER-dependent gene transcription and cell growth. Preclinically, SIM0270 has favorable efficacy and PK properties, including antitumor activity in ESR1-mutant models and brain-metastatic models, along with enhanced efficacy when combined with everolimus which was a mTOR inhibitor. In phase Ia, SIM0270 monotherapy was well tolerated with favorable safety, PK and encouraging antitumor activity and recommended dose of SIM0270 monotherapy was determined as 60mg QD. Here, we present the results from phase Ib of SIM0270 combined

with everolimus in patients with ER+/HER2- advanced breast cancer (NCT05293964).
Methods: Patients with ER+/HER2- aBC shown secondary resistance to prior endocrine therapy were enrolled to Cohort B(the dose escalation part) and Cohort B1 (the dose expansion part). Key inclusion criterias of cohorts B and B1 were same as follows: ≤2 prior chemotherapies and allowed to have received prior fulvestrant or/and CDK4/6 inhibitors in the advanced setting. Serial plasma samples were obtained for PK and ctDNA analysis. Key endpoint of cohort B was DLT, and key endpoints of Cohort B1 included safety and tolerability, PK and preliminary efficacy.
Results: As of June 17,2024, 8 patients were enrolled to Cohort B at SIM0270 60mg PO, QD combined with everolimus 10mg PO, QD, and DLT events were observed in 2 patients (grade 3 blood creatinine increased in 1 patient, grade 3 hypertriglyceridemia and grade 2 interstitial lung disease in 1 patient), no further dose escalation was recommended by the SRC guided by BOIN. Additional 32 patients were enrolled at the same dose in Cohort B1. In total of cohorts B+B1,75.6% patients had visceral disease and 22.0% patients had ctDNA ESR1 mutations detected at baseline. Patients were predominantly ET pre-treated in the advanced setting, 68.3% with AI, 58.5% with fulvestrant, and 68.3% with prior CDK4/6 inhibitors. 43.9% of patients had been treated with chemotherapy in the advanced setting, while 17.1% of patients had received 2 lines of chemotherapy. The incidence of TEAEs was 97.6%. The most common TEAEs (incidence ≥30%) were hypercholesterolemia 65%, hypertriglyceridemia 65%, anemia 55%, AST increased 50%， ALT increased 47.5%, hyperglycemia 45%, decreased appetite 42.5%, WBC count decreased 37.5%, hypokalemia 35%, Sinus bradycardia 35%, proteinuria 32.5%, asthenia/hypoalbuminemia/neutrophil count decreased/stomatitis 30% each. Grade ≥3 TEAEs and grade ≥3TRAEs occurred in 68.3 and 58.5% of patients, respectively. In B+B1, 65.9% patients had dose interruptions and 34.2% had dose reductions due to TEAEs, 4 patients (1 hypertriglyceridemia, 1 arrhythmia, 1 rash, 1 ILD) discontinued study treatment (SIM0270 or everolimus or both) due to TEAEs. The confirmed ORR was 19.4% in 36 response evaluable patients, and CBR was 65.5% in 29 CBR evaluable patients (per RECIST v1.1). Four patients with stable brain metastases were enrolled to cohorts B and B1: 2 confirmed CR and 2 SD (per RANO-BM), and 3 of these 4 patients were still under treatment. After the first dose and daily dosing for 15 consecutive days, the exposure of SIM0270 was not significantly different from that of SIM0270 alone, the exposure of everolimus was consistent with reported data of everolimus monotherapy.
Conclusions: SIM0270 in combination with everolimus showed acceptable safety and tolerability, comparable to BOLERO-2 trial of exemestane +everolimus, along with promising evidence of clinical activity in patients with ER+/HER2- aBC. Further data will be presented at the meeting.

PS14-05: Clinically actionable genomic alterations in breast cancer brain metastases

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Pellegrini, Francesca Zanghi, Maria Cristina Guarascio, Matteo Fassan, William Jacot, Stefano Indraccolo, Amelie Darlix, Valentina Guarneri

Background: Breast cancer brain metastases (BCBM) represent a critical unmet clinical need in metastatic BC, as they are associated with significant morbidity and poor prognosis. The identification of novel therapeutic targets is therefore urgently needed in this context. In this study, we aimed to describe clinically actionable targets in breast cancer brain metastases (BMs) using comprehensive genomic profiling.

Patients and methods: Genomic DNA was extracted from formalin-fixed paraffin-embedded archival BCBM samples from three institutions (all selected for tumor cellularity>30%). DNA samples with adequate quality were then analyzed using the commercially available Agilent SureSelect V6 whole exome sequencing (WES) kit and an Illumina NovaSeq 6000 platform.
Pathogenic alterations were classified as actionable alterations (AA) if they met the updated metastatic BC or tumor-agnostic ESCAT I or II criteria of the ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) (Mosele et al, Ann Oncol 2024). Due to technical limitations, gene fusions and MSI-H status were not assessed.
Hormone receptor (HR) and HER2 status were evaluated on the BCBM by ASCO-CAP criteria. Overall survival (OS) was defined as interval from first BM diagnosis to death or last follow-up, whichever occured first.

Results: Tumor WES data from 56 BCBM samples was available.
Thirty-three BCBMs (59%) were classified as HER2-. Among these, 8 (24%) presented AA of BRCA1 (N=2, 6%), BRCA2 (N=5, 15%), and PALB2 genes (N=1, 3%). These alterations were observed in 26% of HR-/HER2- BCBMs (5/19; N=2 BRCA1 and N=3 BRCA2, respectively) and in 21% of HR+/HER2- BCBMs (3/14; N=2 BRCA2, N=1 PALB2).
ESCAT I/II PIK3CA/AKT1/PTEN-pathway alterations were also present in 50% (7/14) of HR+/HER2- BCBMs: 6 PTEN (2 mutations, 4 deletions) and 1 hotspot PIK3CA mutation. A rare non-hotspot PIK3CA mutation was also detected in an additional HR+/HER2- BCBM sample. Interestingly, we did not detect ESR1 mutations in HR+/HER2- BCBMs.
Twenty-three BCBMs (41%) were classified as HER2+; among these, 3 (13%) presented a hotspot PIK3CA mutation and 7 (30%) presented a PTEN deletion. Among patients with HER2+ BCBMs, the identification of a hotspot PIK3CA mutation was significantly associated with worse prognosis (median OS from BM diagnosis 22.2 versus 53.0 months, log-rank p=0.034).
Overall, an ESCAT I/II AA was detected in 66% (N=37) of all BCBMs, and in particular in 64% of HR+/HER2- BCBMs. Tumor mutational burden (TMB) data will be presented at the meeting.

Conclusions: ESCAT I/II actionable genomic alterations are frequent in BCBMs, with a particularly high rate of tumor BRCA1/BRCA2/PALB2 alterations in the HER2- subgroup. These data highlight the potential for genomically targeted treatments in this setting. Furthermore, the negative prognostic impact of PIK3CA mutations in HER2+ BCBMs

provides the rationale for the potential combination of PIK3CA inhibitors and HER2-targeted agents in this setting.

PS14-06: Gene expression profiling of brain metastases and matched primary breast tumours with focus on the immune system and tumour microenvironment

Anna Thulin, Anika Kovacs, Anne-Vibeke Laenkholm, Henrik Fagma

Background: Despite advances in the treatment of breast cancer, brain metastases (BM) remain a significant clinical problem. Detailed biological information is increasing on extracranial metastases through for example the AURORA program, however the biology of BM is less studied. Immune checkpoint inhibitors (ICI) are efficient in treatment of early and disseminated TNBC but the effect on BM seems to be modest.
Aims: To compare gene expression (GE) signatures between BM and the primary breast tumours (PT) by use of NanoString nCounter 360™ Panel with a focus on signatures involved in the immune system and microenvironment (ME), as well as levels of CD4+, CD8+, CD68+ and Treg cells by immunohistochemistry. We also wanted to compared GE signatures between BM according to mutational status i.e. wildtype (wt) or somatic mutations in TP53 and PIK3CA respectively.
Methods: Patients with surgery of BM between 1994-2014 were identified. Clinical data and presence of TP53 and PIK3CA mutations have been published previously. The Breast Cancer 360TM panel version 2 (nCounter® platform) was used according to the manufacturer's specifications (NanoString Technologies, Inc., Seattle, WA, USA). IHC was performed according to the manufacturer's specifications with the following antibodies: CD4(1:100; IR649), CD8 (1:100; M7103), CD68 (1:200; IR613) (DAKO, Glostrup, Denmark) and FOXP3 (1:200; AB20034) (Abcam, Cambridge, United Kingdom). Stained slides were scanned on Hamamatsu Photonics NanoZoomer s210. Differences between GE signatures in BM and PT were calculated with Anova and Bonferroni post hoc analysis and for IHC with Wilcoxon paired test. Significance was set at 0.05 unless otherwise specified.
Results: Analyses were performed on 60 BM out of which 23 cases had the matched PT and five single PT. The following GE signatures were statistically significantly lower in BM than in PT: PD-L1, PD-1, PD-L2, CD8+ T-Cells, Treg, B7-H3 or CD276 (type I membrane protein with a sequence similar to the extracellular domain of PD-L1), IFN Gamma, MHC2, apM (antigen processing machinery), TGF-Beta, TIS (tumour inflammation signature), TIGIT (T cell immunoreceptor with Ig and ITIM domains), Stroma, IDO1, Mast Cells, Endothelial Cells (all p< 0,001) and Inflammatory Chemokines (p=0,02). The following GE signatures were upregulated in BM compared with PT; SOX2 (p < 0,001), Genomic Risk (p <0,001), BRCA-ness (p < 0,001), Differentiation (p= 0,004), Hypoxia (p <0,001), BC Proliferation (p=0,002) and HRD (p=0,047). The IHC staining showed statistically significant lower expression of CD4+ T helper cells (p=0.007), cytotoxic CD8+ T cells (p=0.04), TILs (p=0.004) and a trend not reaching statistical significance of Treg (p=0.07). No difference was found on the levels of CD68+ (p=0.35). BM with TP53 mutations had higher expression of BC proliferation

(p=0.001), HRD (p=0.007) and mast cells (p=0.001) compared with BM with wt TP53. BM with PIK3CA mutations had higher expression of MHC class 2 (p = 0.027) and BC proliferation (p = 0.033) compared with BM with wt PIK3CA.
Conclusions: We found a downregulation of GE signatures related to the immune system and tumour ME in BM compared with PT. This was validated by the lower levels of TILs, CD4+ and CD8+ T cells in BM. The lower immunogenicity of BM may partly explain the reduced effect by immunotherapy intracranially. New treatment combinations that enhance the immunogenicity of BM deserve further investigations.

PS14-07: Spatial transcriptomics of matched breast cancer brain metastases and primary tumors identifies a brain-specific immune-suppressive transcriptional program

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Background: Breast cancer brain metastases (BCBM) affect up to 50% of patients (pts) with metastatic breast cancer (MBC). However, therapies to specifically activate the intracranial anti-tumor immune response against BCBM are lacking due to incomplete understanding of brain-specific immune alterations. Here we use GeoMX Digital Spatial profiling of samples from 33 pts with BCBM and matching breast primary (n=5) over 62 immune-rich and 124 tumor-rich areas of interest (AOI) to reveal brain-specific transcriptional programs that may drive immune-suppression.
Methods: We previously collated a cohort of pts with matched BCBM and primary tissue at a single institution under an IRB-approved biobanking protocol (DFCI 93-085). A tissue microarray of 1.5 mm cores was generated with each sample duplicated. Nanostring GeoMX Digital Spatial analysis was used for AOI spatial transcriptomics using GeoMX Cancer Transcriptome Atlas gene panel(v1.0). The GeoMx Solid Tumor TME Morphology Kit was used to identify tumor-rich areas (cytokeratin positive) and immune rich areas (CD45+) for AOI identification. Eluted RNAs underwent next-generation sequencing and analyzed using the R packages NanoStringNCTools and GeomxTools. DE comparisons used linear mixed-effects models to account for patient as a random effect and the package clusterProfiler was used for GSEA on the resulting lists ranked by fold change.
Results: After filtering samples for quality, we identified 33 BCBM [HR+ HER2- (n=10), HER2+ (n=10) and TNBC (n=7)], 5 primary breast [HR+ HER2- (n=1), HER2+ (n=1) and TNBC (n=3) and 1 lung metastasis [HR+ HER2-], tissues suitable for immune analysis. 6 additional BCBM patient samples [HR+ HER2- (n=2), HER2+ (n=4)] were available for tumor analysis only. We analyzed 124 tumor-rich and 62 immune-rich AOIs. Principal component analysis (PCA) of the top 1000 variable genes separated immune-rich and tumor-rich AOIs, with greatest separation of tumor AOIs by patient compared to immune AOIs. To identify alterations in BCBM TME we focused on immune-rich AOIs [n=62, 47 brain, 12 breast, 3 lung, 5405 genes]. PCA showed clustering of immune AOI by tissue site. We next focused on immune AOIs in TNBC, given its poor prognosis and potential

sensitivity to immunotherapy. Gene set enrichment analysis (GSEA) found significant enrichment of Estrogen Response Early, Estrogen Response Late, G2M Checkpoint and E2F Targets Hallmark Gene Sets (ES < -0.3, FDR < 0.001) in TNBC brain immune AOIs compared to primary TNBC immune AOIs where Interferon Gamma Response, Allograft Rejection, Complement and Interferon Alpha Response (ES > 0.3, FDR < 0.001) were enriched. S100A1, CD24, ELF5, IGFBP2 were the top 5 differentially expressed genes (DEGs) in immune AOIs from TNBC brain tissue compared to primary tumors where B2M, HLA-B, IGKC, POSTN, HLA-DRA were enriched. Comparing TNBC immune AOIs to HR+ or HER2+ immune AOIs in the brain did not identify significant DEGs. We also compared tumor AOIs between TNBC brain and breast samples. GSEA found significant enrichment of Estrogen Response Early, Estrogen Response Late and KRAS Signaling Down (ES < -0.3, FDR < 0.001) in TNBC brain tumor AOIs compared to primary TNBC tumor AOIs where Interferon Gamma Response, Interferon Alpha Response, MTORC1 Signaling and Epithelial Mesenchymal Signaling were enriched (ES > 0.3, FDR < 0.001). S100A1, HOXC10, CLPSL1, ITGA6, ZG16B were the top 5 DEGs in tumor AOIs from TNBC brain tissue compared to primary tumors where BST2, LGALS3BP, EIF2AK2, TAP1, IFI27 were enriched.
Conclusion: We performed region-of-interest spatial transcriptomics to identify gene expression differences between primary breast tumors and matching BCBM. We identified estrogen receptor signaling as a key immune and tumor signaling pathway that was differentially expressed in BCBM compared to primary tumors in TNBC. These results have potential therapeutic implications and warrant validation in additional cohorts. Further analyses of HR+ and HER2+ tumor and immune AOIs and spatial proteomic analysis will be presented at the conference.

PS14-08: Targeting CXCL1-CXCR2 axis blocks brain metastasis in inflammatory breast cancer

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Background: Brain metastasis is a frequent site of relapse in patients with inflammatory breast cancer (IBC) - a rare, highly aggressive and metastatic variant of breast cancer. We have discovered that soluble E-cadherin (sEcad), an 80-kDa proteolytic fragment of full-length E-cadherin, correlated with increased risk of brain metastasis and death in patients with metastatic IBC. Additionally, we demonstrated that sEcad promotes brain metastasis growth and progression in HER2+ and triple-negative IBC brain metastasis models. However, how sEcad promotes brain metastatic progression is unknown. We hypothesize that sEcad promotes the production of specific cytokines, which, upon extracellular release, promote the activation of astrocytes, priming the brain microenvironment for metastatic growth.
Methods: Stable overexpression of sEcad in IBC cell lines (MDA-IBC3 (ER–/HER2+) and SUM149 (ER–/HER2–) was achieved using lentiviral vectors. We injected MDA-IBC3-sEcad

and control cells (tail-vein) and SUM149-sEcad and control cells (intracardiac) into SCID/Beige mice to assess brain metastasis burden and survival in mice. Human cytokine array was used to examine conditioned medium from sEcad high and control cells. Clinical datasets were used to compare expression and percent risk of brain relapse. Mice were treated with brain-permeable CXCR2 inhibitor in both IBC brain metastasis models. Multiplex quantitative imaging was used to visualize cells of the brain metastatic microenvironment.
Results: Higher serum sEcad levels were significantly associated with reduced OS, earlier metastasis onset, and increased brain metastasis incidence. sEcad is an independent predictor of OS on multivariate analysis (hazard ratio [HR]=2.07 [95% CI 1.19-3.60], p=0.01). Treatment of astrocytes with recombinant sEcad increased reactive astrocytosis, in vitro and in vivo. Cytokine array analysis showed increased levels of pro-inflammatory cytokine CXCL1, DKK1 and CXCL8 in conditioned medium from sEcad-overexpressing IBC cells compared with control cells, which was validated by ELISA. In patient samples, CXCL1, CXCL8 and CXCR2, the receptor for CXCL1 and CXCL8, were expressed higher in brain metastasis compared to other metastases. Additionally, patients with high CXCL1/CXCL8 or CXCR2 expression had reduced brain metastasis relapse. Inhibition of CXCR2 decreased sEcad-mediated induction of reactive astrocytes. Treatment of mice bearing MDA-IBC3-sEcad and SUM149-sEcad brain metastases with the brain permeable CXCR2 inhibitor reduced number of brain metastasis, metastasis burden and prolonged survival of mice. Multiplexed immunofluorescence staining showed a significant reduction of reactive astrocytes in brain metastasis lesions treated with the CXCR2 inhibitor.
Conclusion: Our findings underscore that sEcad drives brain metastasis by promoting an inflammatory brain microenvironment via a targetable CXCL1/CXCL8-CXCR2 axis. Targeting this axis presents a promising therapeutic strategy to effectively block brain metastasis in aggressive breast cancers.

PS14-09: Central Nervous System as the Primary Site of First Relapse in Patients with Triple-Negative Breast Cancer Achieving Pathological Complete Response After Neoadjuvant Treatment

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Introduction: In patients with triple negative breast cancer (TNBC), achieving a pathological complete response (pCR) after neoadjuvant treatment (NAT) is associated with better survival. However, up to 10% of these patients relapse after surgery. Understanding the pattern and determinants of this failure is essential. This study aims to describe the pattern of relapse in patients with pCR after NAT and to compare it with the non-pCR counterpart.
Methods: This retrospective, multicenter study included 898 patients with TNBC (estrogen-

receptor <10%, HER2-negative) treated with NAT between 2001 and 2023 at nine European Institutions. We collected the type of first relapse-free survival (RFS) event (locoregional relapse, distant relapse, death without other event) and the site of metastasis for patients with distant relapse as the first event. The cumulative incidence of specific events was evaluated using competing risk analysis and compared between pCR (ypT0/is ypN0) and non-pCR cohorts.

Results: The study population included 443 (49.3%) patients with pCR and 455 (50.7%) with non-pCR. Patients with pCR had less frequently stage III disease (22.1% vs 33.3%, p<0.001). Median TILs were higher in the pCR vs non-pCR group (median 15% [IQR 7-40] vs 10% [IQR 5-30], p<0.001). As part of NAT, most patients received anthracyclines (95.8%) and taxanes (89.2%), more patients in the pCR group received carboplatin (45.8% vs 38.0%, p=0.017) and immunotherapy (8.6% vs 5.1%, p=0.038). Adjuvant chemotherapy was more common in the non-pCR cohort (46.5% vs 6.3%, p<0.001).

With a median follow up of 4.6 years (95%CI 4.3-4.9),171 events occurred: 30 in the pCR cohort and 141 in the non-pCR cohort. RFS was significantly longer for pCR vs non pCR (5-yr RFS 92.7% vs 67.2%, p<0.001). The cumulative incidence of distant metastasis in the overall population was 15.4% at 5 years, significantly lower in pCR vs non-pCR cohort: 4.1% vs 25.8%, p<0.001. The same was observed for distant metastasis involving visceral site as first event: 5-yr cumulative incidence was 10.6% in the overall population, 3.6% in pCR vs 17.1% in non-pCR cohorts, p<0.001. No difference in the cumulative incidence of CNS relapse without extracranial disease (CNS-only) as first event was observed between the two groups: 5-yr cumulative incidence was 2.6% in pCR vs 2.3% in non-pCR cohorts, p=0.693.

Next, we analyzed the frequency of site-specific first metastasis among patients who experienced a distant metastasis as first event. Over the total of distant relapse events, visceral involvement was more frequent in pCR vs non-pCR cohorts (15/17 88.2% vs 74/110 67.3%, p=0.094). The predominant site of distant relapse in the pCR cohort was CNS-only: 10/17 (58.8%) vs (9/110) 8.2% in non-pCR, p<0.001. The second most frequent site of distant relapse in pCR-cohort was liver (n=5/17, 29.4%) without significant difference vs non-pCR (n= 29/110, 26.4%). Baseline characteristics of patients who developed CNS-only relapse as first event did not differ between pCR and non-pCR cohorts and were as follows: median age 51 years (range 34-72), stage III 52.6%, nodal involvement 73.7%, Grade 3 94.1%, median TILs 13% (IQR 2-25).

Conclusions: In patients with TNBC achieving pCR after NAT, CNS relapse without extracranial disease is the predominant first relapse site. Identifying factors predicting this dismal event in an otherwise low-risk population is urgently needed, as these patients could participate in in targeted follow-up programs and clinical trials investigating more aggressive therapeutic approaches after NAT. Our data also challenge the appropriateness of systematic avoidance of CNS staging in early TNBC at diagnosis, especially in patients at increased risk.

PS14-10: Effects of trastuzumab deruxtecan (T-DXd) on health-related quality of life (HRQOL) & neurological function in patients (pts) w/ HER2+ advanced/metastatic breast cancer (mBC) with or without brain metastases (BM): DESTINY-Breast12(DB-12) results

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Background: In DB-12 (NCT04739761), a prospective Phase 3b/4, single-arm, open-label study, T-DXd exhibited substantial and durable overall and intracranial clinical activity in HER2+ mBC, including pts with BM. BM cohort 12-month (mo) progression-free survival (PFS): 61.6% (95% CI 54.9, 67.6); 12-mo CNS PFS: 58.9% (95% CI 51.9, 65.3). CNS objective response rate: 71.7% (95% CI 64.2, 79.3); 79.2% (95% CI 70.2, 88.3) in stable and 62.3% (95% CI 50.1, 74.5) in active BM. Efficacy in the non-BM cohort was consistent with previous reports. No new safety signals were identified (Harbeck N, et al. Nat Med. 2024). BM and local therapies for BM (eg radiotherapy) can impact HRQOL and neurological function. Here, we report T-DXd effect on HRQOL and neurological function in pts with and without BM.

Methods: Adults with HER2+ mBC and progression on ≤2 prior lines of therapy were enrolled in the BM cohort (stable / active [untreated/treated progressing] BM not requiring immediate local therapy; dexamethasone ≤3 mg daily use or equivalent allowed for symptom control) or non-BM cohort. Design did not allow cross-cohort comparison. Pts received T-DXd 5.4 mg/kg intravenously every 3 weeks. HRQOL was assessed as proportion of pts with clinically meaningful deterioration (change from baseline [BL]: ≤−4 for cognitive functioning, ≤−8 for Global Health Status [GHS], ≤−10 for pain and physical functioning) using EORTC QLQ-C30. Neurological function was assessed as change from BL score to worst score (at any point during treatment) using the NANO scale, and clinically meaningful change (±10 points) from BL for the MDASI symptom diary (brain tumor-specific outcomes; BM cohort only).

Results

Median total treatment duration in the BM cohort (N=263): 11.5 mo; 118 pts were still receiving T-DXd at data cutoff (DCO). EORTC QLQ-C30, NANO, and MDASI compliance: >81.0% at BL and >78.3% overall. For EORTC QLQ-C30, the number (%, time to deterioration [TTD] 25th percentile) of pts with BL values who had clinically meaningful deterioration was 101/223 (45.3, 2.1 mo) for cognitive functioning (CF); 131/223 (53.2, 0.8 mo) for GHS/QOL; 99/224 (44.1, 2.0 mo) for pain; and 99/224 (44.1, 2.7 mo) for physical functioning. Median TTD was not calculated (NC) for any domain. For NANO, 220/254 (86.6%) pts had neurological stability as BL score; of these, 140 had neurological stability and 80 had neurological progression as worst score at any point during treatment. Of 25 (9.8%) pts with neurological progression as BL score, 18 had neurological progression and seven had neurological stability as worst score at any point during treatment. No clinically meaningful mean changes were observed in brain tumor-specific MDASI symptom diary

items (including difficulty concentrating, speaking, and understanding; irritability; seizures; vision).
Median total treatment duration in the non-BM cohort (N=241): 12.0 mo; 95 pts were still receiving T-DXd at DCO. Four pts developed new symptomatic CNS metastasis (incidence rate 0.017 [95% CI 0.00452, 0.04250]) during the study. EORTC QLQ-C30 and NANO compliance: >85.9% at BL and >80.5% overall. For EORTC QLQ-C30, 93/207 (44.9, 2.7 mo) pts had clinically meaningful deterioration of CF; median TTD was NC. For NANO, 217/236 (91.9%) pts had neurological stability as BL score; of these, 172 had neurological stability and 45 had neurological progression as worst score at any point during treatment. Of 16 (6.8%) pts with neurological progression as BL score, ten had neurological progression and six had neurological stability as worst score at any point during treatment.
Conclusion
Overall, HRQOL and neurological function were preserved in most pts with HER2+ mBC, with and without BM, receiving T-DXd.

PS15-01: Utilization of weight management treatment and subsequent cardiovascular events among patients with breast cancer

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Background: Previous studies have suggested that overweight and obese breast cancer (BC) survivors have an increased risk of cancer recurrence along with a higher all-cause mortality including from cardiovascular disease (CVD). CVD is the primary cause of death among patients with BC and CVD risk factors, such as obesity and metabolic disorders, may be more prevalent among BC survivors compared to the general population as BC and CVD share common risk factors. Weight loss medications and bariatric surgery have proven to effectively reduce weight and cardiovascular events (CVE) in the patients who are obese or overweight. We assessed the use of weight modifying agents following a BC diagnosis and evaluated the rates of CVE in these patients.
Methods: We conducted a retrospective cohort study using the MarketScan Commercial Claims and Encounters and Medicare Supplemental Database. Eligibility included aged 18-95 years, overweight or obese per ICD 9 and ICD 10 coding, with a diagnosis of invasive BC between 1/1/2009 – 12/31/2021, who were treated with lumpectomy or mastectomy. Patients who underwent bariatric surgery within one year prior to index date, or had a secondary breast cancer or gastrointestinal cancer prior to surgery were excluded. Patients were categorized by weight status (overweight, obesity class I/II/unspecified, or obesity class III) and by use of weight management treatment (WMT) (yes/no) including nutrition counseling, weight loss medications (Bupropion Hydrochloride/Naltrexone Hydrochloride, Liraglutide, Orlistat, Phentermine Hydrochloride/Topiramate, Semaglutide, and Setmelanotide) and bariatric surgery. We utilized descriptive statistics and conducted a univariate analysis for factors associated with receipt of WMT and rates of CVE by group, defined as cardiovascular complications or myocardial infarction on the same day of

surgery or within 1 year after surgery using ICD-9 or -10 codes. We fit a multivariable logistic regression model to determine factors associated with receipt of WMT.
Results: We identified 35,206 eligible patients, 18.8% (n=6,631) were overweight, 53.7% (n=18,919) were obese class I/II/unspecified, and 27.4% (n=9,656) were obese class III. Of these, 5.3%, 6.4%, and 9.6% received WMT within 1 year of surgery, respectively (p<0.001). Among the 2,484 patients who received WMT, types of therapy utilized were: 72.7% nutritional counseling, 26.7% weight-loss medication, and 4.9% bariatric surgery. Over the years 2009 to 2021, the incidence of WMT significantly increased from 3.7% to 11.3% (p < 0.001), and the use of weight-loss medication increased from 0.3% to 5.1% (p <0.001). In multivariable analysis, young age, 19-40 years old (aOR 2.72 95%CI 2.10-3.52), obesity class III (aOR 1.66 95%CI 1.44-1.90), lumpectomy (aOR 1.14 95%CI 1.04-1.90), more recent year of surgery (aOR 3.04 95%CI 2.22-4.17, referent 2009), comorbidity score of two or greater (aOR 1.24 95%CI 1.09-1.41), and WMT before surgery (aOR 13.56 95%CI 12.19-15.07) were associated with receipt of WMT within 1 year after surgery compared to older age (71-95 years old), overweight classification, mastectomy, earlier year of surgery, comorbidity score of zero, and no prior WMT, respectively. Across all patients, there were 457 CVE within 1 year of surgery; 438 of these patients had not received WMT and 19 patients had received WMT. Patients who received at least one type of WMT had a lower cumulative incidence of CVE, 0.8% compared to 1.3% (p 0.02) in patients who did not receive WMT.
Conclusions: Among newly diagnosed patients with BC, WMT and specifically, weight loss medication use, has increased over time. Among those who received WMT, there was a lower incidence of CVE. Given the known risk of obesity and cardiovascular disease in BC survivors, more work is needed to determine the optimal weight management strategy in this vulnerable population.

PS15-02: Genome-wide association study (GWAS) of aromatase inhibitor musculoskeletal toxicity (AIMT) among early-stage breast cancer (BC) survivors.

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Background: More than half of patients with early BC on aromatase inhibitors (AI) experience AIMT. Inflammatory traits linked with treatment-related estrogen depletion have been associated with AIMT. A potential role for germline variants in genes regulating inflammation, bone turnover, estrogen metabolism and AI pharmacokinetics has also been suggested (Reinbolt 2018, Stearns 2024, Lintermans 2016, Wang 2016, Hertz 2017). Yet study were relatively small and results inconsistent or not validated. Therefore, the understanding of the biological and potential genetic underpinnings of AIMT is still limited. We aimed to comprehensively explore the association of genetic variants with AIMT in

order to identify targets for impactful interventions.

Methods: We included postmenopausal patients with stage I-III BC treated with adjuvant AI from the longitudinal CANTO cohort (NCT01993498). AIMT was evaluated at month 3-6, year (Y) 1, Y3 and Y5 of AI treatment and defined as presence of any grade [G] articular or muscular pain by CTCAE v4.0. All germline variants were assessed on blood samples obtained at BC diagnosis. We performed a GWAS between 1,894,475 single nucleotide polymorphisms (SNPs) (Illumina InfiniumExome24 / Illumina GSA24) and AIMT, using multivariable logistic regressions. Each model was adjusted on pre-specified clinical and behavioral factors, including age, socio-economic status, comorbidities, tumor and treatment-related variables (including type of AI), and patient-reported health outcomes, all assessed at BC diagnosis. Missing data was handled by multiple imputation with a set of 15 complete datasets. The study had approximately 80% power to detect associations with AIMT at Y3 at suggestive GWAS significance alpha level of 10-6 targeting variant with minor allele frequency (MAF) of 0.25 and odds ratio (OR) of 1.5. Therefore, our primary outcome of interest was AIMT at Y3. Given the exploratory nature of these analyses, we report relevant associations at an alpha threshold of 10-4. Genes harboring significant top SNPs were annotated to identify potential explicatory biological pathways.

Results: Overall 4854 patients treated with AI were included. Of these, 3847 had available genetic data. Mean age was 63.9 years (SD 7.2), mean BMI was 27.0 (5.6). 63% patients reported history of articular disease, 41% received chemotherapy. 55% received letrozole, 40% anastrozole and 5% exemestane as first AI. 65% of patients reported AIMT at Y3 (of whom G3 9%), while 86% patients reported AIMT overall (at any time point; of whom G3 18%). Patients with AIMT at Y3 were generally younger, with higher BMI, had previous history of articular or muscular disease, and reported more frequently anxiety or depression at diagnosis. The GWAS identified 9 SNPs as associated with AIMT at Y3: rs4096589 (MAF 0.39; OR 1.36 [95% CI 1.21-1.53]), rs983495 (MAF 0.46; OR 0.76 [0.68-0.85]), rs12964962 (MAF 0.25; OR 0.73 [0.64-0.84]), rs348780 (MAF 0.42; OR 0.77 [0.69-0.86), rs72485589 (MAF 0.13; OR 0.67 [0.56-0.81]), rs2559854 (MAF 0.42; OR 0.78 [0.69-0.87]), rs895876 (MAF 0.16; OR 0.72 [0.61-0.83]), rs2926866 (MAF 0.17; OR 1.40 [1.20-1.66]), rs2240027 (MAF 0.08; OR 1.58 [1.28-1.96]). rs2926866 was also associated with AIMT at Y1 (1.22 [1.05-1.41]), Y5 (1.36 [1.12-1.66]), and overall (1.48 [1.24-1.76]). Relevant molecular functions that were associated with the identified SNPs included protein homodimerization activity (rs4096589), identical protein binding (rs895876), actin- and actin-filament binding (rs2240027).
Conclusions: This was an exploratory study aimed to assess associations between genetic variants and AIMT in postmenopausal patients with early BC. Results of this relatively large prospective cohort identified several SNPs that might be involved in AIMT. Further analyses are warranted for model validation and to better elucidate the mechanistic role of the described genetic variants from a functional perspective.

PS15-03: Retrospective study of GLP-1 Receptor Agonists in Breast Cancer Survivors: Weight Loss and Patient Outcomes

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Background: Obesity in breast cancer (BC) survivors is associated with higher BC recurrence risk and mortality, and weight loss in an important tenet of health promotion. Glucagon-like peptide-1 receptor agonists (GLP1-RA) are incretin mimetics with favorable metabolic effects and approved for type 2 diabetes (DM2) or weight loss. While drug utilization is increasing, the implications in cancer survivors are not well elucidated. This study evaluated treatment patterns of GLP1-RA, weight loss trends, and patient outcomes in a cohort of BC survivors. Methods: We retrospectively analyzed patients with non-metastatic (DCIS or invasive stage I-III) BC treated at MD Anderson Cancer Center (MDACC) who received at least 3 months of GLP1-RA from 2005 - 2024. Data was obtained from the MDACC BC and pharmacy databases. Linear regression models estimated the association between weight change and clinical factors. After excluding patients with DCIS, propensity score matching (1:2 ratio) was used to match patients who received GLP1-RA with those who did not, based on baseline body mass index (BMI), DM2, age, clinical stage, and BC receptor status. Kaplan-Meier estimates and log-rank tests estimated and compared overall survival (OS) and disease-free survival (DFS) between patients who received GLP1-RA and those who did not. Results: In total, 1,022 patients met inclusion criteria. Median age was 54 years (23-86) years, and 56.9% were postmenopausal; 79.0% had DM2. Most (91.4%) had stage I-III invasive BC and 8.6% had DCIS; 80.2% had hormone receptor positive BC and 65.9% received adjuvant endocrine therapy with an aromatase inhibitor (AI) or tamoxifen. GLP1-RA was started following definitive BC therapy (chemotherapy, surgery, radiation) in 87.6%; the median time from BC diagnosis to GLP1-RA initiation in these patients was 4.7 (0.0-34.0) years. The median duration of GLP1-RA use was 1.2 (0.3-8.1) years. Median BMI at BC diagnosis was 33.5 (20.1-56) kg/m2. Baseline median weight and median BMI at GLP1-RA initiation (within 90 days prior) was 86.8 (47.2-175.0) kg and 33.6 (18.9-61.8) kg/m2, respectively. In patients (n=442, 43.2%) who received the drugs approved for weight loss (semaglutide or tirzepatide), median weight loss at 3 (+/- 45 days), 6 (+-/45 days), and 12 (+/- 60 days) months was -1.9% (-13.2%-14.9%), -3.1% (-20.2%-19.0%), and -2.6% (-27.8%-11.5%), respectively. On multivariate regression analysis, no significant association was observed between the 6 month change in weight and clinical factors (DM2, metformin use, endocrine therapy use, duration of GLP1-RA, clinical stage); however, the 12 month change in weight was significantly associated with clinical stage (invasive BC was associated with more weight loss vs DCIS, $p<0.01$) and endocrine therapy (tamoxifen or AI use was associated with weight gain, $p=0.019$). Patients who received endocrine therapy experienced, on average, a 2.83 kg weight gain at 12 months compared to those who did not, after adjusting for other variables in the model. In those with invasive BC (DCIS excluded); there was no significant difference in DFS but there was a significantly improved OS in patients who received GLP1-RA compared with controls (median survival not reached (0-30.9) vs 27.0 years (0-57.3), $p<0.0001$). Conclusion: This is the largest study describing

real world patterns of GLP1-RA use in BC survivors. These medications were associated with modest weight loss; however, endocrine therapy may decrease this impact. An improved all-cause survival was observed, but there was no difference in DFS. Clinical trials are needed to investigate the role of these agents for weight loss as an adjunct to lifestyle interventions in cancer survivors. Further exploration of potential anti-cancer biological effects for BC risk reduction and cancer control may also be warranted.

PS15-04: Impact of a dietitian and nurse-led survivorship clinic utilizing ePRO collection on body composition and muscle strength in early-stage breast cancer: Results from the Linking You to Support and Advice (LYSA) Randomized Control Trial

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Background: Breast cancer history and adjuvant endocrine therapy use are associated with unfavorable body composition alterations, including increased body weight and adipose tissue mass and decreased muscle mass and strength. Qualitative research highlights that breast cancer survivors are unlikely to receive optimal support to manage these alterations. We evaluated the impact of a dietetic intervention utilizing electronic patient reported outcomes (ePROs) on body composition in a randomized control survivorship trial.
Methods: LYSA (n=200) was a multisite randomized control trial with parallel arms (experimental and active comparator) which assessed the feasibility of a complex survivorship intervention incorporating ePROs in early-stage hormone receptor (HR)-positive breast and gynecologic cancer (NCT05035173, DOI: 10.1200/JCO.2024.42.16_suppl.1505). Amongst those with HR-positive breast cancer (n=168), endocrine therapy use (n=163) included aromatase inhibitors (55%, n=90), tamoxifen (36%, n=59) and ovarian function suppression combinations (8%, n=14). Although not powered to assess between arm differences, a secondary outcome of the trial explored the impact of a dietetic intervention on body composition. Body composition assessments were conducted at baseline and 12 months in both arms, including lean body mass and adipose tissue mass via bioelectrical impedance analysis; muscle strength via handgrip strength, waist circumference and body mass index. The experimental arm completed ePROs bi-monthly from baseline to 12 months including body weight and weight gain concern. ePROs triggered a nurse and dietitian-led personalized symptom management pathway.
Results: In the experimental arm, 63 breast cancer participants initiated 211 dietetic consultations through ePROs. At baseline, weight gain concern was reported by 67% (n=55, active comparator) and 66% (n=52, experimental). At 12 months, weight gain concern increased to 72% (n=56) in active comparator arm but reduced to 52% (n=39) in experimental arm. In the experimental arm, there was a consistent numerical decrease in weight gain concern over the study period: 55% (n=43) at month 2 versus 46% (n=32) at

month 10. Weight remained stable in both arms at 12 months (p=0.057, -1.12 [95% CIs -2.27 to 0.03]). There were non-significant increases in lean tissue mass in the experimental arm versus the active comparator arm at 12 months (35kg [range: 26-60] vs 34kg [22.5 to 49], p=0.718 [95% CI -1.08 to 0.75]). Handgrip strength decreased over 12 months in both arms. However, the experimental arm maintained more muscle strength versus the active comparator arm at 12 months (24kg [11-41] vs 23kg [6.5 to 35.5], p=0.126 [95% CI -0.23 to 1.86]). In all arms, an exploratory analysis showed that participants with stronger handgrip strength were likely to have a higher lean tissue mass.
Conclusion: A dietetic intervention, within a randomized survivorship trial incorporating ePRO collection, appeared to reduce weight gain concern in patients with HR-positive breast cancer. Impact on changes in weight and lean tissue mass should be explored in future trials powered to detect a difference in these endpoints.

PS15-05: Breast Cancer Patients with High-Risk Disease Characteristics have Proportionately Higher Risk of Mortality from Non-Breast Cancer Related Causes in both Non-Metastatic and Metastatic Disease

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Background: Breast Cancer (BC) is the most common cancer among females with significant improvement in survival in both metastatic (mBC) and non-metastatic (nmBC) setting. Prolonged survival increases the risk of acquisition of other comorbidities which may or may not be linked to primary BC, and results in non-breast cancer related mortality (NBCRM). The purpose of this study is to quantify the causes of NBCRM and identify related high-risk disease characteristics.
Methods: Patients with BC diagnosed as first primary between 2010-2019 were identified from SEER 17 registries database 2023, with last follow up till 12/31/2021 and with at least 1-month survival. Demographic and clinical data were extracted. Cause of mortality was based on death certificates of the patient and were extracted from SEER database. We calculated BC related mortality (BCRM) and NBCRM, separately for nmBC and de-novo mBC. Multivariate cox proportional hazard model was used to identify high-risk disease characteristics (based on tumor histology, biomarkers, stage and demographics of the patients) and quantify its association with cause-specific mortality.
Results: 457,655 patients with BC (4.73% de-novo mBC) were identified. 83,797 patients died during the study period with 15,130 (18.05%) death in the mBC. The median OS for mBC was 34 months (95% CI 33-35). Median OS not reached for nmBC. Proportion of BCRM was significantly higher in mBC (87.76%) compared to nmBC (43.44%, p-value < 0.05). Risk of BCRM was higher for older adults (Age >65 Years) (mBC HR 1.47, p <0.05; nmBC HR 1.72, p <0.05) compared to age < 50 years, Non-Hispanic African American (NHAA) (mBC HR 1.32; nmBC HR 1.43, p <0.05) compared NH-White, Triple Negative (mBC HR 2.81, p <0.05; nmBC HR 2.47, p <0.05) compared to HR+/Her2-, Stage II (HR 4.03, p <0.05) and Stage III (HR 14.90, p <0.05) compared to Stage I. Compared to ductal histology, Lobular histology had lower risk of BCRM for nmBC (HR 0.90, p <0.05) and no difference

were seen for mBC (0.99, p 0.94). For NBCRM, risk was higher for older adults (nmBC HR 10.83, p <0.05, mBC HR 3.27, p <0.05) and age group 51-65 years (nmBC HR 2.57, p <0.05, mBC HR 1.64, p <0.05) compared to age ≤ 50 years. TNBC (nmBC HR 1.42, p <0.05; mBC HR 2.26, p <0.05) had higher risk compared HR+/Her2-, and higher risk for Stage II (HR 1.61, p <0.05) and Stage III (HR 2.81, p <0.05) compared to Stage I. Lobular histology had lower risk of NBCRM for nmBC (HR 0.84, p <0.05) and no difference were seen for mBC (HR 0.93, p ~ 0.27). Patient who received radiation had both lesser risk of BCRM (nmBC HR 0.60, p <0.05, mBC HR 0.94, p <0.0003) and NBCRM (nmBC HR 0.54 p <0.05, mBC HR 0.81, p <0.05). Similarly, patients who received chemotherapy had lesser risk of BCRM (nmBC HR 0.94, p <0.05, mBC HR 0.66, p <0.0003) and NBCRM (nmBC HR 0.48 p <0.05, mBC HR 0.52, p <0.05). Cardiovascular (30.76%) and subsequent malignancies (19.62%) were the most common causes of NBCRM for nmBC. For mBC, subsequent malignancies (30.24%) and Cardiovascular (24.78%) were the 1st and 2nd most common causes.
Conclusions: With the rising number of BC survivors, it is imperative to identify high-risk disease characteristics which can lead NBCRM. TNBC, ductal histology (for nmBC), and late stage confers higher risk for both BCRM and NBCRM and conveys need for better treatment strategies. Cardiovascular death and subsequent malignancies are significant irrespective of stage of diagnosis and so adherence to preventative health guidelines and lifestyle modifications is crucial. Our study reflects the need to focus on survivorship specifically for older adults and NHAA.

PS15-06: Ability to comply with placebo predicts overall survival in randomized trial

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Background: Poor compliance with medications has been linked to inferior health outcomes. Inability to complete therapy is often attributed to adverse effects from the treatment or its financial burden. However, other less easily measurable factors (e.g. trust in the recommendation, fear of future adverse events, nocebo effect, misinformation, convenience, competing life priorities, etc.) can also influence patients' willingness and ability to follow medical recommendations. In this study, we compared the survival of patients who completed versus not the everolimus placebo pill assigned in the control arm of the S1207 randomized trial.
Methods: The S1207 trial compared 1 year of adjuvant everolimus versus 1 year of placebo added to standard of care adjuvant endocrine therapy. No benefit of everolimus was seen for invasive disease-free (IDFS) or overall survival (OS1. Treatment completion rates were 48% in the everolimus arm and 73% in the placebo arm. In this exploratory analysis, we assessed in the control arm if iDFS and OS differed between patients who could complete 1 year of placebo versus those who could not. The Kaplan-Meier method was used to estimate survival from 1 year (i.e. end of treatment period) and the log rank test to compare the groups. Multivariable Cox regression model included age, performance status, and

prognostic risk group1 as covariates.
Results: This 1-year OS landmark analysis included 823 patients in the placebo arm. Median age was 54 years, 86% were White, 32% premenopausal, and 80% were in prognostic risk groups 3 (i.e. ≥ 4 positive nodes at diagnosis) and 4 (≥ 1 positive nodes after neoadjuvant chemotherapy). Two hundred and twenty-one patients (18%) did not complete placebo defined as taking < 75% of total planned placebo pills. Grade 1-2 adverse events (AE) were reported in 77% (515 out of 670) of the patients in the completer cohort and in 63% (133 out of 211) ($p < 0.0001$) of the non-completer cohort. Grade ≥3 AEs were reported in 5% and 13% ($p < 0.0001$) respectively. After placebo discontinuation, endocrine therapy continued for 89% of patients in the completer and 85% of patients in the non-completer cohort (p=0.30). The 1-year iDFS landmark analysis included 782 patients in the placebo arm. The estimated 5-year iDFS rates from 1-year were similar, 76.4% and 78.2% respectively (HR=0.95, 95% CI: 0.64 –1.41, p=0.81). At median follow-up of 74 months, the 5-year OS rate from 1-year among placebo completers and non-completers was 88.6% and 67.4%, respectively (HR=0.32, 95%CI: 0.22-0.44, p<0.0001). In multivariable analysis, only completer status was associated with improved OS (HR=0.31, 95%CI 0.22-0.44, p<0.0001).
Conclusion: Patients who were not able to complete the assigned placebo drug in the control arm of the randomized S1207 trial had significantly lower OS compared to those who completed the assigned placebo treatment.
Reference: 1. Chavez-MacGregor, M., et al. Phase III Randomized, Placebo-Controlled Trial of Endocrine Therapy±1 Year of Everolimus in Patients With High-Risk, Hormone Receptor–Positive, Early-Stage Breast Cancer. J. Clin Oncol, JCO.23.02344 DOI:10.1200/JCO.23.02344.

PS15-07: Late effects of chemotherapy on patient-reported falls among older breast cancer survivors

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Introduction: It is unknown whether adjuvant chemotherapy increases the risk of falls in older breast cancer (BC) survivors. We evaluated the long-term impact of chemotherapy receipt on patient reported balance problems and falls among older BC survivors.

Methods: Using the Texas Cancer Registry (TCR)-Medicare linked data, BC survivors aged 65 years and older at diagnosis, with local/regional disease, and diagnosed between 2012–2013 were identified. Self-administered questionnaires collected information on patient-reported outcomes (including treatment side effects), demographic and clinical variables between April 2018 and October 2019. Rao-Scott Chi-square tests were used to examine the association between chemotherapy receipt after initial cancer diagnosis and patient-reported falls in the 12 months prior to survey completion, balance problems and receipt of fall prevention interventions (such as assistive devices or physical therapy). Multivariable logistic regression models were used to evaluate the factors associated with patient-

reported falls and balance problems.

Results: Of the 1,493 BC survivors who completed the survey, majority were non-Hispanic white (83.8%), with a history of hormone receptor positive (HR+) BC (77.1%) and localized stage disease (76.6%). 28.3% received adjuvant chemotherapy, and 89% of those received a taxane. Patients who received chemotherapy were more likely to report severe numbness/tingling (12.3% vs 5.1% p <0.0001). However, falls in the past 12 months (32.0% vs 32.9%, p = 0.77), and balance problems (50.3% vs 49.8%, p = 0.86) were similar between chemotherapy vs non-chemotherapy treated groups. In adjusted analysis, taxane use was not significantly associated with falls (aOR: 1.15; 95% CI 0.87–1.52), but older age and higher comorbidity scores were. Patients treated with a taxane were more likely to report problems with balance and walking than those who did not receive chemotherapy (aOR: 1.36; 95% CI 1.04–1.78). Black (aOR: 1.64; 95% CI 1.00–2.69) and Hispanic (aOR: 1.69; 95% 1.14–2.50) BC survivors were more likely to receive an intervention from their health care provider to prevent falls or treat balance problems compared to White survivors.

Conclusion: Chemotherapy use is associated with persistent numbness/tingling more than 5 years after treatment in older BC survivors and patients treated with taxanes are more likely to report balance and walking problems than survivors who did not receive chemotherapy. However, these persistent symptoms and chemotherapy use were not associated with a higher risk of falls among older breast cancer survivors.

PS15-08: Factors and trends associated with alcohol intake in late survivorship for patients with breast cancer

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Background: Alcohol intake (AI) has consistently been shown to increase the risk of breast cancer (BC), and AI during survivorship may impair prognosis. The American Cancer Society, American Institute for Cancer Research, and National Comprehensive Cancer Network guidelines recommend that female survivors abstain from or limit AI to no more than 1 drink per day. Yet many US cancer survivors self-report regular AI, including some who display excessive drinking behaviors. As AI is potentially modifiable, a better understanding of factors associated with AI in BC survivors may inform public health strategies to reduce risk of recurrence. This study aimed to describe sociodemographic and treatment-related predictors of AI near the time of BC diagnosis, and approximately four years after the same.
METHODS:
Adult patients newly diagnosed (within 1 year prior) with BC (stage I-III) and seen at Mayo Clinic Rochester were invited to enroll in the Mayo Clinic Breast Disease Registry. Those

who consented to participate between 12/4/2014 and 4/16/2018 (N=3252) were asked to complete self-reported questionnaires at baseline and at four years after diagnosis. These questionnaires included questions about weekly AI, education, and other demographic factors. Respondents reported weekly AI as follows: none, 1-4, 5-9, 10-14, 15-19, 20-29, 30-39, or 40+ drinks per week, with one drink defined as 5 ounces of wine, 12 ounces of beer or 1 ounce of liquor. Three patients with recurrences were excluded (N=734). Clinical data were abstracted from medical records by a trained nurse abstractor. Questionnaire data from year 2 were used to understand how posttraumatic symptoms, assessed by the Impact of Event Scale (IES-R), as well as depression, assessed by the PHQ-2, might be associated with later AI. Univariate models were used to assess which factors were associated with higher AI (5+ drinks per week) at year 4. Associations of AI with variables of interest were assessed using Cochran Mantel Haenszel tests for trend. This longitudinal cohort study was approved by the institutional review board.

Results: Among 734 participants who reported their weekly AI at both timepoints (mean age 58.5 years, 98.9% female, 97% white), 176 (24%) and 137 (18.7%) reported AI of ≥5 drinks weekly at baseline and year 4, respectively. Higher AI at baseline was strongly associated with higher AI at year 4 ($p<0.001$). There was a decrease in total AI over time across the cohort ($p=0.003$). In univariate models, younger age at cancer diagnosis ($p<0.001$), not having received radiotherapy ($p=0.05$), and carrying a known deleterious BRCA mutation ($p=0.05$) were associated with greater AI at year 4. Smoking was associated with greater AI at both baseline and year 4 ($p<0.001$). More minutes of moderate intensity exercise (described as “not exhausted”, example fast walking) and mild intensity exercise (described as “minimal effort”, example easy walking), higher Godin activity scores, and greater physical health at baseline and year 4 were also associated with increased AI. The IES-R total score at year 2 was strongly positively associated with high AI at year 4 ($p<0.001$), as were IES-R subscale scores for intrusion ($p=0.008$), avoidance ($p=0.006$), and hyperarousal ($p<0.001$). Higher depression scores at year 2 also were linked to greater AI at year 4 ($p=0.04$). Financial stability, educational status, having received chemotherapy, having received endocrine therapy, tumor stage and type of surgical treatment pursued were not associated with AI at year 4.

Coclusion: While overall AI decreased over time in this cohort of BC survivors, AI at baseline was still associated with AI at 4 years. Young patients and those with deleterious BRCA1/2 mutations, as well as those who reported posttraumatic distress and depressive symptoms at year 2, were more likely to report AI at year 4 after a BC diagnosis. These findings may inform targeted public health interventions to mitigate AI and improve BC outcomes.

PS16-01: Longitudinal validation in the UK Biobank of a breast cancer risk assessment tool that combines a polygenic score for all ancestries with traditional risk factors

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Background: Polygenic risk scores (PRS) have been shown to improve predictive accuracy when incorporated into traditional breast cancer (BC) risk assessment tools. However, most PRS have demonstrated suboptimal performance among women of non-European ancestry. We improved a previously reported multiple-ancestry PRS (MA-PRS) to create a second-generation MA-PRS consisting of 56 ancestry-informative and 329 BC-associated single-nucleotide polymorphisms (SNPs). MA-PRS achieved accuracy for diverse populations by characterizing genetic ancestry at each BC SNP and applying ancestry-specific SNP risks and frequencies. Here, we present longitudinal validation of a combined risk score (CRS) that integrates the second-generation MA-PRS with Version 8 of the Tyrer-Cuzick (TC) model using data from the UK Biobank (UKBB).
Methods: The study cohort comprised 198,872 female participants from the UKBB with no history of cancer at the time of study enrollment. Data was dispensed on September 20, 2023. The primary outcome was the time from enrollment to diagnosis of invasive BC or censoring. CRS calibration was assessed by comparing observed (O) to expected (E) incident BCs. The ability of CRS and TC to discriminate between women diagnosed with BC versus women who were unaffected throughout study follow-up was measured by Cox proportional hazards models adjusted for age and ten genetic principal components. We used Kaplan-Meier analysis to examine BC incidence for patients classified as high-risk or low/moderate-risk according to a 5% 10-year risk threshold. Analyses were conducted in both the full cohort and in a subset of 11,563 female participants of self-reported non-White ancestry.
Results: After a median follow-up of 11.8 years, 7,127 (3.6%) participants from the overall cohort and 315 (4.4%) non-White participants were diagnosed with invasive BC. The CRS was well calibrated in the overall cohort (O/E 1.00; 95% CI 0.97-1.02) and in the non-White sub-cohort (O/E 0.91; 95% CI 0.81-1.02). The incorporation of MA-PRS led to significantly improved discriminatory accuracy of CRS compared to TC in both the overall cohort (p=8.2´10^-320) and in the non-White sub-cohort (p=3.1´10^-04). More participants were identified as high risk by CRS (13.0%) than by TC alone (8.4%). Among those classified as high risk by TC, 39.3% were low/moderate risk by CRS, and of those classified as low/moderate risk by TC, 8.6% were high risk by CRS. In cases where CRS and TC classifications disagreed, CRS was more accurate in predicting incident BC.
Conclusion: The CRS, incorporating a second-generation MA-PRS, was well-calibrated in predicting BC and significantly improved upon a traditional risk factor model in UKBB participants unaffected with BC at the time of enrollment. Clinical use of CRS has the potential to improve BC survival through more accurate identification of individuals at high risk.

PS16-02: Polygenic risk score as an aid for risk stratification in benign breast disease

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Background: Pathologic diagnoses of benign breast disease (BBD), subclassified by histologic impression as non-proliferative disease (NP), proliferative disease without atypia (PDWA) and atypical hyperplasia (AH), predict increasing breast cancer risk (BC) in populations; however improved methods to predict individual risk are needed. Research has established that a polygenic risk score (PRS) based on variation in 313 single nucleotide polymorphisms (SNPs) predicts breast cancer (BC) risk in the general population, but data among women with BBD are limited. Thus, we evaluated a PRS in a large single institutional study to assess associations with BBD subtype and BC risk.

Methods: With IRB approval, we evaluated 3,943 subjects in our institutional BBD cohort with existing genotyping data or germline buccal DNA that was genotyped with Illumina's Global Screening Array; these comprised a sample from the underlying BBD cohort of 20,380 women from years 1967-2013, for whom followup data were available on later BC events.

PRS was computed using 261 of the published 313-PRS SNPs that were commonly available across all datasets. The PRS was a weighted linear combination of the genotypes and odds ratios from the largest BC genome-wide association study (GWAS) to date. PRS was standardized within genotyping platform and evaluated per one standard deviation. BBD histologic impression was based on pathology review as NP, PDWA, or AH. Odds ratios (OR) and 95% confidence intervals (CI) for the PRS and histologic impression were calculated using logistic regression, adjusted for age and genotyping platform. Models were examined for histologic impression alone, PRS alone, and the two factors combined. Also, associations between PRS and histologic impression were assessed as well as potential interactions between the two variables on BC risk.

Results

Of the 3,943 women with BBD and PRS data, 857 were cases (i.e., developed BC after BBD) and 3086 were controls. Across the entire sample, histologic impression was NP in 56%, PDWA in 35%, and AH in 9%. The PRS scores after normalizing by platform across the 3,943 women in the sample had a mean of -0.007 with a standard deviation of 1.001 and a range of -3.67 to 3.94. In multivariable models estimating risk of BC based on a referent of NP and mean PRS, factors associated with risk included: PRS (per-SD OR=1.52, 95% CI: 1.39-1.66); PDWA (OR=1.63, 95%CI: 1.35-1.97) and AH (OR=1.71, 95%CI: 1.26-2.30), all significant at $P<0.001$. An interaction term between BBD histologic impression and PRS was not statistically significant ($p>0.70$).

Conclusions: Our analysis suggests that both BBD histologic subtype and PRS are independently associated with BC risk; thus, PRS information may be helpful for personalizing risk assessment in the setting of BBD.

PS16-03: Isoform-level analyses of breast cancer and its subtypes uncover extensive genetic risk mechanisms undetected at the gene-level

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Integrating genome-wide association studies (GWAS) and transcriptomic datasets can identify potential mediators for germline genetic risk of breast cancer. However, traditional methods have been largely unsuccessful at most loci because of an overreliance on total gene expression. These approaches overlook alternative splicing, which can produce multiple isoforms from the same gene, each with potentially different effects on cancers. Here, we integrate genetic and multi-tissue isoform-level expression from the Genotype Tissue-Expression Project (GTEx, N = 838) with publicly available European-ancestry GWAS summary statistics to identify both isoform- and gene-level risk associations with overall breast cancer and estrogen receptor-positive (ER+) and -negative (ER-) subtypes. We employ our recently developed isoform-level Transcriptome-Wide Association Study (isoTWAS) framework that jointly models germline genetic variation, all isoforms of a gene, and phenotypic associations to identify transcriptomic mediators of genetic risk loci. Directly modeling isoform expression substantially increases discovery of transcriptomic mechanisms underlying genetic associations, compared to traditional methods leveraging total gene expression.

Across the 3 indications, isoTWAS identified 2,304 more unique genes than TWAS, reflecting an 34.3% increase (jackknife SE = 3.8%) in effective sample size. In total, we find that isoTWAS-prioritized genes are enriched for pathways involved in DNA repair and damage responses, apoptosis, DNA and transcription factor binding, and cell cycle regulation. In addition, these genes are enriched for transcription binding sites of oncogenic transcription factors, like RUNX2, ESR1, and MYC. Critically, isoTWAS is able to pinpoint an isoform-specific mediation of the ESR1 and FOXA1 loci using isoform expression of non-cancerous breast tumor.

Next, isoTWAS associations are enriched for genes that are evolutionary constrained and showing intolerance to protein-truncating variation (pLI score 0.90), prioritizing 587 constrained genes compared to 153 by TWAS (P = 2.8 x 10-9). This result suggests that isoform associations capture more likely disease signal. Of the 309 independent GWAS loci across the 3 indications, isoTWAS tagged 42% additional GWAS loci with transcriptomic associations (221 loci with isoTWAS, 156 with TWAS, 124 in common). Relatedly, the cis-genetic component of isoform expression (20.8 ± 7.3%) mediates an estimated 92.4% more of cancer risk SNP heritability compared to that of gene expression (10.8 ± 2.6%), using Mediated Expression Score Regression. Lastly, we highlight several salient and novel isoTWAS associations that exhibit isoform but not gene expression colocalization, including BABAM1 and FDPS, associated with overall breast cancer risk, and L3MBTL3 and CASP8, associated with ER- breast cancer risk.

Our results strongly demonstrate that modeling isoform expression is crucial to maximize discovery of genetic risk mechanisms for breast cancer that are testable and actionable.

PS16-04: Differences in breast cancer phenotype by germline TP53 variant functional classification.

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Introduction: TP53 is a multi-functional tumor suppressor gene, with several important roles in tumorigenesis. Females who harbor germline TP53 pathogenic/likely pathogenic variants (GPV) have a very high lifetime risk of developing breast cancer (BC), especially hormone receptor positive and HER2 positive tumors. Specific TP53 variants have different functional consequences for molecular pathways and somatic aberrations in cancers, leading to the hypothesis that the type of TP53 GPV could modulate breast tumor phenotype.
Objective: To examine differences in TP53 GPV and their predicted functional impact on age at first BC diagnosis and BC subtype (ER and HER2 status).
MATERIAL AND METHODS: This multicenter international cohort was comprised of female TP53 GPV carriers diagnosed with any invasive BC (non-metastatic or de novo metastatic). We included TP53 variants classified as GPV in ClinVar, or classified as GPV by at least one major laboratory, or truncating variants. Carriers of a second GPV in other BC genes and carriers of TP53 GPV downgraded to a variant of uncertain significance by the TP53 ClinGen-VCEP were excluded. Clinical data were abstracted from medical records. TP53 GPV were classified according to mutation type: nonsense, frameshift, missense affecting the DNA binding domain (Missense_DBD) or the tetramerization domain (Missense_TD), variants affecting splicing, and copy number variations. For this report, functional classification was performed using Fortuno's classification based on two germline TP53 databases from commercial labs. Functional classification categories included: missense variants with dominant negative effect (Missense_DNE) and without DNE (Missense_notDNE), truncating and hotspots variants. The bivariate association between tumor phenotype and each of the variables (mutation type and Fortuno's functional classification) was assessed using two-sample Wilcoxon test for continuous and Fisher's exact test for nominal categorical variables.
Results: Among 301 females who met study criteria,mean age of BC diagnosis was 37.0 years (SD 10.46), 187 (62.1%) met Li-Fraumeni syndrome clinical criteria (revised Chompret or Classic criteria), and 41 (13.6%) had bilateral synchronous BC. The distribution of BC subtypes was: 108 (35.9%) ER+/HER2-, 79 (26.2%) ER+/HER2+, 62 (20.6%) ER-/HER2+, 20 (6.6%) ER-/HER2-, and 32 (10.6%) ER unknown and/or HER2 unknown. Most TP53 GPV were missense variants (n=217; 141 Missense_DBD, 76 Missense_TD). In comparison to Missense_TD, Missense_DBD carriers had a younger age at BC diagnosis (35.7 vs 42.3, p<0.01), lower rates of ER+ disease (63.1% vs 80.3%, p<0.01), and higher rates of HER2+ disease (48.9% vs 27.6%, p<0.01). When considering ER and HER2 subtypes, Missense_TD carriers had more ER+/HER2- BC (51.3% vs 34.8%, p=0.02) and Missense_DBD carriers had more ER-/HER2+ tumors (22.0% vs 5.3%, p=<0.01).

According to Fortuno's classification, 89 (29.6%) TP53 GPV were Missense_notDNE, 59 (19.6%) Missense_DNE, 80 (26.6%) truncating, 49 (16.3%) hotspots variants, and 24 (8.0%) variants unclassified by the method. Among all these categories, Missense_notDNE variants were associated with older ages of BC diagnosis (51.7% above 40 years; $p<0.001$), higher rates of ER+ disease (79.8%; $p<0.001$), lower rates of HER2+ tumors (29.2%; $p<0.001$), and lower rates of bilateral synchronous BC ($p=0.003$).
CONCLUSIONS: These findings suggest that TP53 GPV functional status can influence age at breast cancer presentation and tumor ER/HER2 status which can potentially improve targeted treatment strategies and inform risk prediction and risk reduction strategies.

PS16-05: Primary breast cancer prevention using oral endoxifen

Per Hall, Stephen Nash, Magnus Bäcklund, Jenny Bergqvist, Mattias Hammarström, Mikael Eriksson, Steven Quay, Kamila Czene

Estrogens play a crucial role in regulating the growth and differentiation of glandular cells in the breast. Tamoxifen blocks the effect of estrogens through binding to the estrogen receptor thereby altering downstream signaling. Tamoxifen was approved by the U.S. Food and Drug Administration (FDA) in 1977 for the treatment of breast cancer. In 1997 the FDA accepted tamoxifen for prevention of breast cancer. Theapproval for prevention was based on results from several large randomized controlled trials targeting women aged 35 years or older with a 5-year breast cancer risk of 1.67% or higher.
Despite tamoxifen's success in reducing the recurrence risk of breast cancer, its systemic side effects have led to generally low acceptance. Also, the effect is heterogeneous because tamoxifen acts as a pro-drug that needs to be metabolization in the liver, primarily via the CYP2D6 enzyme. The most potent metabolite is endoxifen.
Recent studies have shown that breast cancer patients who are poor or ultrarapid metabolizers have a worse outcome than intermediate and normal metabolizers. Poor metabolizers have too low endoxifen levels, while ultrarapid metabolizers often discontinue therapy due to unacceptable side effects. Additionally, common medications, such as selective serotonin reuptake inhibitors, influence CYP2D6 activity, altogether leading to a heterogeneity in tamoxifen efficacy. Endoxifen has been proposed as an alternative, not needing activation and not influenced by drug interactions.
It has been shown that tamoxifen-induced mammographic density decrease is strongly associated with tamoxifen therapy response. Thus, a change in mammographic density could be used for evaluating the effect of endoxifen and / or tamoxifen. A density decrease could also theoretically be used to increase the sensitivity of a mammogram since density influences the ability to identify breast cancer on a mammogram.
The ATOS-016R trial is a phase 2, randomized, double-blinded, placebo-controlled, study of oral (Z)-endoxifen (2 mg, 1 mg, and placebo; 80 women in each arm) in premenopausal women with measurable breast density. The trial invited women aged 40-55 years from three hospitals in Stockholm, Sweden.
The primary objective is to determine if six months of daily (Z)-endoxifen can lead to a relative reduction in mammographic breast density area (cm^2). The secondary objective is

to assess the safety and tolerability of daily oral (Z)-endoxifen. The exploratory objectives are to: i) determine if daily oral (Z)-endoxifen for a maximum of six months results in at least a one-category reduction in the BI-RADS scale, ii) study the durability of (Z)-endoxifen effect on breast density response at 24 months post-standard of care screening mammogram, and iii) evaluate the levels of endoxifen over time and correlate endoxifen values to density change.
Standard of care mammograms was used for the screening mammogram. Two study mammograms (each equivalent to the radiation dose of half a normal mammogram) was performed at 3 and 6 months. Clinical labs, vital signs, adverse events, and responses to questionnaires was used to assess safety and tolerability.
The study was launched in December 2021, and the end of follow-up for the last of the 240 women is scheduled for June 14, 2024. Early results are expected in the fall of 2024.
The ATOS-016R trial is a follow-up study to the KARISMA Low Dose Tamoxifen trial presented at SABCS in December 2023. In that trial, we showed that substantially lower doses of tamoxifen (2.5 mg) reduced mammographic density to the same extent as the established 20 mg dose. Severe side effects were reduced but reports of menopausal-like side effects associated with tamoxifen exposure were still substantial.

PS16-07: Quantitative breast density measures and radiomic parenchymal phenotypes improve breast cancer risk prediction among Black and White women undergoing mammography screening

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Breast parenchymal patterns identified on mammograms are associated with breast cancer risk. Breast density, which is clinically and visually assessed based on the BI-RADS lexicon, is a well-recognized risk factor for breast cancer that has modest reproducibility. Black women have, on average, lower BI-RADS categorized density than White women, despite having higher volumes of dense breast tissue when density is measured quantitatively. Beyond breast density, we recently identified and validated six parenchymal phenotypes, based on 390 radiomic texture features extracted from 2-D full-field digital mammograms performed from 2011 through 2014 from a racially diverse population of 30,000 women. The six phenotypes were also significantly associated with breast cancer risk after adjusting for BI-RADS density among Black and White women. The purpose of this study was to evaluate whether the combination of quantitative breast density measures (dense volume and volumetric percent density) with the parenchymal phenotypes improves breast cancer risk prediction, particularly among Black women. Eligible women were aged ≥35 with no prior history of breast cancer who underwent routine screening mammography at two large academic medical centers. Invasive breast cancer cases were identified and matched to controls in a 1:3 ratio based on age within 5 years, race, timing of images, and

mammogram site. The six phenotypes were estimated on the 1055 invasive breast cancer cases and 2764 controls, of whom 144 cases and 390 controls were Black and 875 cases and 2298 controls were White. BI-RADS density was clinically assessed according to the 4th or 5th edition lexicon. Dense volume (DV) and volumetric percent density (dense volume / total breast area, VPD) were estimated using Volpara software. Conditional logistic regression was used to estimate the associations of BI-RADS density, quantitative density, and radiomic parenchymal phenotypes with breast cancer risk, adjusted for age and BMI. Area under the receiver operating curve (AUC) was also calculated for each model. Both dense volume and radiomic phenotypes were significantly associated with breast cancer risk among both Black and White women ($p<0.05$). Among White women, age and BMI adjusted models including BI-RADS density (AUC=0.600), volumetric percent density (AUC=0.596), and dense volume (AUC=0.601) yielded similar AUCs. For Black women, BI-RADS density had higher AUC (0.630) than VPD (AUC=0.599) or dense volume (AUC=0.607). The addition of parenchymal phenotypes to the models increased discrimination to a greater extent for Black women (BI-RADS + radiomics AUC=0.679, 95%CI=0.632-0.725, VPD + radiomics 0.666, 95%CI=0.619-0.713, and DV + radiomics 0.679, 95%CI=0.632-0.725) than for White women (BI-RADS + radiomics AUC=0.609, 95%CI=0.589-0.629, VPD + radiomics AUC=0.607, 95%CI=0.588-0.627), and DV + radiomics AUC=0.623, 95%CI=0.603-0.643). The model containing age, BMI, BI-RADS density, radiomic parenchymal phenotypes yielded significantly higher AUC among Black women (AUC=0.692, 95% CI 0.646-0.737) compared with White women (AUC= 0.625, 95%CI=0.606-0.645). These results suggest that incorporation of parenchymal phenotypes based on radiomic features improves breast cancer risk prediction and that the addition of quantitative breast density measures further improves risk prediction particularly among Black women.

PS16-08: Trends in LCIS Incidence from 2000-2020 Mirror USPSTF Screening Guidelines: A SEER Registry Analysis

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Introduction: Lobular carcinoma in situ (LCIS) is a proliferative breast lesion that is associated with an increased risk of developing breast cancer. The United States Preventative Task Force (USPSTF) screening mammography guidelines have had a significant impact on screening practice. This is especially true for women aged 40-49 who were recommended screening in the 2002-2009 guidelines but not in the 2009 guideline. The incidence and population level impact of LCIS has not been characterized in association with imaging utilization, specifically changes in the USPSTF screening mammography guidelines. We sought to determine if variations in the incidence of LCIS in the US population correlated with changes in mammographic screening guidelines.

Methods: Utilizing the Surveillance, Epidemiology and End Results (SEER) 22 Registries

2000-2020, we identified all diagnoses of LCIS from 2000-2020 using the ICD-0-3 Hist/behave code 8520/2. Patients <20 years of age and those with a prior breast cancer diagnosis were excluded. Age adjusted incidence per 100,000 was analyzed for trends over the study period. Simple linear regression models were used to determine significance in trend with set at 0.05. Joinpoint version 5.0 was utilized to evaluate the average annual percent change (APC) in age-adjusted incidence over time. Inflection points were set at the years USPSTF screening mammography guidelines changed (2002 and 2009) to evaluate temporal trends before and after the change in guidelines.

Results: 71,588 documented cases of LCIS were identified within the SEER database between 2000-2020. Since 2000, the age-adjusted incidence of LCIS increased from 2.8 per 100,000 to 3.0 per 100,000 in 2020 (p=0.006), with the highest incidence recorded in 2009 at a rate of 3.7 per 100,000. Between 2002 and 2009, during which time USPSTF guidelines recommended starting screening mammography at age 40, joinpoint analysis identified a significantly increased rate of LCIS diagnosis with an APC of 3.6 (confidence interval [CI] 2.2-4.7, p<0.01). Prior to this change in guidelines, from 2000-2002, APC of LCIS incidence was 0.06 (CI -5.5-2.8, p=0.84). Following 2009, when the USPSTF guidelines recommended against routine screening for women ages 40–49 and the reduced screening frequency for women ≥50, APC was -1.2 (CI –[1.8-0.31], p<0.01). Subset analysis was performed separating patients by age group given the differences in screening recommendations by age over the study period. From 2002-2009, the incidence of LCIS was found to increase the greatest in women age 40-49 (APC 5.6, CI 4.8-7.1, p<0.01), although the incidence also increased in women age 50-59 (APC 2.5, CI 1.1-3.3, p<0.01) and those aged 60-69 (APC 0.65, CI -0.01-1.6, p=0.052). From 2009-2020, however, the incidence of LCIS declined in women age 50-59 (APC -1.5, CI –[2.1-0.51], p<0.01) and age 60-69 (APC -2.0, CI –[2.5-1.7], p<0.01), while the incidence of LCIS was unchanged in women age 40-49 (APC -0.39, CI -1.1-0.28, p=0.22).

Conclusions: Incidence of LCIS has increased from 2000 to 2020 with rate of change mirroring changes in USPSTF screening guidelines, with higher incidence identified when guidelines recommended initiating screening at age 40. This suggests that early initiation of screening may lead to diagnosis of LCIS at a younger age, which may allow for earlier initiation of risk-reducing interventions. Given the recent USPSTF guideline changes recommending screening mammography initiation at age 40, we anticipate a future increase in the incidence of LCIS diagnoses in screened populations and subsequently increased opportunity and demand for education, tailored supplemental screening, and risk management for these individuals with LCIS.

PS16-09: A decision support intervention to promote the use of preventive therapy among women at high risk for invasive breast cancer

Inimfon Jackson, Lisa Lowenstein, Parijatham S. Thomas, Therese Bevers, Viola Leal, Jurnie Hinde, Robert J. Volk, Abenaa M. Brewster Background: Clinical trials have reported

significant breast cancer risk reduction with preventive therapy among women at high risk for invasive disease, but these medications remain underutilized. We developed risk communication and decision support tool comprised of an educational video and graphic display of benefits of preventive therapy for providers to use during patient consultations. The primary aim was to increase the uptake of preventive therapy among high-risk women.
Methods: Women aged 35–69 years with a history of lobular carcinoma in situ (LCIS) or atypical hyperplasia (AH) receiving care at MD Anderson Cancer Center, Houston, Texas, were eligible to participate. After cognitive testing, a field test was performed on two patient populations before (pre-implementation) and after incorporating the tool into clinical practice (implementation). Study participants completed self-administered questionnaires including knowledge about preventive therapy, treatment preferences, decisional conflict, shared decision-making process and Ottawa acceptability scales; physicians completed surveys on their experiences with the decision support tool. Descriptive analyses and standard tests of association were performed.
Results: Of the 48 female participants who completed surveys, 21 were in the implementation group. Majority of the participants were non-Hispanic (80.8%), White (75%), with a college degree or more (63.8%) and mean age of 53 years. Most participants had good knowledge about the role of preventive therapy but only 10% pre-implementation and 15% in the implementation group correctly identified that taking preventive therapy can reduce the risk of breast cancer by up to 50%. Overall, 65.2% of participants were leaning towards taking preventive therapy. Compared to those in the implementation group, women in the pre-implementation group were more likely to be unsure about their decision (34.6% vs 20.0%, p=0.088). Participants in the implementation group were less likely to take preventive therapy (57.1% vs 70.4%, p=0.428). Decision making process scores were high (3.26 vs 3.65, p=0.122) and decisional conflict was low in both groups (12.9 vs 16.1, p=0.498). While participants in the implementation group agreed that the amount of information provided by the tool was just right (80%), they found the materials slanted towards taking preventive therapy (75%). Using a psychometric assessment, physicians gave high ratings for acceptability (mean 4.1, SD 0.6), feasibility (mean 4.4; SD 0.60) and appropriateness (mean 4.2, SD 0.6) of the tool and were satisfied/very satisfied (83.3%) with the tool.
Conclusion
Although study participants had good health literacy, the majority were unaware of the significant benefit of preventive therapy in reducing breast cancer risk. A greater percentage of women in the pre-implementation group were unsure about their decision compared to women who received the tool but after receiving the tool, women in the implementation group were less likely to agree to preventive therapy. These findings suggest that the decision support tool might reduce the proportion of patients uncertain about preventive therapy but increase preference for not starting treatment. The next steps are to enhance provider discussions on the benefits of preventive therapy, test the decision support tool in less educated and underrepresented minority populations, and track adherence to preventive therapy in patients who receive the tool.

PS17-01: Spatial transcriptomics identifies molecular patterns predictive of response to neoadjuvant chemotherapy in triple-negative breast cancer

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Background: Triple-Negative Breast Cancer (TNBC) is a highly aggressive subtype of breast cancer and only ~40% of TNBC patients achieve pathological complete response (pCR) following neoadjuvant chemotherapy (NACT). Since molecular heterogeneity of TNBC contributes to NACT response, we investigated their histology and spatial transcriptomics (ST) patterns.
Methods: In total, 97 core biopsies, taken prior to (n = 92) or during (n = 5) treatment, and 44 post-treatment surgical resections were collected from 96 women with TNBC receiving NACT from archival samples and the ongoing FORCE (NCT03238144) clinical trial at Guy's Hospital, London, UK. Each tissue section was H&E stained, imaged, and annotated by a pathologist. A 6-digit classifier was designed to capture the histology of each region of interest (ROI), characterising the tumour region, epithelial type and immune cell localisation, population, distribution, and abundance. The annotations were used to guide ST profiling of serial sections, using the GeoMx Digital Spatial Profiler. Tissue sections were stained with fluorescent antibodies against PanCK and CD45. Tumour and immune-enriched compartments, defined as PanCK+/CD45- and PanCK-/CD45+, respectively, were acquired. Data analysis was performed in R 4.3.1, including quality control, batch correction, differential and topographical gene expression, immune cell deconvolution and gene set enrichment analysis (GSEA). Epithelial states (ES) were defined by identifying gene modules of co-expressed genes and annotating these with enriched pathways.
RESULTS: To date, we have performed ST profiling of 120 TNBCs from 85 patients (40 Non-Responders; 45 Responders), totalling 4506 tumour or immune-cell enriched regions. Genes signatures known to predict pCR of NACT in women with TNBC were first assessed by generating a pseudo-bulk dataset produced in silico by combining gene counts from all regions. Two gene signatures, namely the PAM50 proliferation signature and the GeparSixto Immune signature, were found to be predictive (PAM50: OR = 0.12, p-value = 0.06; GeparSixto: OR= 0.19, p-value = 0.03) in our data. Seven transcriptionally distinct epithelial states (ES), each underpinning unique molecular functions, were defined by identifying gene modules of tumour-enriched regions. The relative abundance of ES2 (DNA repair mechanisms) and ES4 (deregulated ECM, increased epithelial-mesenchymal transition and TGF-b signaling) in pre-NACT core biopsies was significantly associated with pCR (OR=1.03, p-value = 0.02) and residual disease (OR = 0.98, p-value = 0.05), respectively. Within the immune compartment, seven clusters of tumour-immune microenvironments (TIMEs), were obtained by unsupervised hierarchical clustering of immune cell proportions. A high relative abundance of TIME1 (naïve CD4 T cells and plasma cells) and TIME5 (naïve CD4 T cells, naïve B cells, memory B cells and plasma cells) were significantly associated with pCR (TIME1 OR = 1.05, p-value = 0.01; TIME5 OR = 1.04, p-value = 0.05). We further investigated rates of co-occurrence between each ES and TIME, identifying combinations

that were statistically most probable to occur and identified TIME as a confounding factor in the relative risk associated with the presence of individual ES. Moreover, we identify potential ligand-receptor interactions between distinct ES and TIMEs which may provide new insights into the mechanisms driving differential responses to NACT in TNBC.

Conclusion: To date, we have collected one of the largest spatial transcriptomics data sets with detailed histological annotation. The abundance of epithelial states and tumour-immune microenvironments is predictive for pCR in NACT TNBC. The spatial location, colocalization and ligand-receptor interactions in ES and TIME provide novel insights into the molecular heterogeneity of TNBCs and mechanisms regulating responses to NACT.

PS17-02: Molecular and Immune Landscape of Metaplastic Triple Negative Breast Cancer Compared with Invasive Ductal Triple Negative Breast Cancer

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Background: Metaplastic Breast Cancer is rare and aggressive form of BC with majority having triple-negative receptor status. There are no standard therapeutic approaches for MBC and patients are treated similar to invasive ductal triple negative breast cancer (ID-TNBC) but with worse outcomes in comparison to ID-TNBC. Hence, there is an urgent need for development of new drug targets and therapies to improve outcomes in these patients. Here, we characterize the molecular and immune signature of metaplastic TNBC (M-TNBC).
Methods : 455 BC samples (M-TNBC, n=91; ID-TNBC, n=364) were analyzed by next-generation sequencing (592, NextSeq; WES, NovaSeq), Whole Transcriptome Sequencing (WTS; NovaSeq) (Caris Life Sciences, Phoenix, AZ). Tumor mutational burden (TMB) totaled somatic mutations per tumor (high>10 mt/MB). Microsatellite-instability (MSI) was tested by IHC and NGS. Immune cell fractions were calculated by deconvolution of WTS: Quantiseq. Pathway enrichment was determined by GSEA (Broad Inst). Statistical significance was determined using chi-square and Mann-Whitney U test and p-value <0.05 was considered significant.
Results: M-TNBC had a higher frequency of PIK3CA (50.0% vs 14.5%), PTEN (16.1% vs. 8.4%), EGFR, (2.2% vs 0%), PIK3R1 (14.4% vs. 5.5%), TERT (29.7% vs. 0.5%), but lower frequency of TP53 (70.6% vs. 89.7%) compared to ID-TNBC (all p<0.05). There was no difference in the frequency of TMB-high (3.3% vs 5.2%, p=0.58), dMMR/MSI-H (2.2% vs 1.1%, p = 0.34) and PD-L1 positivity (22c3) (50.0% vs 42.6%, p=0.38) between M-TNBC and ID-TNBC. M-TNBC had lower AR protein expression (11.0% vs 24.8%) and lower frequency of fusion variant-AR (0% vs 4.9%) compared to ID-TNBC (all p < 0.05). Analysis of inferred immune cell infiltrates showed that M-TNBC had increased infiltration of M2 macrophages (3.3% vs. 2.9%) and neutrophils (5.9% vs. 2.6%) but decreased infiltration of B cells (3.9% vs. 4.5%), T regulatory cells (Treg) (1.0% vs. 1.9%), Dendritic Cells (DC) (1.7% vs. 3.2%)

and CD8 T cells (0% vs. 0.5%) (all $p < 0.05$). M-TNBC had decreased IFNγ score (-0.31 vs -0.24, $p=0.05$), but increased MAP kinase pathway activity score (0.9 vs 0.08, $p<0.05$). ID-TNBC had higher expression of immune checkpoint genes (FOXP3, IDO1; FC: 1.3-1.5), cell cycle genes (CDKN1B, E2F1, CCNE1; FC: 1.2-1.4), inhibition of apoptosis genes (BIRC3, BIRC6, BCL2; FC: 1.1-1.3), but lower expression of stem cell-related genes (CD44, ALDH1A2, KLF4, SOX2; FC: 1.2-2.3) (all $p < 0.05$). M-TNBC had gene set enrichment of epithelial to mesenchymal transition (EMT) pathway (NES: 1.5, FDR<0.25).
Conclusion: These data indicate that M-TNBC is associated with an aggressive disease biology with higher frequency of PIK3CA, PTEN, PIK3R1, TERT, EGFR and gene set enrichment of EMT pathway. Higher expression of stem cell-related gene expression in M-TNBC indicates their association with therapy resistant phenotype. Also, M-TNBC had increased infiltration of M2 macrophages and neutrophils, decreased infiltration of B cells, Treg, DC and CD8 T cells and, lower IFNγ score and MAP kinase activity score suggesting differential tumor immune microenvironment compared to ID-TNBC However, these findings warrant further validation in larger studies.

PS17-03: Real World Adjuvant Capecitabine Utilization and Patient Outcomes Among Patients With Triple-Negative Breast Cancer with Residual Disease after Neoadjuvant Chemotherapy

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Background: Residual disease (RD) after neoadjuvant chemotherapy (NAC) for patients diagnosed with triple-negative breast cancer (TNBC) identifies a high risk population with increased rates of recurrence and poor prognosis. Adjuvant therapy (AT) with capecitabine is a standard management for patients with TNBC-RD based on the CREATE-X study. Alternative approaches, such as adjuvant capecitabine for unselected patients with TNBC (GEICAM 2003-11/CIBOMA 2004-01) have not shown benefit. Real-world utilization of adjuvant capecitabine for patients with TNBC-RD and the association of capecitabine with survival outcomes remains largely unknown. To address this gap, we investigated the utilization of capecitabine and other adjuvant therapies and association with outcome in a large cohort of patients with TNBC receiving NAC in the era prior to routine use of immunotherapy.
Methods: Patients with stage I-III TNBC (including estrogen receptor and progesterone receptor <10%) who received NAC and underwent surgery between January 2016 and June 2019 were identified from databases from 3 comprehensive cancer centers. Treatment in the adjuvant setting was categorized as: capecitabine, other systemic therapy, or no AT. Propensity score methods were used to control potential confounding effects from baseline covariates (site, age at diagnosis, race, clinical stage, histology type, genetic status, residual

cancer burden/RCB class, HR at diagnosis, HER2 at diagnosis, HR at surgery, HER2 at surgery, nodal disease at surgery, neoadjuvant anthracycline/taxane receipt). Survival outcomes were estimated using Kaplan-Meier and Cox proportional hazards models.
Results: Among 977 patients with TNBC, the NAC regimen was primarily anthracycline-taxane (n=611; 62.5%), anthracycline-taxane-platinum (180; 18.4%), or taxane-platinum (39; 4.0%), while 122 (12.5%) patients received single-agent therapy or an alternate regimen. In the total population, 316/977 (32.3%) experienced pathologic complete response (pCR) while 661/977 (67.6%) had RD. Among patients with TNBC-RD, omission of AT was common: 45.1% (298/661) of patients with TNBC-RD did not receive any AT. Adjuvant therapy regimens among patients with TNBC-RD were diverse with 202/661 (30.6%) receiving capecitabine, 115/661 (17.4%) other chemotherapy, and 46/661 (7.0%) only targeted therapy. The primary objective was to evaluate survival outcomes among patients with TNBC-RD who received capecitabine versus no AT. At 3.5 years median follow-up, receipt of capecitabine was associated with significantly improved recurrence-free survival (RFS; hazard ratio/HR 0.70 95% confidence interval/CI 0.54-0.91, p=0.008), distant recurrence-free survival (DRFS; HR 0.71, 95% CI 0.54-0.93, p=0.01), and overall survival (OS; HR 0.66; 95% CI 0.49-0.90, p=0.009). As a sensitivity analysis, receipt of adjuvant capecitabine without any other chemotherapy (n=189) versus no AT demonstrated similar significant association with RFS, DRFS, and OS. Receipt of other adjuvant chemotherapy (n=109; with 67/109 single agent anthracycline, taxane, or platinum) versus no AT did not demonstrate significant association with RFS (HR 0.90, 95% CI 0.57-1.42, p=0.7), DRFS (HR 0.84, 95% CI 0.52-1.34, p=0.5), or OS (HR 0.63, 95% CI 0.37-1.08, p=0.08).
Conclusion: In this large cohort of patients with TNBC-RD, omission of adjuvant therapy was common, with less than a third of patients receiving adjuvant capecitabine. However, receipt of adjuvant capecitabine was associated with significantly improved RFS, DRFS, and OS. These results highlight the importance of evaluating adjuvant capecitabine benefit in the immunotherapy era.

PS17-04: Implications of Adjuvant Chemotherapy in Small Triple-Negative Breast Cancer

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Background: The role of adjuvant chemotherapy in triple-negative breast cancer (TNBC) is critical given the absence of effective targeted therapies. Current guidelines strongly recommend adjuvant chemotherapy for patients with T1cN0 TNBC (primary tumor over 1 cm), yet the potential benefits for T1ab (up to 1 cm) TNBC remain unclear.
Methods: This study evaluates the efficacy of adjuvant chemotherapy across early stages of TNBC (T1abc) using our institution's cancer registry and survival data. We collected patients' demographics and clinical manifestations. Kaplan-Meier analysis was used for disease-free survival (DFS) and overall survival (OS). Cox multivariate analysis was used to identify independent predictors of survival outcomes.

Results: A total of 389 patients were diagnosed with stage I TNBC between 2000-2023. Clinical and survival data were available for 280 patients from the main campus, while 109 patients from the satellite site had survival data only. The median age at diagnosis was 60. Among these patients, 8 out of 48 (16.7%) with T1a, 61 out of 116 (52.3%) with T1b, and 143 out of 225 (63.6%) with T1c received adjuvant chemotherapy. Our data suggest that adjuvant chemotherapy significantly improved OS in patients with T1b and T1c TNBC ($p=0.048$ and $p<0.001$, respectively), while no difference was observed in OS for T1a TNBC. There were no significant differences in DFS in T1abc, likely due to the small number of events. Cox multivariate analysis revealed that patients older than 50 years (HR 3.53, $p=0.003$) and those with T1c stage tumors (HR 3.20, $p=0.031$) had significantly poorer OS. Conversely, adjuvant chemotherapy was associated with a marked improvement in OS (HR 0.36, $p=0.001$). Notably, patients with significant comorbidities were less likely to receive chemotherapy, potentially confounding OS outcomes.
Conclusion: Our study provides crucial insights into the potential benefits of extending adjuvant chemotherapy to small TNBC tumors. These findings advocate for further research to precisely define which early-stage TNBC patients can significantly benefit from chemotherapy, thereby refining treatment protocols and improving patient outcomes.

PS17-05: Use and benefit of neoadjuvant versus adjuvant chemotherapy in node-negative, T1 triple negative breast cancer.

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Purpose: Neoadjuvant chemotherapy (NACT) is frequently used in node-positive and/or large (>2cm) triple-negative breast cancer (TNBC). However, there are scarce data about use and benefit of NACT in small size, node-negative TNBC. We examined survival differences of patients with T1N0 TNBC that were treated with NACT vs. adjuvant chemotherapy (ACT).
Methods: This is a retrospective, cross-sectional study using data from the Surveillance, Epidemiology, and End Results database of patients with T1N0 TNBC diagnosed between 2010-2020. Logistic regression analysis was used to identify factors associated with the use of NACT. Cox regression models were used to compare overall survival (OS) for ACT and NACT cohorts, adjusting for demographic, clinicopathological, treatment and socioeconomical variables. Cumulative incidence functions (CIF) and cause-specific hazard models were used to compare the risk of breast cancer death between ACT and NACT cohorts. We also evaluated pathological complete response (pCR) rate in patients treated with NACT.
Results: We found 8,146 patients treated with ACT and 1,263 patients treated with NACT. Age <50 years, mastectomy and radiation therapy were associated with higher odds of receiving NACT. Age >65 years, non-metropolitan residency, histology other than ductal, and having small tumor size were associated with lower odds of receiving NACT. When adjusted for demographic, clinicopathological, treatment, and socioeconomic covariables,

NACT was associated with worse OS (HR, 1.34; 95% CI, 1.04–1.73; p=0.023) and breast cancer specific survival (BCSS) [HR, 1.52; 95% CI, 1.11–2.08; p=0.008] than ACT. The worse OS and BCSS with NACT were mainly driven by T1a tumors as patients with T1a tumors had worse OS (HR, 9.13; 95% CI, 2.40–34.75) and BCSS (HR, 19.70; 95% CI, 3.82–101.67) when treated with NACT when compared to those treated with ACT; whereas patients with T1c tumors had no difference in OS and BCSS when treated with NACT or ACT. pCR in all T1 tumors was associated with improved OS (HR, 0.28; 95% CI, 0.15 – 0.52; p<0.001) and BCSS (HR, 0.21; 95% CI, 0.01 – 0.46).
Conclusion: Patients with T1b and T1c tumors benefit equally from the use of ACT and NACT. The reasons for worse outcome in patients with T1a tumors treated with NACT compared to ACT are unknown and need further investigation. pCR was associated with improved outcomes in patients with T1N0 TNBC who received NACT.

PS17-06: Neutrophil-to-lymphocyte ratio (NLR) predicts long-term survival in early triple negative breast cancer (TNBC) treated with neoadjuvant chemotherapy (NACT)

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Introduction: Biomarkers for long-term survival in early TNBC are needed to improve neoadjuvant strategies. This study aimed to evaluate the role of neutrophil-to-lymphocyte ratio (NLR) in predicting survival after NACT.
Methods: We retrospectively reviewed our institutional database to identify patients (pts) who underwent NACT for early-stage TNBC (II-III) from 2012 and 2024 and collected data from medical records. Event-free survival (EFS) was calculated from the first cycle of NACT until an event occurred. EFS events were defined as death, disease recurrence or disease progression that precluded surgery. EFS and overall survival (OS) were estimated with the Kaplan-Meier method and Cox regression model was used to calculate Hazard Ratio (HR). Logistic regression was used to verify association between NLR and pathological complete response (pCR). NLR was calculated from the complete blood count before NACT initiation.
Results: Among the 692 pts with available information identified, 62.9% had stage III disease, 61.1% grade 3 disease and 77% ki67 >50%. Median age was 48 years and the most common NACT regimen used was AC-T (86.2%). The overall pCR rate was 28.3% and pts with NLR ≤ 2 had an increased probability of achieving pCR (32.6% vs 22.7%, p = 0.005). After a median follow-up of 59.6 months, NLR ≤ 2 was associated with improved 5-year EFS in the overall population (51% vs 66%, HR 0.59, 95 % Confidence Interval [95CI] 0.46 - 0.75, p<0.001), in pts with stage II disease (69% vs 81%, HR 0.49, 95CI 0.29 - 0.85, p=0.01), stage III (43% vs 55%, HR 0.70, 95CI 0.53 - 0.93, p=0.01), residual disease (42% vs 54%, HR 0.65, 95CI 0.50 - 0.84, p=0.001) and a trend for improved survival for pts with pCR (82% vs 92%, HR 0.45, 95CI 0.18 - 1.1, p=0.08). 5-year OS was also improved in the overall population with NLR ≤ 2 (58% vs 73%, HR 0.56, 95%, 0.42 - 0.74, p<0.01]), stage II disease

(75% vs 86%, HR 0.42, 95CI 0.22 - 0.81, p=0.009), stage III disease (50% vs 62%, HR 0.68, 95CI 0.50 - 0.93, p=0.015) and in pts with residual disease (50% vs 64%, HR 0.62, 95CI 0.46 - 0.82, p=0.001), but not in pts who achieved pCR (82% vs 91%, HR 0.50, 95CI 0.20 - 1.24. p=0.1). In multivariate analysis, including pCR status and clinical stage, NLR ≤ 2 remained statistically significant for improved OS (p= 0.002) and EFS (p=0.002).
Conclusion: NLR >2 is an independent risk factor for poorer survival in pts with TNBC who received NACT. As a readily available biomarker, NLR should be further explored in current neoadjuvant protocols for early TNBC.

PS17-07: Comparison of an Atezolizumab monotherapy window followed by Atezolizumab and chemotherapy vs. Atezolizumab and chemotherapy alone in high-risk triple-negative breast cancer (TNBC) – a subgroup analysis of the neoadjuvant neoMono trial

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Background: The neoMono trial prospectively analyzed whether the addition of a preceding Atezolizumab monotherapy window prior to Atezolizumab and neoadjuvant chemotherapy (CTX) improves pCR rates among patients with early TNBC (eTNBC). pCR rates in the investigational arm (i.e., Atezolizumab monotherapy window) were 91.5% in the PD-L1 (IC)-positive group and 56.1% in the PD-L1 (IC)-negative group, corresponding pCR rates in the control arm were 82.2% and 64.5%, respectively. In multivariate analysis of the ITT population (including tumor size, nodal status, tumor grade, age and PD-L1 status) the odds ratio (OR) for achieving a pCR was 4.77 (p< 0.001) for PD-L1-positive tumors indicating a significant impact of the PD-L1 status on pCR-rates in the neoMono trial. The goal of the following subgroup analysis was to explain the influence of different clinical risk profiles on the role of PD-L1 as a biomarker in eTNBC for efficacy of neoadjuvant ICI-containing therapy by analysis of patients with high risk (tumor size 2 cm and higher and/or N+) eTNBC.
Methods: NeoMono is a phase 2 randomized multicenter trial planned to recruit a maximum of 458 female and male pts with primary TNBC (defined as ER/PR < 10% and HER2 negative) with tumor stages cT1c – cT4d (cN0 and cN+). PD-L1 status had to be identifiable by central pathology by means of the VENTANA PD-L1 (SP142) assay and was defined by PD-L1 expression on immune cells (IC). Neoadjuvant treatment in both study arms consisted of Atezolizumab 1200 mg every 3 weeks in addition to neoadjuvant CTX (12 x Carboplatin/Paclitaxel q1w followed by 4x Epirubicin/Cyclophosphamide q3w), in arm A preceded by an Atezolizumab monotherapy window of 840 mg once two weeks prior to initiation of combination therapy. The neoMono statistical design uses Bayesian posterior probabilities (uniform prior distribution) and logistic regression. In this subgroup analysis we included only patients with a tumor size of 2 cm and higher and/or involved lymph

nodes
Results:
115 pts in arm A and 121 in arm B from 34 study sites were included in this subgroup analysis. Demographics and baseline characteristics as well as drug exposure were well-balanced in both arms. In the analysis stratified by PD-L1 (IC) status (negative: <1% versus positive: ≥ 1%), pCR rates in arm A were 88.9 % in the PD-L1 (IC)-positive group and 50.6 % in the PD-L1 (IC)-negative group, the corresponding pCR rates in arm B were 80.0 % and 59.3 %, respectively. In a multivariate analysis including therapy arm, PD-L1 status, tumor size, nodal involvement, grade 3 and age only PD-L1 (IC) status (OR 4.42; p <0.001) and grade 3 (OR 3.49; p=0.014) interacted significantly with pCR.
Conclusion:
In this analysis, the association between PD-L1 (IC) status and pCR rates could be reproduced in a subgroup of patients with =/> cT2 and/or N+ tumors. These results are in line with exploratory results of the NeoTRIP trial and the IMPASSION031 trial demonstrating an association of PD-L1 status with pCR rates after neoadjuvant Atezolizumab + chemotherapy in patients with high risk eTNBC similar to the population in this subgroup analysis. Our analysis in the largest group of patients with high risk eTNBC treated with neoadjuvant Atezolizumab + chemotherapy published to date demonstrated a significant association of PD-L1 status and pCR rates. Further investigations are needed to better understand the clinical implications of this finding.

PS17-08: Association of antibiotic exposure with pathologic complete response in patients with non-metastatic triple-negative breast cancer receiving neoadjuvant chemotherapy and pembrolizumab

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Background: Results of the KEYNOTE-522 trial established the role of immunotherapy in combination with neoadjuvant chemotherapy (NAC) for early-stage triple negative breast cancer (TNBC). In this trial, the addition of the immune checkpoint inhibitor, pembrolizumab, when used in combination with paclitaxel-carboplatin/doxorubicin-cyclophosphamide NAC achieved higher pathologic complete response (pCR) and improved event-free survival compared to NAC alone and thus, this regimen has become standard of care in this population. As the use of immunotherapy for early stage TNBC increases, it is critical to assess factors that impact treatment efficacy. Emerging data suggests antibiotic exposure may decrease rates of pCR among patients with solid cancers receiving NAC + immunotherapy, however, there is minimal data in breast cancer and there is heterogeneity in defined windows of antibiotic exposure. We therefore sought to assess the association of antibiotic exposure and window of antibiotic exposure on rates of pCR among patients with TBC treated with the KEYNOTE-522 regimen.
Methods: Patients with non-metastatic TBNC who received NAC + pembrolizumab and completed breast surgery at our institution were identified. Clinicopathologic and treatment data including patient age, BMI, clinical stage, histology, chemotherapy regimen,

receipt of antibiotics, number of pembrolizumab doses received, and final pathology details were collected. Pathologic complete response was defined as no residual invasive carcinoma in the breast or nodes (ypT0/isN0). Differences between the cohort of patients who did or did not receive antibiotics were assessed by Chi-squared tests. Rates of pCR were compared between those with antibiotic exposure vs none at the following time points: antibiotics concurrent with NAC + pembrolizumab, antibiotics within 30 days of first pembrolizumab dose, and antibiotics within 60 days of first pembrolizumab dose using logistic regression.
Results: 42 women received NAC + pembrolizumab followed by surgery for non-metastatic TNBC from 2021-2024. Patient demographics are as follows: mean age 51 years, 90% cT2-4 tumors, 50% node positive at presentation, 93% invasive ductal histology with 7% metaplastic tumors. Overall, 20 (47.6%) patients received antibiotics concurrent with NAC + pembrolizumab, 13 (31%) within 30 days of first pembrolizumab dose, and 20 (47.6%) within 60 days of first pembrolizumab dose. There were no significant differences in age, BMI, clinical stage, tumor history, or number of pembrolizumab doses received between those who did or did not receive antibiotics. The overall pCR in the cohort was 59.5%. Rates of pCR for those who did or did not receive antibiotics concurrently with pembrolizumab were 55% vs 63.6%, p=0.6, respectively. Similarly, pCR rates for those who received antibiotics within 30- or 60- days of initiation of NAC + pembrolizumab compared to those who did not were 53.9% vs 62.1%, p=0.6 and 60% vs 59.1%, p=0.95, respectively.
Conclusions: Use of antibiotics concurrently or within 30 days of initiation of NAC + pembrolizumab was associated a non-significant trend of reduced pCR among non-metastatic TNBC patients. While this series is limited due to the small patient cohort, these patterns align with data from other cancer types highlighting the need for additional study of the impact of antibiotic exposure and efficacy of immunotherapy for this high-risk patient population.

PS17-09: Immune landscape and clinical features of acquired resistance to immune checkpoint blockade in triple negative breast cancer

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Introduction: A substantial number of patients with metastatic triple negative breast cancer (mTNBC) relapse after having an initial response to immune checkpoint blockade (ICB). Our understanding of acquired resistance to ICB in solid tumors is limited and data is lacking for TNBC. Here, we present an in-depth analysis of the immune and genetic landscape of patients with mTNBC who developed acquired resistance during treatment with anti-PD1.

Methods: We identified patients with acquired resistance from the TONIC [NCT02499367] and TONIC-2 [NCT04159818] trials, in which patients with mTNBC were treated with anti-PD1 with or without a short induction treatment with low dose chemotherapy or irradiation. Acquired resistance was defined as (oligo)progression despite continuous

dosing of anti-PD1 following an initial response with a duration of at least 6 months (complete or partial response according to iRECIST1.1). Biopsies were taken at baseline, during response to anti-PD1 and at time of acquired resistance. Nine baseline tumor samples were available for whole exome sequencing (WES), bulkRNAseq and multiplexed ion beam imaging (MIBI). Paired biopsies at baseline and time of acquired resistance were available for six patients.

Results: Among 24 responders out of a total of 158 evaluable patients, we identified 14 patients with acquired resistance and 7 patients with a durable response > 1 year (median 169 weeks). Patients with acquired resistance had median tumor infiltrating lymphocyte (TIL)-levels at baseline of 14% (range 1% – 60%), and 10/14 patients had a PD-L1 positive tumor (CPS ≥10). Half of the patients had visceral metastases, predominantly lung (n=4), and only 1 patient had liver metastasis. Among patients with a durable response, median TIL-score was 10% (range 1%-80%), 4/7 patients had a PD-L1 positive tumor and only 1 patient had liver metastasis.
Our exploratory analyses revealed a decrease in infiltrating CD8+ T cells and MHC-II-related gene set expression at time of acquired resistance compared to the time of ongoing response ($p < 0.05$). In addition, we observed an increase in expression of gene MAL2 ($p < 0.05$), which is known to be involved in several processes among which endocytosis and degradation of MHC-I complexes.
In contrast to patients with progression of all tumor lesions, patients with only one progressive lesion (oligo-progression) had an increase in immune checkpoint molecules (PD-L1, PD1, CTLA4, TIM3, LAG3, TIGIT) and infiltration by T cell subsets (CD8+, helper T- and regulatory T cells) accompanied by IFN-y signaling pathway upregulation at time of acquired resistance compared to baseline. CTLA4-expression already increased during response with further increase at time of acquired resistance in this subset of patients. Newly acquired mutations related to immune escape or -invasion were not yet identified, except for a mutation in IFN-alfa16 in 1 patient at time of resistance. Analyses of MIBI-data, and a comparison between patients with acquired resistance and primary resistance is currently ongoing. Results will be presented at SABCS.

Conclusion: Our exploratory analyses based on longitudinal tumor samples suggest that patients with TNBC who develop acquired resistance to anti-PD1 might have a decreased T cell activity in the TME and hampered antigen presentation at time of acquired resistance. These findings inform novel approaches for overcoming acquired resistance, which occurs in a substantial group of mTNBC patients with initial response to anti-PD1.

PS17-10: Genomic and transcriptomic analyses of residual invasive triple-neg breast cancer after neoadjuvant chemotherapy in prospective MIRINAE trial (a randomized phase II trial of adjuvant atezolizumab + capecitabine versus capecitabine; KCSG-BR18-21)

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Background: Neoadjuvant chemotherapy (NAC) is the preferred treatment approach for clinical stage II/III triple-negative breast cancer (TNBC). Pathologic complete response (pCR) rates ranges from 30% to 65%. The MIRINAE trial (KCSG-BR18-21) is a randomized phase II trial evaluating the efficacy and safety of adjuvant atezolizumab plus capecitabine versus capecitabine in TNBC patients who do not achieve pCR after NAC without immunotherapy (NCT03756298). This study aims to characterize residual TNBC following NAC through comprehensive tumor microenvironment (TME) and genetic analysis in a prospective multicenter trial.
Methods: After anthracycline and taxane-based NAC, surgically resected residual tumors were collected, and analyzed for stromal tumor-infiltrating lymphocytes (TILs), FoundationOne®CDx, and RNAseq. A 30% cut-off was used to classify samples into TIL-high and TIL-low. RNAseq-based molecular subtyping was performed to determine intrinsic subtype via PAM50, as well as for TNBC and TME subtypes. Immune cell deconvolution was conducted using CIBERSORTx. Fisher's exact test was used to analyze the association between TILs and molecular subtypes, with significance set at a $p < 0.05$. Differentially expressed genes were identified using glmFit function in edgeR package.
Results A total of 311 patients (median age 48; range, 28-74) were analyzed. ypTNM stages were stage I (28.0%), II (48.7%), and III (23.3%). TILs were evaluated in 299 (96.1%) samples, with 27.1% classified as TIL-high. FoundationOne®CDx was performed in 255 (82.0%) samples. The most frequently mutated genes were TP53 (87.8%), PIK3CA (19.6%), BRCA1 (8.6%), and PTEN (6.7%). Pathogenic alterations in the PI3K/AKT pathway (PIK3CA, PTEN, AKT1, and PIK3R1) were observed in 28.6%, while homologous recombination repair-related genes (BRCA1, BRCA2, and PALB2) were mutated in 12.1%. RNAseq was performed in 221 (77.8%) samples. According to the PAM50 classification, most patients (51.1%) were classified as Basal-like, 19.0% as Luminal A, 5.0% as Luminal B, 14.0% as HER2-enriched and 10.9% as Normal-like. TNBC molecular subtyping revealed that 36.7% of the tumors were categorized as mesenchymal (MES), followed by basal-like immune suppressed (BLIS) at 30.3%, basal-like immune activated (BLIA) at 20.8%, and luminal androgen receptor (LAR) at 12.8%. Among 213 patients with both RNAseq and TIL data available, we found that TIL-high group was significantly enriched with BLIA subtype (37.5%) and TME subtypes including IE (immune-enriched, 45.3%) and IE/F (immune-

enriched/fibrotic, 31.3%). Immune-suppressive subtypes D (immune-depleted, 49.7%) and F (fibrotic, 29.5%) were significantly enriched in TIL-low. CIBERSORTx immune cell deconvolution analysis showed greater proportions of plasma cells, CD8+ T-cells, CD4+ memory resting T-cells, follicular helper T-cells, activated NK-cells, and M1 macrophages in TIL-high tumors (all adjusted $p < 0.01$). TIL-high samples showed upregulation of immune cell-related genes (CD3D, CD4, CD8A, CD38, IRF4, MZB1 and GZMK). Chemokine and immune-checkpoint genes (CXCL9, CXCL13 and LAG3) were also higher in TIL-high. VEGFA, NDRG1 and IRS4 were highly expressed in TIL-low (all adjusted $p < 0.0001$). Gene sets related to immune response and interferon-gamma response were significantly enriched in TIL-high group, while TIL-low tumors showed enrichment for hypoxia related gene sets (all adjusted $p < 0.05$).
Conclusion: Residual invasive TNBC after standard NAC in the MIRINAE trial were predominantly MES and BLIS subtype with a high frequency of TP53 mutations. TIL-high tumors were associated with immune-enriched cancer including BLIA and TME subtypes. Ongoing analysis of invasive disease-free survival as the primary endpoint in each arm of MIRINAE trial and the role of atezolizumab in association with genomic features will provide deeper insights into the role of ICIs as adjuvant therapy.

PS18-01: Spatial Transcriptomics-Derived Classification of Invasive Lobular Carcinoma: Associations with Clinical, Genomic Characteristics, and Prognosis

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Background: Invasive lobular carcinoma (ILC) is the second most prevalent histological subtype of breast cancer. This study presents four newly identified ILC subtypes associated with tumor microenvironment (TME) heterogeneity and examines their associations with clinical characteristics and prognosis.
Methods: Spatial transcriptomics (ST, Visium 10X) was performed on frozen tumor samples from 43 primary hormone receptor positive (HR+), HER2-negative (HER2-) ILCs. H&E slides were morphologically annotated, and ST spots were clustered by gene expression. By integrating morphology and sequencing data, ILCs were classified based on TME heterogeneity using the intNMF algorithm. Subtypes were annotated using morphology (image analysis), pathway enrichment analysis (GSEA), and cell type composition from single-cell deconvolution (CARD software). Gene signatures for each subtype were derived and used to retrieve the subtypes in METABRIC (ILC cohort, n = 122) and SCAN-B (ILC cohort, n = 853) microarray/bulk RNA-sequencing datasets. Statistical analyses included chi-square and Kruskal-Wallis tests to investigate associations with clinical characteristics and Cox proportional hazard models for univariate and multivariate survival analyses in both METABRIC and SCAN-B.
Results: Patient-level classification revealed four ILC subtypes, namely proliferative (P),

normal-stroma enriched (NSE), metabolic (M), and metabolic-immune enriched (MIE). In our ST cohort, the P subtype (n = 12) was enriched in tumor cells and proliferation-related pathways; NSE subtype (n = 10) was associated with more fibroblasts, carcinoma in situ, and heightened expression of EMT-related pathways; the M subtype (n = 9) was enriched in endothelial cells, metabolic-related pathways, and heightened AR gene expression; the MIE subtype (n = 10) was enriched in adipose tissue, endothelial cells, M2 macrophages, and metabolic-related pathways. All four subtypes were identified in METABRIC (NSE = 40, P = 34, MIE = 17, M = 31) and SCAN-B (NSE = 291, P = 226, MIE = 150, M = 186), with GSEA showing consistent differences at the gene expression level. In METABRIC, the P and MIE subtypes were associated with high and low cellularity, respectively ($p = 0.042$), and P was also linked to higher tumor grade ($p = 0.0187$). Notably, the P subtype exhibited more copy number aberrations (CNA) compared to other subtypes ($p = 0.0159$), but no differences in tumor mutational burden. In SCAN-B, the P and NSE subtypes were associated with larger and smaller tumors, respectively ($p < 0.001$). The P subtype was also linked to higher tumor grade ($p < 0.001$), lymph node involvement ($p = 0.02$), and high Ki67 ($p < 0.001$). In METABRIC, univariate analysis showed NSE and P subtypes to be associated with good and poor prognosis, respectively, for relapse-free interval (RFI) (HR = 0.56, $p = 0.027$, FDR = 0.055; HR = 1.8, $p = 0.019$, FDR = 0.055). Multivariable analysis confirmed NSE's association with good prognosis (HR = 0.47, $p = 0.03$, FDR = 0.12 for RFI), even when correcting for clinical features. In SCAN-B, univariate analysis revealed an association between NSE and longer RFI (HR = 0.42, $p = 0.0018$, FDR = 0.0035) and between P and shorter RFI (HR = 2.2, $p = 0.0014$, FDR = 0.0035). When correcting for clinical characteristics, NSE remained associated with longer RFI (HR = 0.54, $p = 0.035$, FDR = 0.11), while a trend was observed for P and shorter RFI (HR = 1.6, $p = 0.079$, FDR = 0.13).
Conclusions: Spatial transcriptomics revealed four ILC subtypes describing TME heterogeneity. These subtypes were successfully identified in microarray/bulk-RNA sequencing datasets and associated with different clinical characteristics. Importantly, the subtypes showed differences in disease outcomes, refining prognosis in ILC.

PS18-02: E-cadherin inactivation shapes tumor microenvironment specificities in invasive lobular carcinoma

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Background: Invasive lobular carcinoma (ILC) shows distinct clinicopathological features compared to invasive breast carcinoma of no special type (IBC-NST), including specific stromal characteristics. One of them concerns the singular infiltration pattern of lobular tumor cells, which induces minimal stromal reaction. In addition, a key function of tumor microenvironment has been previously demonstrated reporting that tumor-infiltrating lymphocytes (TIL) are not associated with good prognosis in ILC, in contrast to IBC-NST. So far, the precise impact of E-cadherin inactivation in tumor cells on these stromal peculiarities remains largely unknown.

Materials and Methods: In this context, we performed an in-depth characterization of heterogeneity, function, and interactions of Cancer-Associated Fibroblasts (CAF) with both immune and tumor cells in ILC compared to IBC-NST. To do so, we leveraged a well characterized retrospective series of 251 patients, who underwent surgery at Institut Curie for a primary ILC between 2005 and 2008, for whom frozen tumor samples were available for RNA sequencing. We established tissue microarrays (TMA) for 158 ER+ ILC from this cohort and 77 primary ER+ IBC-NST from our institute. Based on the TMA cohort, we did comparative immunohistochemical analysis of specific markers of different CAF populations, in particular FAP+ CAF, and immune cells. In addition, we characterized six classical ILC by single cell RNA sequencing and we performed spatial transcriptomic analysis of 16 ILC from the retrospective series. Finally, we studied the interactions of ER+ breast cancer cell lines with CAFs and CD8 T lymphocytes in vitro, using co-culture experiments and selective inactivation of E-cadherin (in tumor cells) or some of its heterologous receptors (especially in CAFs).
Results: By combining these analyses, we found that (1) CAF content of ILC is heterogeneous, notably depending on two tumor histopathological features (ILC histological type and tumor cellularity), (2) ILC have more inflammatory CAF and less myofibroblastic CAF than ER+ IBC-NST, (3) this particular feature is dependent on a previously undescribed mechanism involving E-cadherin in CAF plasticity, (4) CAF content in ILC determines the pattern of TIL infiltration which in turn has an independent prognostic impact in this cancer and finally, (5) the lack of good prognostic value of TIL in ILC may be partly explained by a cytotoxic CD8 T cell-related immune escape mechanism associated with E-cadherin inactivation in tumor cells.
Conclusion: In conclusion, we have demonstrated for the first time that E-cadherin has a direct effect on CAF differentiation via a previously undescribed mechanism, explaining some of the morphological specificities of the ILC stroma. Furthermore, inactivation of E-cadherin determines both the spatial organization pattern of TILs and the escape of tumour cells from CD8 T cell cytotoxicity. Our data provide an unprecedented insight into the ILC stroma and E-cadherin-mediated modulation of breast cancer tumour microenvironment.

PS18-03: GeoMx DSP and CosMx single cell spatial transcriptomics for molecular characterization of invasive lobular breast cancer cells and their microenvironment

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Invasive lobular breast cancer (ILC) represents 10-15% of all breast cancer cases but remains understudied. Lack of e-cadherin is thought to be the basis for the distinct single-file growth pattern of ILC. ILC exhibits late recurrence, commonly metastasizing to the peritoneum and bone with poor outcome. Primary ER+ ILC tumor cells exhibit wide variation in nuclear GR expression, ranging from negative to strongly positive. We previously reported better patient outcome in ER+ breast cancers with high glucocorticoid

receptor (GR) expression (Pan et al 2011). We hypothesize that GR expression may also impact outcome in ILC.

Crosstalk between tumor and stromal cells leads to evolution of a tumor microenvironment (TME) favorable for growth and/or invasion. Because of the characteristic histology of ILC, we expected the TME to be unique compared to other breast cancers. We recently reported that GR+ ILC shows reduced proliferation to GR- but exhibit an enhanced metastatic phenotype (Porter et al 2023). Hence, we predicted that tumor cell-intrinsic GR expression will impact the ILC TME.

We used an integrated approach to explore in-depth the molecular profile of primary ILC and to study interactions between tumor and neighboring cells. We measured average gene and protein expression in regions of interest on primary ILC tissue sections using nanoString GeoMx DSP with whole transcriptome (WTA), protein modules and IO Proteome Atlas. We also performed single-cell spatial transcriptomics for a subset of those patients leveraging the CosMx platform.

GR+ ILC tumor cells exhibited distinct transcriptional profiles with enrichment of RNA processing, translation pathways and oxidative phosphorylation. MHC class II antigen presentation pathways were downregulated. The most significantly enriched pathways in GR- tumor cells and TME were collagen synthesis and extracellular matrix (ECM) remodeling. Sflomos et al previously reported that ILC cells contribute to their own extracellular matrix, but we were surprised to observe the magnitude of increased expression in cells without GR. Gene expression in the TME strongly suggested of a much greater presence of cancer-associated fibroblasts (CAFs) surrounding GR- ILC. Proteome assays supported the WTA data, showing more fibronectin and type I collagen in GR- tumors. Also consistent with transcriptomic data, protein DSP indicated suppression of antigen presentation in GR+ ILC evidenced by lower expression of type I and type II MHC proteins.

Next, CosMx single cell in situ transcriptomics allowed us to reaffirm observations from DSP, and to examine expression at the single cell level, assign cell types and examine interactions between cells. GR+ and GR- tumor cells were transcriptionally distinct, as shown by clustering, and the abundance of certain cell types in their surrounding microenvironment were notably different. We confirmed a higher presence of CAFs transcriptionally similar to myofibroblasts that were in close proximity to GR- tumor cells. We also observed downregulation of MHC class I and II genes at the single cell level in GR+ compared to GR- tumor cells and low representation of antigen presenting cells in the stroma.

Our combined data supports repression of antigen presentation pathways in GR+ ILC accompanied by a relative exclusion of CAFs. Evidence suggests that tumor cells as well as stromal cells contribute to the modeling of the ECM, and crosstalk between them leads to the establishment of a favorable environment to support ILC tumor cell survival, growth and invasion.

PS18-04: Tumor intrinsic and extrinsic characteristics of invasive lobular carcinomas

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Background: It is now well-established that invasive lobular carcinoma (ILC) is a distinct disease with higher risk of distant recurrence after 10 years compared to invasive ductal carcinoma (IDC) (Pestalozzi et al. J Clin Oncol 2008). However, little is known about the underlying mechanisms of relapse in ILC cases. Additionally, recent large landmark studies have revealed heterogeneity at the morphological, molecular, and microenvironmental levels (Ciriello et al. Cell 2015, Michaut et al. Sci Rep 2016, Desmedt et al. J Clin Oncol 2016) suggesting that the overarching category of ILC may encompass several distinct subgroups. A more detailed understanding of the underlying genomic drivers, mechanisms of immune evasion, and evolution may inform more effective and personalized treatment. Previously, across histological types, we established a genome-driven breast cancer classification scheme that defines 11 integrative subgroups (ICs) of disease with distinct copy number aberrations (CNAs), transcriptional profiles, and clinical outcomes. Specifically, we identified four subgroups of ER+ disease (IC1, IC2, IC6, IC9) with a persistent risk of lethal distant relapse up to two decades after diagnosis, each with focal copy number drivers and two distinct subgroups of triple-negative disease (Curtis et al. Nature 2012; Rueda et al. Nature 2019). These findings nominate new therapeutic strategies, however, it is not known how mutational processes and genomic architecture differ between IDC and ILC in this context to sculpt the evolution of disease, nor how their microenvironments differ. Methods: To interrogate the genomic and immune landscape of invasive lobular carcinomas, we collected a meta-cohort of 401 primary invasive breast tumors, including 254 with whole-genome or whole-exome sequencing, 350 with whole-transcriptome sequencing, and 203 with both modalities. This meta-cohort includes the clinically curated METABRIC dataset with 20 years of clinical follow-up. Additionally, we established a single-cell spatially resolved transcriptomic meta-cohort of 54 primary ILCs, including 7 metastatic ILCs with primary and matched metastatic lesion(s). Results: Analysis of this large clinically curated dataset reveals a distinct pattern of relapse for patients with ILC tumors. Patients with ILC exhibited a higher 5 year recurrence risk (39% vs. 30%) and modestly higher cumulative recurrence risk (62% vs. 54% at 20 years) in the ER+ High-risk group, while this difference was even more striking amongst ER+ Typical-risk tumors, where ILC cases exhibited higher rates of late recurrence than IDC (55% vs. 37% at 20 years). Paradoxically, ILC tumors are enriched for the genomic stable ER+ Typical-risk subgroup that displays quiet genomes compared to other IC subgroups. In particular, ER+ High-risk and HER2+ tumors are associated with focal amplifications, extrachromosomal DNA, and coincident structural variants, whereas triple-negative tumors are characterized by global genomic instability and tandem duplications. Consistent with their quiet genomes and higher proportion of CDH1 mutations, ILC tumors demonstrated a lower proportion of whole-genome doubling, lower ploidy, lower fraction of copy number aberrations, lower

proportion of TP53 mutations and replication stress compared to IDC. Moreover, the ILC ER+ Typical-risk tumors were enriched for immune-depleted and fibrotic TMEs compared to ER+ Typical-risk IDCs tumors. Spatial transcriptomic profiling suggests an overall lower level of interaction and cell-cell communication between tumor, immune and stromal cells in ILC compared to IDC. Conclusion: Taken together, our data uncover tumor-intrinsic molecular characteristics of ILC and implicate tumor-extrinsic factors in disease aggression.

PS18-05: Clinical Management and Oncological Outcomes of Pure Pleomorphic and Florid Lobular Carcinoma in Situ of the Breast: Results from the MultiLCIS Study

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Background:
Pure pleomorphic (PLCIS) and florid (FLCIS) lobular carcinoma in situ, without concurrent invasive carcinoma (IC) or ductal carcinoma in situ (DCIS), are rare and poorly understood. Their treatment is not standardized due to limited outcome data and lack of management guidelines. We aimed to gather the first large multicentric cohort of cases and analyze oncological outcomes to identify the optimal therapeutic approach.
Methods:
This international multicentric study (ClinicalTrials.gov ID NCT06133465) collected data from 23 centers. Patients diagnosed with pure PLCIS and/or FLCIS on core biopsy and/or final specimen pathology were included, with concurrent presence of classic (C-) LCIS permitted. Cases with associated (micro-) IC and/or DCIS were excluded. An internal slide review by a dedicated pathologist was required. A minimum follow-up of 2 years was guaranteed.
Results:
We collected 327 patients diagnosed with pure P-/FLCIS on core biopsy and/or on surgical specimen between 01/2004-06/2022. Of these, 58 showed P-/FLCIS only upon final histology, including 44/58 preoperative B3 lesions (17 CLCIS) and 14/58 misdiagnosed DCIS. The remaining 269 had a preoperative diagnosis of pure P-/FLCIS; of these, 82/269 cases (30.5%) upgraded on surgical specimen, with 72/82 (87.8%) to IC and 10/82 (12.2%) to DCIS ($p<0.001$). The upgrade rate to IC was 32.4% for PLCIS and 14.9% for FLCIS ($p=0.002$). Factors significantly associated with upgrade included radiological mass-like

aspect, size and multicentric distribution of the lesion, irregular margins on ultrasound, and presence of necrosis on core biopsy.
Our analysis focused on the 245 (74.9%) cases diagnosed as pure PLCIS (152/245, 62%) or FLCIS (93/245, 38%) on surgical specimen. Median age was 58 years, and 79.2% of the patients were diagnosed through screening mammogram. MRI was performed in 46% of cases, with 68% showing a non-mass-like enhancement. Median lesion size was 13 mm (IQR 7.5-22). Mastectomy was performed in 22 cases, while 223/245 (91%) patients underwent lumpectomy. Margin involvement (defined as P-/FLCIS on ink) occurred in 52/223 cases (23.3%), leading to 21 re-excisions, 4 mastectomies, and no further intervention in 27 cases. Factors significantly associated with positive margins included pleomorphic form, calcifications and architectural distortion on mammogram, a radiological mass-like aspect, and multicentric distribution. Sentinel lymph node biopsy was performed in 62/245 (25.3%) cases, with no positive lymph nodes found. Adjuvant radiotherapy was administered to 38/245 (15.5%) patients, while 50/245 (20.4%) received adjuvant endocrine therapy.
With a median follow-up of 76 months (IQR 34-91), local recurrence (LR) occurred in 35/245 (14.3%) cases, including 16 IC, 2 DCIS and 17 P-/FLCIS. Involved margin ($p<0.001$) and multicentric radiological distribution ($p=0.001$) were associated with LR. Adjuvant therapies did not influence recurrence rates (RR) in patients with clear margins ($p=0.3$), nor in those with involved margins ($p=0.06$). Among patients with positive margins, local RR was significantly higher in patients who did not have a re-excision (22/27, 81.5%) compared to those who did (2/25, 8%) ($p<0.001$). RR were similar between pure PLCIS and FLCIS cases (15.1% vs. 12.9%, $p=0.427$). The 5-year recurrence free survival was 84.6% for the entire cohort (95%CI 78.8-89%).
Conclusion:
In this cohort, yet the largest collected to date, the upgrade rate of pure P-/FLCIS to IC/DCIS was 30.5%, suggesting careful preoperative assessment. Axillary surgery should be omitted. Involved margins were associated with LR (invasive in nearly half of cases) while adjuvant therapies were not. Surgery with clear margins, and not adjuvant therapies, seem to be the mainstay of treatment for this disease.

PS18-06: Primary results and the transcriptomic analysis of PELOPS, a randomized phase II study of neoadjuvant palbociclib with or without endocrine therapy for breast cancer patients with invasive lobular carcinoma or invasive ductal carcinoma

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Background: Invasive lobular carcinoma (ILC) is biologically distinct from invasive ductal carcinoma (IDC). Pre-clinical studies in human cell lines demonstrate that ILC has a unique

epigenetic state and estrogen receptor (ER) transcriptional axis, resulting in relative resistance to tamoxifen (T). Retrospective analyses of phase III clinical trials of adjuvant endocrine therapy (ET) (BIG 1-98, SOFT and TEXT) also support this hypothesis, with a greater difference between tamoxifen (T) and an aromatase inhibitor in ILC compared to IDC; PELOPS was designed to prospectively test this hypothesis.
Methods: PELOPS was a multi-center randomized phase II trial with both a window and treatment phase. Postmenopausal patients (pts) with early-stage ER+ breast cancer were randomized to a 2- week window of neoadjuvant letrozole (L) or T. Subsequently, pre and postmenopausal pts were randomized 2:1 to 6-cycles of neoadjuvant palbociclib (P) plus ET or ET alone. Histological subtype was among the stratification factors. Primary endpoints included 1) change in Ki67 after two weeks of T or L in the window phase within cohorts of pts with different histological subtypes, and 2) residual cancer burden (RCB) scores between arms in the treatment phase. Wilcoxon-rank sum test with two-sided alpha of 0.05 was used for both endpoints. Event-free survival (EFS) was defined as time from registration to the first event of disease progression precluding surgery, locoregional ipsilateral invasive recurrence, contralateral invasive breast cancer, distant recurrence or death from any cause. Biopsies were collected at baseline, after 2 weeks of ET and at surgery, and were subjected to RNAseq. Gene set variation analysis (GSVA) pathway analysis was performed.
Results: A total of 116 pts were evaluable for the window phase of the study (T=58, L=58). The Ki67 log-scale fold change (FC) was significantly lower with L compared to T among pts with both IDC (T: N=27, median FC=-0.121, L: N=28, median FC =-1.26, p=0.0045) and ILC (T: N=25, median FC=-0.452, L: N=25, median FC=-1.8, p=0.0161). In the treatment phase, 188 pts were evaluable (P+ET=128 and ET=60). The RCB scores in the two arms were comparable (median RCB 3.13 and 3.09, p-value = 0.9288). Median follow up was 4.65 years (IQR: 3.66 – 5.56 years), five-year EFS rate was 79.4% (95% CI: 71.2% - 88.5%) for P+ET and 80.9% (95% CI: 70.8% - 92.4%) for ET (hazard ratio 0.97 [95% CI, 0.47-2.00], p=0.926). RNAseq was completed on biopsies from a subgroup of pts highly representative of the entire population (279 biopsies from 97 pts). Most pts had luminal B breast cancers. Hierarchical clustering of baseline samples showed a distinct ILC transcriptome compared to IDC (Fisher's exact test, $p<0.001$). Pathway analysis revealed a significant reduction in expression of the cell cycle pathways after two weeks of T in IDC pts (paired T-test, $p<0.05$). In contrast, T did not suppress the cell cycle in ILC pts (lowest p=0.2). Interaction tests of GSVA changes after T treatment between ILC and IDC met significance or near significance (E2F targets p=0.051; Mitotic Spindle p=0.002; G2M checkpoint p=0.05, unequal variances t-tests). L treatment significantly decreased cell cycle pathways in both ILC and IDC, but other gene expression changes were disparate, underscoring the unique ER axis in ILC.
Conclusions: In the neoadjuvant setting, two-week treatment with letrozole achieved a superior reduction in Ki67 compared to tamoxifen in IDC and ILC, however, the addition of palbociclib to ET for 6 cycles did not improve RCB scores. Comprehensive molecular studies of biopsies from PELOPS provides the first prospective evidence of differential responses to T in ILC versus IDC. These results highlight the importance of molecular analyses in neoadjuvant ET trials and warrant trials for the optimization of ET specifically in ILC.

Additional gene expression analyses, including data from ET+/- palbociclib will be presented.

PS18-07: Differential odds of response in ILC versus IDC correlate with changes in the TIME in a phase II trial of pre-operative fulvestrant with or without enzalutamide.

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Background: The majority of ER+ breast cancers (BC) express androgen receptors (AR). In a randomized phase II trial for women with ER+/HER2- primary BC T2 or greater, neoadjuvant fulvestrant (Fulv) alone or with enzalutamide (Combo) was given for 4 months prior to surgery. A total of 59 patients were evaluable: 33 on Combo and 26 on Fulv. The addition of AR blockade to Fulv reduced residual tumor at time of surgery as measured by modified preoperative endocrine predictive index (PEPI) score. Fresh tumor biopsies were required at study entry (baseline), after 4 weeks on therapy (W5), and at surgery. The Combo arm achieved PEPI=0 more frequently (24%: 8/33) than Fulv (8%: 2/26). Interestingly, the odds of response were 4.6-fold (95% CI: 0.9-22) higher for patients with invasive lobular cancer (IDC) versus invasive ductal (IDC). Results: When examining all tumors, gene expression analyses showed significantly decreased estrogen response and cell division gene sets in tumors in both arms; however, only Combo treated tumors exhibited significant enrichment of immune activation genes sets, including interferon gamma, complement, inflammation, antigen processing, and B and T cell activation. AR protein was significantly reduced by time of surgery in the Combo arm only ($P<0.05$) as measured by immunohistochemistry. AR was also significantly lower at time of surgery in the PEPI=0 as compared to PEPI >0 ($P<0.05$) and in the Ki67 responders versus non-responders ($p<0.02$). Because of the 4.6-fold higher odds of PEPI=0 response in ILCs versus IDC, we examined phosphoproteins that changed with treatment in these two histologic groups by reverse phase phosphoprotein assay (RPPA) from frozen tumor sections. Both AR and phosphoS650 AR showed significantly more decrease from BL to W5 in ILC versus IDC. Cyclin D1, S6RP, HIF-1 alpha, ATP citrate lyase, were significantly lower in ILC than IDC, while CHK1 and ALK were higher. As would be expected with the higher odds of response in ILC, cell cycle proteins decreased significantly more with treatment in this histologic subtype, as did growth/survival and metabolism proteins. Polaris Multiplex immunofluorescence, used to study the tumor immune microenvironment (TIME), revealed that the number of tertiary lymphoid structures (TLS) per area surrounding resected tumor at time of surgery was higher in the Combo arm and was significantly higher near tumors that achieved PEPI=0 ($P<0.03$). Additionally, the average number of TLS/mm2 was higher in the ILC versus IDC ($P < 0.059$). T regulatory cells were reduced in the Combo arm

($p<0.0002$) and in Ki67 responsive tumors ($p<0.004$); however, T regs were not significantly different in ILC versus IDC. Tumor-associated macrophages decreased by time of surgery only in the Combo arm ($p<0.0001$), only in tumors that achieved PEPI=0 ($p<0.0005$) and trended towards decreased numbers in ILC vs IDC. Conclusions: AR inhibition in combination with a SERD activates the immune system in ER+ BC, particularly in ILC.

PS18-08: MDA iLobulaRx: An Advanced Clinico-Patho-Therapeutic Tool for Risk Stratification in Early-Stage Invasive Lobular Carcinoma

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Background: Invasive lobular carcinoma (ILC) is a distinct subtype of breast cancer that differs from the more common invasive ductal breast cancer (IDC) in its unique characteristics and arguably inferior prognosis. Recent studies have shown that ILC is associated with worse long-term outcomes compared to IDC. Risk stratification is essential in early breast cancer for tailoring treatment to optimize outcomes. We developed the MDA iLobular prognostic tool to incorporate clinical and pathological features to predict recurrence and survival in early-stage ILC (eILC). Recent studies suggest that the choice of adjuvant endocrine therapy (ET) significantly impacts recurrence and survival in eILC. We present an improved tool, “MDA iLobulaRx,” which includes clinical, pathological, and planned ET to enhance prognostic accuracy.
Methods: A retrospective analysis was performed on patients treated at MD Anderson Cancer Center with a diagnosis of stage I-III ILC in our prospectively collected and curated electronic database. Primary endpoints were overall survival (OS) and distant recurrence-free survival (DRFS). Univariate and multivariate Cox Proportional Hazard (PH) regression models assessed the significance of variables. Univariate Cox analysis identified prognostic factors for further multivariate analysis. Backward and stepwise multivariate Cox PH regression identified significant factors for final models. Hazard ratios and 95% confidence intervals were estimated, considering $P < 0.05$ significant. We evaluated model performance using Harrell's C-index, dividing data into two-thirds training and one-third test datasets.
Results: The study included 4,216 female patients with a median age of 56 years. The median pathological tumor size was 20 mm, and the median number of lymph nodes involved was one. Among these patients, 70% were post-menopausal. The ET used were tamoxifen (32%), non-steroidal aromatase inhibitor (NSAI) (30%), and no therapy (38%). The training cohort had 2,950 patients, and the test cohort had 1,266 patients. The best prognostic model for OS and DRFS had a Harrell's C-index of 0.727 and 0.713, respectively. The model included age, lymph nodes, tumor size, ER status, tumor grade, ILC histology, LCIS presence, and choice of adjuvant endocrine therapy data. (Table 1).
Conclusion: MDA iLobulaRx represents a significant advancement, offering the first dedicated clinico-patho-therapeutic tool for risk stratifying eILC.

PS18-09: Endocrine Response in Women with Invasive Lobular Carcinoma (TBCRC 037): A Multicenter Randomized Clinical Trial

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Background: Adjuvant endocrine therapy (ET) recommendations for patients (pts) with invasive lobular carcinoma (ILC) are no different than for other breast cancer subtypes. However, due to unique differences in estrogen pathway signaling of ILC, identifying biological markers of ET sensitivity or resistance could have a major impact on its clinical management. Pre-clinical studies suggest that fulvestrant may be more effective than anastrozole or tamoxifen for ILC. Here we test reduction in the proliferation marker Ki67 as a surrogate for treatment response to ET.
Methods: This open label, randomized, controlled, multicenter phase 0 window of opportunity trial (NCT02206984) was conducted at 12 TBCRC institutions between 10/8/15 and 7/28/23. Postmenopausal women with previously untreated hormone receptor-positive (HR+), HER2 negative ILC, centrally histologically confirmed on diagnostic core needle biopsy (CNB), measuring ≥ 1 cm, were eligible. Stage IV was excluded. Pts were randomized 1:1:1 to fulvestrant (500 mg IM on days 1 and 15), anastrozole (1mg/day) or tamoxifen (20 mg/day). After 21-27 days of treatment, pts underwent operation (from which a CNB of residual tumor bed was taken) or a post-treatment image-guided CNB. The primary endpoint was change in Ki67 immunohistochemistry at post-treatment compared to baseline. Given the log-normal distribution of Ki67 measurements generally observed in this population, values were log-transformed for statistical analysis [log (post/pre)]. Group medians were compared using a quantile regression linear model adjusting for institutional-level random effects. A linear mixed model (glmmTMB R package) was fit that allowed for treatment level error variance. Post-hoc pairwise comparison of log(post/pre) were performed using Tukey adjustment for multiple comparison.
Results: 201 women were randomized, 172 completed the assigned treatment, and 138 (fulvestrant n=37; anastrozole n=49; tamoxifen n=52) had evaluable pre- and post-treatment tissue. No serious adverse events or deaths occurred. Pre-treatment demographic and tumor characteristics were well-balanced between the treatment groups. Median [range] age was 67 [48-86] years. Race was reported as Asian in 2 (1.5%), Black in 15 (10.9%), other in 4 (2.9%) and White in 117 (84.8%). Presenting clinical stage was IA in 54 (39.1%), IB in 4 (2.9%), IIA in 51 (37%), IIB in 19 (13.7%), IIIA in 4 (2.9%), IIIB in 5 (3.6%), and IIIC in 1 (0.7%). Median H-score [IQR] was ER 260 [50], PR 140 [215]. In the fulvestrant, anastrozole and tamoxifen groups respectively, pre-treatment Ki67 [IQR] was 13.8 [13.5], 13.6 [8.7], and 12.6 [11.9]; and post-treatment Ki67 [IQR] was 4.8 [5.4], 4.5 [5.6], and 5.7 [10]. A statistically significant reduction in Ki67 was found favoring fulvestrant vs tamoxifen (p=0.0419). No significant difference after adjustment was seen between anastrozole and tamoxifen (p = 0.2602) or between fulvestrant and anastrozole (p = 0.6643).

Conclusions: A greater reduction in Ki67 was seen post-treatment in CNB of pts with ILC treated with fulvestrant vs tamoxifen. Interestingly, tamoxifen treatment resulted in a reduction in Ki67 of a similar magnitude to anastrozole, despite clinical concerns about its reduced efficacy in ILC. Planned correlative studies will determine whether Ki67 reduction is associated with alterations in expression of ER and ER-regulated genes and/or in other novel pathways, potentially opening avenues for improved treatment strategies in ILC.

PS19-01: Medication Nonadherence and Financial Toxicity Among Patients with Metastatic Breast Cancer on Cyclin Dependent Kinase 4/6 Inhibitors

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Background: Existing research on adherence to breast cancer (BC) medications commonly focuses on the adjuvant treatment of early-stage BC. With the growing availability of novel oral therapies for metastatic BC (MBC), there is a gap in knowledge regarding adherence to medications for MBC. First-line treatment for hormone-positive MBC typically consists of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) combined with endocrine therapy (ET). CDK4/6i often require a complex dosing schedule, have a less favorable side-effect profile than ET alone, and can result in high patient out-of-pocket costs. These characteristics are known to affect medication adherence, yet little is known about the prevalence and drivers of nonadherence to CDK4/6i.
Methods: We identified a convenience sample of patients with MBC from the BC clinic of our urban academic medical center with at least 1 prescription for ribociclib or palbociclib between 3/2017 (FDA approval) and 5/2024. We did not include patients on abemaciclib, as it is often prescribed in the adjuvant setting. We assessed pharmacy fill adherence for both CDK4/6i (as a class) with 2 approaches to calculate the proportion of days covered (PDC): (i) using EHR-linked fill data exclusively; and (ii) integrating order data to determine the first continuous period of medication use. We followed patients from their first CDK4/6i fill or first fill after their first order for 180 days (or up until the start of a 30-day gap in orders or a 180-day gap in fills). We measured the overall prevalence of nonadherence, defined as either order or fill PDC <80% (adjusted for dosing schedule), and conducted a bivariate demographic subgroup analysis using Fisher's exact and Chi-squared tests. We examined the results of a patient-reported financial toxicity screener (2 questions from the COmprehensive Score for financial Toxicity [COST]), administered through the patient portal at appointment registration starting in 3/2021, which assesses financial certainty and financial worry. We performed a bivariate analysis of the association between nonadherence and financial toxicity.
Results:
We identified a total of 159 MBC survivors on CDK4/6i with pharmacy fills between 4/2017 and 5/2024 (mean age 66 yo; 43.4% non-Hispanic White, 13.8% non-Hispanic Black, 23.9% Hispanic). Of these, 129 (81.1%) had at least 1 palbociclib fill and 45 (28.3%) at least 1

ribociclib fill. With nonadherence defined as either order of fill PDC <80%, the overall nonadherence prevalence was 21.0% for CDK4/6i. On subgroup analysis, we did not identify any statistically significant difference in adherence prevalence by age, race, ethnicity, or language. A total of 64 patients included in our study population also responded to the financial toxicity screener. Of these, 58 (90.6%) screened positive for financial toxicity, reporting financial uncertainty (for 57 patients [89.1%]) and/or worry about finances (for 46 patients [71.8%]) due to cancer care costs. This is significantly higher than the 66.5% prevalence of financial toxicity in the overall breast oncology population at our BC clinic during this time ($p<0.001$). Of the 58 patients on CDK4/6i who reported financial toxicity, 10 (17.2%) were non-adherent based on PDC calculations. Of the 6 patients who screened negative for financial toxicity, none were nonadherent.
Conclusions:
Patients with MBC on CDK4/6i are at risk for medication nonadherence, with over 1 in 5 patients nonadherent based on fill or order data. They are also highly vulnerable to the financial toxicities of cancer treatment compared to the general breast oncology population. Further research is needed to clarify the impact of financial constraints on adherence to costly oral therapies and identify other possible drivers of nonadherence that can be targeted in future interventions.

PS19-02: Economic Impact of Concurrent Tissue and Circulating Tumor DNA Molecular Profiling In Advanced Breast Cancer Patients

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Background: The use of molecularly-directed targeted therapies has become a vital tool in treating advanced breast cancer patients. While solid tumor testing is standard of care for biomarker detection, the use of circulating tumor DNA (ctDNA), a noninvasive technology for molecular profiling is becoming more common. Concurrent testing, or the use of simultaneous solid tissue and ctDNA testing, has the potential to increase detection of actionable findings, as well as reduce adverse events from repeat biopsies compared to solid tissue testing alone. A large real-world study of patients tested concurrently identified an additional 20% of patients with advanced breast cancer with actionable findings which were missed by tissue-based testing alone. We use these data to assess the economic impact of concurrent testing compared to tissue-only testing in a simulation of patients with advanced Breast Cancer in the United States.
Methods: This microsimulation compares concurrent testing to solid tumor testing alone in patients with advanced breast cancer. Frequency of actionable biomarkers (BRCA1, BRCA2, ERBB2, ESR1, PIK3CA, and MSI) detected by testing modality were derived from a real-world data study of patients tested concurrently. Other model inputs, including detection rates of testing modalities, rates of rebiopsy due to inadequate tissue, and rates of adverse events were derived from literature. Total costs modeled included cost of NGS, biopsy costs, and management of adverse events due to biopsies. We assumed the cost of solid tumor and

the cost of liquid biopsy were each $2,919. The primary economic endpoint measured was the total cost per patient, while the primary clinical endpoints included percentage of patients with actionable variants identified, total biopsies performed per cohort, and serious biopsy events avoided per cohort.
Results: In a simulated cohort of 1,000 patients, real-world data suggest that 587 patients will carry an actionable variant. In the model simulations, concurrent testing identified 93 more patients with actionable findings than solid tumor testing alone (586 patients detected via concurrent testing vs. 493 patients detected by solid tumor testing), while also reducing the number of additional biopsies by 24. This reduction translates to a number needed to test (NNT) with concurrent testing to prevent one additional biopsy of 42 patients.The improved outcomes came at an increased incremental cost per patient of concurrent testing of $2,803 ($6,853 compared to $4,050 for solid tumor testing alone), $106 less than the cost of an additional liquid biopsy test.
Conclusions: Concurrent testing in advanced breast cancer identifies a higher proportion of patients with actionable variants than tissue testing alone, at a per-patient cost that is less than the actual cost of the liquid biopsy. This increased identification, coupled with a reduction in repeat biopsies, highlights a role for concurrent testing in the management of advanced breast cancer.

PS19-03: Contemporary patterns of Medicare Utilization and Spending on Herceptin and its Biosimilars in Breast Cancer

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Introduction: A biosimilar is a biological product that is highly similar to and has no clinically meaningful differences from an existing FDA-approved reference product. Trastuzumab biosimilars are significant in HER2-positive breast cancer due to their cost savings and increased access to HER2-targeted therapy in early-stage and metastatic cases. The six FDA approved Herceptin biosimilars are: Ogivri, Herzuma, Ontruzant, Trazimera, Kanjinti, and Hercessi. Our objective is to analyze the trends of utilization, spending and cost of these biosimilars as compared to Herceptin (transtuzumab).
Methodology: We analyzed publicly available datasets from Centers for Medicare & Medicaid Services (CMS) within Medicare Part D. We extracted total spending, spending per claim, the number of beneficiaries, and spending per beneficiary for trastuzumab and its biosimilars. Kanjinti, Ontruzant, Trazimera were approved in 2019, so we examined changes between 2020 and 2022. All costs were adjusted for inflation and represented in 2022 US dollars. Because all data were deidentified and publicly available, the study was deemed exempt from institutional review board.
Results: From 2020 to 2022, total spending on Herceptin significantly decreased from $13,633,376 to $5,953,812, while spending on all biosimilars increased. Trazimera had the steepest increase ($70,717.28 in 2020 to $2,940,700.78 in 2022). Kanjinti had the highest spending among biosimilars in 2022 at $4,193,266.07. The total dosage units for Herceptin

decreased by 50% (from 7,184 in 2020 to 3,586 in 2022), while Trazimera saw an increase from 18 to 1,659 units.
Herceptin claims dropped by 48% (from 1,995 in 2020 to 1,044 in 2022), whereas biosimilar claims increased, with Kanjinti having the highest number in 2022 at 881 claims, and Herzuma the lowest at 38 claims. In 2022, Herceptin had the highest average spending per claim at $5,702.89, followed by Herzuma at $5,566.46. Kanjinti and Ontruzant had lower average spending per claim at $4,759.67 and $4,418.89, respectively.
The number of beneficiaries for Herceptin decreased by 44.5% (from 292 in 2020 to 162 in 2022), while it increased significantly for Kanjinti (from 34 in 2020 to 138 in 2022) and Trazimera (from 34 in 2021 to 115 in 2022). On the other hand, the beneficiaries for Ogivri fluctuated slightly (from 45 in 2020 to 58 in 2022). Average spending per beneficiary for Herceptin was $36751.93 in 2022 (vs 45299.84 in 2020). In comparison, the average spending per beneficiary in 2022 for all biosimilars was lower ($24359.03 for Ogivri; $25571.31 for Trazimera; $30385.99 for Kanjinti; $32110.63 for Ontruzant).
Conclusion: There has been a significant decline in Herceptin utilization and spending, accompanied by a shift towards biosimilars; reflecting changing trends in HER2-positive breast cancer treatments. Kanjinti and Trazimera, in particular, experienced substantial increases in spending, dosage units, claims, and beneficiaries, indicating growing acceptance and usage. Despite these increases, total spending and claims for Herceptin remain higher than for its biosimilars. The average spending per beneficiary and per claim is higher for Herceptin compared to its biosimilars. However, the decrease in these costs from 2020 to 2022 possibly indicate a favorable outcome as a result of market competition from biosimilars. The diversification provided by biosimilars has expanded patient options, potentially reduced costs and improved access to essential therapies. Continuous monitoring and analysis will be crucial to understanding the long-term impacts on healthcare costs and patient outcomes.

PS19-04: Exploring the broad societal value of pembrolizumab in triple-negative breast cancer in Canada

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Background: The impact of breast cancer is multifaceted; for each patient there are personal, social, and financial consequences which have wider societal and economic consequences. Cost-effectiveness analyses for health technology assessment (HTA) purposes are predominantly conducted from the traditional payer perspective and often do not capture these wider impacts. Pembrolizumab, a programmed death receptor-1 (PD-1)-blocking antibody indicated for the treatment of triple-negative breast cancer (TNBC), has demonstrated substantial survival benefits to patients. Our study analyzes the traditional societal and broader societal net monetary benefit (NMB) of pembrolizumab-based therapies for early TNBC (eTNBC) and metastatic TNBC (mTNBC) in a Canadian setting. We incorporate novel elements of value described in the third ISPOR Special Task Force Report alongside new elements considered relevant to the disease. METHODS: Two validated and

HTA approved cost-effectiveness models for pembrolizumab in eTNBC and mTNBC were expanded to include elements constituting the broad societal perspective. For the traditional societal perspective, this included productivity. For the broad societal perspective, the additional elements included caregiver burden, insurance value, value of hope, real option value, severity of disease, out-of-pocket expenses, and fertility treatment costs. A targeted literature review was conducted to identify inputs for each element. A standard of care comparator, consisting of chemotherapy, was generated for each indication; formed of a weighted average of the various chemotherapy comparators by indication based on market share. Canadian list prices were used for all treatment acquisition costs. An overall NMB was generated across indications by weighting individual results by prevalence and using a willingness to pay threshold of CAD$100,000. Probabilistic sensitivity analysis (PSA) and scenario analyses were implemented to analyze the robustness of results to plausible variation.

Results: The results showed that adopting a broad societal perspective resulted in more favourable cost-effectiveness results. Specifically, the overall broad societal NMB was CAD$1,113,858, almost four times greater than the traditional payer (CAD$282,644) and traditional societal NMB (CAD$279,534). For mTNBC, the NMB was positive for the broad societal perspective whereas the two traditional perspectives yielded a negative NMB. For eTNBC, pembrolizumab consistently resulted in a positive NMB for all three perspectives, however the broad societal perspective resulted in a substantially higher NMB. This was largely driven by the substantial insurance value impact on quality-adjusted life years. The next most influential element was the value of hope. PSA was consistent with deterministic results. Scenario analysis demonstrated that the choice of input source used to inform the broad elements of value had a substantial impact on NMB.

Discussion: The inclusion of broader societal elements of value resulted in a significantly higher NMB than the traditional payer perspective for both indications, indicating that society as a whole may place a substantially greater value on access to treatment than suggested from a traditional payer perspective. However, the paucity of disease-specific input data and the uncertain estimation of some of the broad societal perspective elements of value result in challenges with interpretation. Overall, the research highlights the importance of considering alternative and broader perspectives of analysis in health technology evaluation, but more research is needed to robustly parameterize novel elements of value. Notably, this study indicates that the traditional payer and societal perspectives commonly used for HTA may be missing key elements of value, and thus underestimate the value that general societal places on access to innovative therapies.

PS2-01: Dalpiciclib versus placebo in combination with letrozole or anastrozole as first-line treatment for women with HR+/HER2- advanced breast cancer: prespecified final analysis of progression-free survival of the phase 3 DAWNA-2 trial

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Background: Dalpiciclib (a highly selective and potent CDK4/6 inhibitor) plus letrozole or anastrozole as first-line treatment led to a statistically and clinically significant improvement in progression-free survival (PFS) compared with endocrine therapy alone in women with HR+/HER2- advanced breast cancer, according to the interim analysis of the DAWNA-2 trial (NCT03966898; Xu et al., Lancet Oncology, 2023). In this report, we present the prespecified final analysis of PFS and an updated safety analysis after more than one year of additional follow-up.

Methods: The DAWNA-2 study was a randomized, double-blind, multicenter phase 3 trial conducted at 42 centers in China. The DAWNA-2 trial enrolled patients aged 18–75 years, of any menopausal status, who had an ECOG performance status of 0–1 and pathologically confirmed HR+/HER2- untreated advanced breast cancer. Patients were randomly assigned in a 2:1 ratio to receive oral dalpiciclib (150 mg per day for three weeks, followed by one week off) or a matching placebo. Both groups also received continuous endocrine therapy, either 2.5 mg letrozole or 1 mg anastrozole, administered orally once daily. The primary endpoint was investigator-assessed PFS. The data cutoff for the updated analysis was March 31, 2024.

Results: Between July 19, 2019, and December 25, 2020, 456 eligible patients were enrolled and randomized into two groups: 303 in the dalpiciclib group (dalpiciclib plus letrozole or anastrozole) and 153 in the placebo group (placebo plus letrozole or anastrozole). At the data cutoff, the median follow-up duration was 40.6 months (IQR 30.3-45.1) for the dalpiciclib group and 38.9 months (IQR 27.5-44.6) for the placebo group. Post-discontinuation therapy was received by 154 (50.8%) patients in the dalpiciclib group and 101 (66.0%) in the placebo group, with endocrine therapy being the most common (32.7% in the dalpiciclib group and 49.7% in the placebo group). Disease progression or death occurred in 160 (52.8%) patients in the dalpiciclib group and 109 (71.2%) in the placebo group. Dalpiciclib plus letrozole or anastrozole significantly prolonged investigator-assessed PFS compared to placebo plus letrozole or anastrozole (median PFS: 33.4 months [95% CI 29.6-39.0] vs. 19.3 months [95% CI 16.5-22.5]; hazard ratio [HR] 0.56 [95% CI 0.44-0.72]; one-sided p <0.0001). The PFS benefit with dalpiciclib was consistent in both postmenopausal women (HR 0.54 [95% CI 0.40-0.74]) and pre- or perimenopausal women (HR 0.63 [95% CI 0.41-0.95]). Per investigator assessment, 181 patients (59.7%) in the dalpiciclib group and 76 (49.7%) in the placebo group achieved an objective response; the

median duration of response was 36.1 months (95% CI 29.4-45.8) and 16.4 months (95% CI 13.8-24.8), respectively. Dalpiciclib showed benefits beyond the initial study treatment, as indicated by the time to first subsequent chemotherapy (median not reached [NR] vs. 47.4 months [95% CI 31.4-NR]; HR 0.76 [95% CI 0.56-1.02]). As of the data cutoff date, 80 (26.4%) patients in the dalpiciclib group and 46 (30.1%) in the placebo group had died. The median overall survival (OS) was not yet mature in both groups. Serious AEs occurred in 17.5% of patients in the dalpiciclib group and 7.8% in the placebo group. Treatment discontinuation due to adverse events (AEs) was reported for 4.6% and 2.0% of patients, respectively.
Conclusion: The addition of dalpiciclib to letrozole or anastrozole continues to demonstrate PFS benefits with no new safety signals identified after prolonged follow-up, further supporting this regimen as an alternative option in the current treatment landscape for patients with HR+/HER2- advanced breast cancer, regardless of menopausal status.

PS2-02: Tibremciclib (BPI-16350) plus fulvestrant versus placebo plus fulvestrant for patients with HR+/HER2- advanced breast cancer after progressing on endocrine therapy: Updated analysis of the phase III study

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Background: Tibremciclib (BPI-16350; TIB), a novel highly potent CDK4/6 inhibitor, improved progression-free survival (PFS) when combined with fulvestrant (FUL) versus placebo (PBO) plus FUL in patients with HR+/HER2- advanced breast cancer (ABC) that progressed on prior endocrine therapy in an interim analysis of a randomized, double-blind, phase III study (NCT05433480). Here, we report the updated analysis from the phase III study.
Methods: Patients with post-menopausal status (natural or artificial) who had recurred during or within 12 months from the end of neo-adjuvant or adjuvant endocrine therapy or who progressed on the first-line endocrine therapy for metastatic disease were randomly assigned to receive TIB (400 mg orally once daily) or matching PBO plus FUL (500 mg intramuscularly on days 1 and 15 of 28 days of cycle 1, then day 1 of every subsequent cycle), in a 2:1 ratio between patients with TIB and PBO. The primary endpoint was investigator (INV)-assessed PFS, and key secondary endpoints were independent review committee (IRC)-assessed PFS, INV-assessed objective response rate (ORR), disease control rate (DCR), clinical benefit rate (CBR); overall survival (OS) and safety.
Results: A total of 274 patients were randomly assigned to receive TIB plus FUL (N = 184) or PBO plus FUL (N = 90). By the data cutoff date of March 31, 2024, the median follow-up was 12.88 months for either group. The median INV-assessed PFS was significantly longer with TIB plus FUL versus PBO plus FUL (16.53 months [95% CI 12.85-16.59] versus 5.59 months [95% CI 4.53-9.20], HR 0.37 [95% CI 0.27-0.52]; $p < 0.0001$). Improvement in PFS was confirmed by the IRC analysis, with an HR of 0.37 (95% CI 0.26-0.54; $p < 0.0001$). The

INV-assessed confirmed ORR was 39.1% versus 10.0% ($p < 0.0001$), DCR was 89.1% versus 76.7% ($p=0.0053$), and CBR was 74.5% versus 42.2% ($p < 0.0001$) in the TIB group and PBO group, respectively. Median OS for the study has still not been reached (19.7% OS data maturity). Safety profiles showed no new safety signals from the interim analysis. The most common treatment-emergent adverse events (TEAEs) in the TIB group were diarrhea (79.3%; versus 13.3% for the PBO group), neutropenia (75.5%; versus 15.6%), and leukopenia (73.9%; versus 16.7%). The most common grade 3 or higher TEAE was neutropenia in the both groups (15.2% versus 5.6% for the TIB group and PBO group). The incidence for grade 3 or higher diarrhea was 4.9% in the TIB group and 1.1% in the PBO group. Serious adverse events (SAEs) occurred in 24 patients (13.0%) in the TIB group and 9 patients (10.0%) in the PBO group. TEAEs leading to dose discontinuation occurred in 4 patients (2.2%) for the TIB group and none for the PBO group. No treatment-related death in the TIB group and 1 death (1.1%) in the PBO group.
Conclusions: Tibremciclib plus fulvestrant continued to demonstrate a statistically significant and clinically meaningful improvement in PFS for patients with HR+/HER2- ABC who had progressed on endocrine therapy. Safety prolife was aligned with the previous interim analysis, with no new safety findings identified.

PS2-03: Comparative overall survival of CDK4/6is plus an aromatase inhibitor (AI) in HR+/HER2- MBC in the US real-world setting

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Background: Palbociclib (PAL), the first CDK4/6i, in combination with endocrine therapy (ET) was approved for HR+/HER2- advanced/metastatic breast cancer (MBC) in 2015. Two additional CDK4/6is, Ribociclib (RIB) and Abemaciclib (ABE), were approved in 2017. CDK4/6i combination therapy has become standard of care for 1st line HR+/HER2- MBC. Randomized clinical trials (RCT) demonstrated that the 3 CDK4/6is plus ET vs ET plus placebo all significantly prolonged patients' progression free survival (PFS, primary endpoint). However, the 3 CDK4/6is have inconsistent 1st line RCT overall survival findings (OS, secondary endpoint). In the absence of head-to-head RCTs, real-world data (RWD) is an important complementary source of evidence. Several small RWD studies have evaluated the relative effectiveness between CDK4/6is, and their findings are inconsistent. Large RWD studies are needed to understand the effectiveness of the 3 CDK4/6is. This study compared OS of 1st line PAL vs RIB and ABE plus AI for HR+/HER2- MBC in routine US clinical practice.

Methods: We conducted a retrospective comparative effectiveness study of CDK4/6is plus AI in HR+/HER2-MBC using the US nationwide Flatiron Health electronic health record (EHR)-derived deidentified Panoramic database, comprised of >650k patients with breast cancer. Patients included had HR+/HER2- MBC, were ≥18 years, started index treatment (PAL+AI, RIB+AI, or ABE+AI) as 1st line therapy within 90 days of MBC diagnosis between

February 2015 and September 2023 (index period), and did not participate in clinical trials. Patients were assessed from start of index treatment to March 2024, death, or last medical activity, whichever came first. OS was defined as months from start of index treatment to death. Patients were balanced via exact 1:1 matching on age, gender, race and ethnicity, practice type, disease stage at initial diagnosis, ECOG, time from initial to MBC diagnosis, visceral disease, bone-only disease, and number of metastatic sites. Kaplan-Meier method and Cox proportional hazard regression model were used to analyze OS.

Results: Of 9770 patients eligible for the analysis, 7563, 1130, and 1077 patients received PAL+AI, RIB+AI, and ABE+AI, respectively. Median follow-up was 32.9 months for PAL+AI, 16.5 months for RIB+AI, and 21.3 months for ABE+AI treated patients. Compared with RIB and ABE groups, PAL group was 1-2 years older and had a lower proportion of patients with ECOG=0. After 1:1 matching, baseline demographics and clinical characteristics were well balanced between PAL vs RIB pairs (n=942) and PAL vs ABE pairs (n=857). In PAL-RIB pairs, 2- and 3-year OS rates were 81.3% and 67.6% for PAL group vs 79.8% and 69.7% for RIB group. In PAL-ABE pairs, 2- and 3-year OS rates were 76.8% and 63.0% for PAL group vs 75.7% and 67.4% for ABE group. Compared with PAL+AI, RIB+AI was not significantly associated with prolonged OS (unadjusted HR=0.90, 95%CI=0.80-1.02, p=0.097; matched HR=0.98, 95%CI=0.84-1.15, p=0.823). Similarly, ABE+AI vs PAL+AI was not significantly associated with prolonged OS (unadjusted HR=0.95, 95%CI=0.84-1.07, p=0.376; matched HR=0.90, 95%CI=0.77-1.06, p=0.212). Further analyses with additional follow-up, treatment duration, subsequent therapies, and time to chemotherapy will be reported.

Conclusions: Our findings suggest that there is no significant OS superiority of first-line RIB+AI and ABE +AI compared to PAL+AI for HR+/HER2- MBC patients in routine clinical practice in the US. Although the sample size precludes a formal non-inferiority analysis and short follow up may limit interpretation, this study represents the largest real-world comparative analysis of OS between the CDK 4/6is in combination with AI conducted to date.

PS2-04: Health related quality of life with first- versus second-line CDK4/6 inhibitor use in advanced breast cancer: results from the phase 3 SONIA trial (BOOG 2017-03)

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Background: Adding cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) to endocrine

therapy improves outcome in patients with hormone-receptor positive, HER2-negative advanced breast cancer. The phase 3 SONIA trial randomised 1,050 patients to receive CDK4/6i in first- versus second-line in addition to aromatase-inhibitor in first- and fulvestrant in second-line. No significant difference between the strategies was observed in progression-free survival after two treatment lines (PFS2; HR 0.87; 95% CI 0.74 to 1.03) while first-line CDK4/6i use increased toxicity (74% more grade ≥3 adverse events, most often neutropenia) and costs. We now present the health-related quality of life (HRQoL) outcomes in the first- versus second-line CDK4/6i group.

Patients and methods

HRQoL was assessed using the Functional Assessment of Cancer Therapy-Breast (FACT-B) questionnaire (version 4) at baseline, 12, 24, 48 and 72 weeks on both first- and second-line treatment and at SONIA treatment discontinuation. FACT-B total scores along with subscale scores for physical (PWB), social (SWB), emotional (EWB), functional well-being (FWB) and the breast cancer subscale (BCS) were calculated for all completed questionnaires within 60 days after SONIA treatment discontinuation. Missing baseline scores were imputed using the mean baseline score. For missing post-baseline timepoints, multiple imputation was employed separately for each score and arm using the predictors age at randomisation, ECOG performance status, baseline score, progression or a serious adverse event within last 3 months and the timepoint of the questionnaire. Mixed linear regression models with a random intercept were used to compare HRQoL over time between the two arms. Response variables were FACT-B total and individual subscales scores and covariates included treatment arm, time (continuous), and baseline HRQoL. We also tested for an interaction between arm and time. A difference of 7-8 points in FACT-B total score and of 2-3 points in subscales was defined as a clinically meaningful difference.

Results: Across arms, questionnaire completion rates were high at baseline (88%), as well as over the different time points during first- (82%) and second-line treatment (73%), and at treatment discontinuation (52%). The average difference in FACT-B total and subscale scores between the two arms was close to zero (i.e. no difference) and we found no evidence of an interaction with time. Over time, the observed mean differences using CDK4/6i in second-line as reference, were -0.49 (95% CI -1.88 to 0.90) for the FACT-B total score, -0.19 (95% CI -0.58 to 0.20) for PWB, 0.40 (95% CI -0.04 to 0.83) for SWB, 0.00 (95% CI -0.34 to 0.35) for EWB, -0.05 (95% CI -0.48 to 0.38) for FWB and -0.36 (95% CI -0.77 to 0.05) for BCS.

Conclusions: Health-related quality of life, as measured by the FACT-B total score and all subscales, was comparable between the CDK4/6i-first and CDK4/6i-second group during the two protocol-defined treatment lines in SONIA. These data indicate that adding CDK4/6i in first-line compared to second-line does not result in improved HRQoL, providing further justification for deferring the use of CDK4/6i to second-line treatment.

Clinical trial registration: NCT03425838

PS2-05: PRESERVE 2: A randomized, phase 3, double-blind trial of trilaciclib or placebo in patients (pts) receiving first-line gemcitabine/carboplatin (GCb) for locally advanced or metastatic triple-negative breast cancer (mTNBC)

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Background: TNBC is an aggressive malignancy for which chemotherapy is a backbone of treatment. Trilaciclib is an intravenous (IV), selective, and reversible cyclin-dependent kinase 4/6 inhibitor. In a randomized phase 2 trial in pts with previously treated mTNBC, trilaciclib administered prior to GCb resulted in a clinically meaningful improvement in overall survival (OS) versus GCb alone, regardless of programed death-ligand 1 (PD-L1) status, potentially via protection and direct activation of immune function. The multinational, randomized, double-blind, placebo-controlled, phase 3 PRESERVE 2 trial (NCT04799249) was designed to further evaluate efficacy and safety of trilaciclib prior to GCb in previously untreated pts with mTNBC. Here, we report final data from pts receiving first-line therapy, regardless of tumor PD-L1 status (cohort 1).
Methods: Eligible pts had locally advanced, unresectable, or metastatic TNBC, an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0/1, received no prior systemic therapy in the advanced/metastatic setting, and were ineligible/unable to receive treatment with a programmed cell death protein-1 (PD-1)/PD-L1 inhibitor. Pts were stratified according to tumor PD-L1 status (positive vs negative), disease-free interval (DFI; ≥ 6 months and < 12 months vs ≥ 12 months or de novo mTNBC), and country, and randomized 1:1 to receive IV placebo or trilaciclib 240 mg/m2 prior to gemcitabine (1000 mg/m2) and carboplatin (AUC 2) on days 1 and 8 of 21-day cycles. Primary endpoint: OS; secondary endpoints: OS according to PD-L1 status, progression-free survival (PFS), antitumor response (including overall response rate [ORR]), quality of life measures, myeloprotection, and safety/tolerability.
Results: Overall, 187 pts from 11 countries were randomized to trilaciclib (n = 96) or placebo (n = 91) prior to GCb. Median age was 58 years (range, 30–82), and 39.6% of pts had PD-L1–positive disease (trilaciclib, 38.5%; placebo, 40.7%). DFI was < 12 months in 20.9% of pts and > 12 months (or de novo disease) in 79.1%. Pts completed a median of 6 (range, 1–41) treatment cycles. Median (95% CI) OS was 17.4 (12.4–21.3) versus 17.8 (13.3–22.5) months (hazard ratio [HR], 0.91 [95% CI, 0.62–1.33]; P = 0.884) in the trilaciclib and placebo groups, respectively, with a median follow-up of 12.1 and 14.9 months. In pts with PD-L1–positive disease, median OS with trilaciclib versus placebo was 23.1 versus 21.8 months (HR, 1.0 [95% CI, 0.51–1.96]; P = 0.923), and in pts with PD-L1–negative disease was 15.7 versus 14.9 months (HR, 0.92 [95% CI, 0.57–1.49]; P = 0.913), respectively. Median (95% CI) PFS was 6.3 (4.4–8.3) months with trilaciclib and 6.4 (5.2–8.1) months with placebo (HR, 1.17 [95% CI, 0.80–1.69]; P = 0.434), and ORR was 29.5% and 38.2%,

respectively. Overall, safety was comparable between treatment arms; 128 pts (92.3%) had an adverse event (AE), including 143 pts (78.6%) with grade ≥ 3 AEs. Trilaciclib-/placebo-related AEs were reported in 109 pts (59.9%). The most common (≥ 40% of pts) any-grade AEs, irrespective of causality, were anemia (trilaciclib, 58.1%; placebo, 62.9%) and neutrophil count decreased (trilaciclib, 40.9%; placebo, 39.3%). Compared with placebo, fewer pts receiving trilaciclib had grade 4 neutropenia (8.6% vs 25.8%), any-grade thrombocytopenia (38.7% vs 53.9%), and chemotherapy dose reductions (49.5% vs 64.0%).
Conclusions: Data from PRESERVE 2 show that compared with placebo, administering trilaciclib prior to GCb did not improve survival in pts with mTNBC. The safety profile of trilaciclib was consistent with previous studies. Some myeloprotective effects were observed, consistent with the known mechanism of action of trilaciclib.

PS2-06:First-line (1L) ribociclib (RIB) + endocrine therapy (ET) vs combination chemotherapy (combo CT) in clinically aggressive HR+/HER2- advanced breast cancer (ABC): a subgroup analysis of RIGHT Choice by intrinsic subtype & gene & signature expression

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Background: The phase 2 RIGHT Choice study showed a statistically significant median progression-free survival (mPFS) benefit with 1L RIB + ET vs combo CT (HR, 0.61; 95% CI: 0.43-0.87; P = .003) in pts with clinically aggressive HR+/HER2− ABC (Lu Y-S, et al. J Clin Oncol. Published online May 21, 2024). This exploratory analysis of the RIGHT Choice study examined efficacy of RIB + ET vs combo CT by intrinsic subtype and gene/signature expression levels.
Methods: Pre/perimenopausal pts with no prior systemic therapy for aggressive HR+/HER2− ABC were randomized 1:1 to RIB + letrozole/anastrozole + goserelin or physician's choice of combo CT. Baseline tumor samples (n = 49) from pts enrolled in the trial were microdissected and underwent gene expression profiling by NanoString nCounter BC360 and PanCancer IO 360 panels. High and low baseline gene/signature expression levels were defined as greater and less than or equal to the median level, respectively.
Results: Of the available samples from the RIB arm (n = 27), 6 (22.2%) were luminal A, 14 (51.9%) were luminal B, 6 (22.2%) were HER2E, and 1 (3.7%) was basal-like; in the CT arm (n = 22), 11 (50.0%) were luminal A, 9 (40.9%) were luminal B, and 2 (9.1%) were HER2E. The mPFS in the RIB vs CT arms was 32.5 mo vs not reached (NR) (HR, 0.88; 95% CI: 0.20-3.91) in luminal A, 38.0 vs 21.7 mo (HR, 0.64; 95% CI: 0.18-2.20) in luminal B, and 17.4 vs 8.0 mo (HR, 0.23; 95% CI: 0.03-1.66) in HER2E subtype. In the combined luminal B/HER2E set, which are known to be associated with poor prognosis, the mPFS was 38.0 vs 18.4 mo with RIB + ET vs combo CT (HR, 0.58; 95% CI: 0.21-1.62). In the RIB arm, pts with high ESR1 expression showed longer mPFS vs pts with low ESR1 expression (high vs low

expression, mPFS, HR [95% CI]; 38.0 vs 32.5 mo, 0.52 [0.16-1.66]). Pts in the RIB arm with low expression of tumor inflammation or immune-related genes/cells, such as tumor inflammatory signature (TIS; BC360 panel) and T-cell (IO 360 panel) expression, had a longer mPFS than pts with high expression of these signatures (low vs high expression, mPFS, HR [95% CI]: TIS, NR vs 11.4 mo, 0.17 [0.04-0.76]; T cells, NR vs 10.3 mo, 0.2 [0.05-0.76]). In contrast to the results in the RIB arm, pts with low expression of immune-related genes/cells in the CT arm had a shorter mPFS than those with high expression of these genes (low vs high expression, mPFS, HR [95% CI]: TIS, 15.0 mo vs NR, 2.08 [0.62- 6.93]; T cells, 12.8 vs 18.4 mo, 1.8 [0.57-5.71]). For most other immune-related genes/cells expressions and signatures, a comparable consistent trend in PFS benefit for low vs high expression levels was observed, with a higher PFS benefit for low gene expression in the ribociclib arm, and a higher PFS benefit for high gene expression in the CT arm. Conclusions: This exploratory analysis showed a trend towards PFS benefit with RIB + ET vs combo CT in pts with luminal B/HER2E intrinsic subtypes, which are associated with poor prognosis. This analysis also suggest a more pronounced trend for PFS benefit with RIB + ET in pts with low baseline expression levels of immune-related genes. Contrary results in the CT arm suggest that these signatures warrant further studies on their potential predictive value. These data are hypothesis generating and should be interpreted with caution due to small sample sizes.

PS2-07: Intrinsic Subtype at Progression to CDK4/6 Inhibitors Plus Endocrine Therapy in Hormone Receptor-Positive/HER2-Negative Metastatic Breast Cancer (MBC)

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Background: Biomarkers after progression to CDK4/6 inhibitors plus endocrine therapy (CDKi+ET) are needed to guide the use of ET-based therapies versus chemotherapy (CT). Here, we explored the prognostic and predictive value of the 4 major intrinsic subtypes (IS) of breast cancer (i.e., Luminal A [LumA], Luminal B [LumB], HER2-enriched [HER2E], Basal-like [BL]) in tumor samples of patients with HR+/HER2- MBC progressing to CDKi+ET.

Methods: This retrospective/prospective observational study included 63 patients with HR+/HER2- MBC treated at the Hospital Clinic of Barcelona between 2018-2024 with at least one line after CDKi+ET and an available tumor biopsy obtained at progression from CDK4/6 inhibition. The primary objective was to determine the progression-free survival (PFS) and overall survival (OS) after CDKi+ET, according to IS determined at progression from CDKi+ET. PFS and OS within luminal (Lum) vs. non-Lum IS according to type of therapy was explored. A paired biopsy (before starting CDKi+ET and at progression to

CDKi+ET) was available in 39 (61.9%) cases. IS was assessed using a research-based PAM50 assay on the nCounter platform. Survival analyses were conducted with the Kaplan-Meier method and Cox regression models. Significance was established at p≤0.05.

Results: The median age was 57.6 years. Overall, CDKi+ET had been administered in 1st, 2nd and ≥3rd line in 65.1%, 14.8% and 20.1% cases, with 54.0% patients progressing to ribociclib as CDKi and 57.1% progressing to letrozole as ET. Median PFS to CDKi+ET was 13.6 months (95% CI 10.2-19.2). In tumor samples obtained at CDKi+ET progression, 27 (42.9%) were Lum (LumA+LumB) and 36 (57.1%) non-Lum (HER2E+BL+normal-like). With a median follow-up of 35.2 months (95% CI 22.5-53.3) after progressing to CDKi+ET, PFS was 5.5 months (95% CI 3.8-7.9), and OS was 21.3 months (95% CI 16.8-28.7). Subtypes at progression to CDKi+ET were prognostic for PFS (p<0.001) and OS (p=0.004) with LumA tumors displaying the best median PFS (7.9 months) and OS (43.3 months), followed by LumB (5.4 and 23.8 months), HER2E (4.8 and 21.4), and BL (4.6 and 10.3). The PFS and OS hazard ratios (HR) between LumA versus others, adjusted for post-CDKi treatment, were 0.39 (p=0.032) and 0.49 (p=0.202), respectively. Patients with non-Lum tumors received more CT +/- targeted therapy (63.9% vs 18.5%) and less ET-based therapies (25.0% vs 66.6%) than patients with Lum tumors (p<0.01). Type of therapy (CT-based versus ET-based) was not found significantly associated with PFS (p=0.585) and OS (p=0.516). However, CT-based therapies within non-Lum disease showed better PFS compared to ET-based therapies (HR=0.44, p=0.039). No difference in OS was observed (p=0.266). Within Lum disease no difference in PFS and OS was observed according to therapy. Finally, in 39 paired tumor samples, subtype switching occurred in 61.9% of the cases. Tumor samples obtained at progression to CDKi+ET were significantly enriched in HER2E disease (51.3% vs 35.9%) and showed less Lum IS (41.0% vs 56.4%), with a consistent increase in the HER2E PAM50 score (p=0.005) and mRNA levels of genes associated to proliferation or HER2E biology, e.g. MKI67 (p=0.009) and FGFR4 (p=0.035), despite no ERBB2 mRNA levels' changes (p=0.841). Similar findings were observed in a subgroup of baseline Lum tumors shifting to HER2E.

Conclusions: Subtype switching towards less ET-sensitive IS, especially the HER2E, occurs under CDKi+ET and has prognostic value. Post-CDKi LumA disease showed the best outcomes, regardless of treatment type. This group of patients might be the ideal group to be treated with ET-based therapies. Non-Lum IS performed better with CT-based approaches. Overall, these findings suggest the necessity to profile tumor samples at progression to CDKi+ET to better tailor treatments.

PS2-08: Association of MammaPrint® with gene expression pathways predictive of resistance to cyclin-dependent kinase inhibition

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Background: Cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) have become a first-line targeted treatment in combination with endocrine therapy for patients with recurring HR+HER2- breast cancer (BC) and have recently been added to the adjuvant setting for the treatment of clinically high-risk early-stage BC. Trials have demonstrated that approximately 30% of advanced stage breast cancers are resistant to CDK4/6i, but no biomarkers exist to inform which patients are least likely to respond. The commercially available MammaPrint genomic signature, characterizing risk of distant recurrence (UltraLow, Low, High 1, and High 2) in early-stage BC, has demonstrated utility in predicting treatment response to chemotherapy as well as targeted immunotherapies. We investigated the utility of MammaPrint in identifying tumor subtypes that predict resistance to CDK4/6 pathway inhibition, using gene expression patterns driven by cellular proliferation pathways that bypass CDK4/6 function.
Methods: All patients with early-stage HR+HER2- tumors enrolled in the ongoing prospective, observational FLEX Trial (NCT03053193), were included in this study (N = 5657). Correlation of 'Rbsig' genomic signature, measuring Retinoblastoma (Rb) loss-of-function and subsequent E2F-dependent cell proliferation, to MammaPrint Risk groups was performed. MammaPrint Risk groups were then correlated to an 11-gene signature profile that measures absence of CDK4 phosphorylation, corresponding to Rb loss-of-function and, therefore, predictive of resistance to the CDK4i, Palbociclib. Differences in clinical characteristics were evaluated by Chi-Squared test or Fisher's exact test. The association of MammaPrint and 'Rbsig' was assessed using Spearman's rank correlation. Differences in 11-gene signature predicted resistance within MammaPrint groups were assessed using Fisher's exact test.
Results: Patients with HR+HER2- EBC included in this analysis had a mean age of 60 (SD ± 12) and were more likely to be post-menopausal (74.4%). Nearly 31% of patients had T2 stage or larger, 19% of patients had nodal involvement, and 17% had Grade 3 tumors. Among all HR+HER2- tumors, 14.6% were classified as UltraLow, 38.1% Low, 37.4% High 1, and 9.9% High 2 Risk. A linear correlation was observed with increasing MP Risk and increasing 'Rbsig' scores ($p < 0.001$), suggesting MammaPrint High 2 tumors demonstrate highest probability of loss of Rb function. The 11-gene signature comparison demonstrated MammaPrint High 2 as having the highest proportion of tumors predicted to have CDK4 driven loss of Rb function (43.0%), in comparison to UltraLow (0.1%), Low (0.5%), and High 1 (1.8%) tumors ($p < 0.001$).
Conclusion: These data identify High 2 tumors as least likely to respond to CDK targeted inhibition compared to other MammaPrint Risk groups. The increasing scores of Rb loss-of-function signature, 'Rbsig,' was closely correlated with MammaPrint High 2. The 11-gene signature profile, defined by absent CDK phosphorylation and high cellular proliferation, was significantly more likely to be associated with MammaPrint High 2 Risk tumors. These data support previous studies demonstrating that increasing MammaPrint index is closely associated with Cyclin E (CCNE1 and CCNE2) and 8q22-24 (CCNE2, MTDH, TSPYL5) genomic expression, that are hypothesized to identify tumor proliferation driven by activity downstream CDK4/6 function in HR+ BC. To our knowledge, MammaPrint is the only clinically used genomic signature that may identify patient subgroups potentially resistant

to CDK4/6 inhibitors. Further research is warranted to distinguish which clinically high-risk, non-High 2 breast cancers are most likely to benefit from CDK4/6i treatments.

PS2-09: Liquid biopsy DNADX assay in advanced ER+/HER2-negative breast cancer after progression on CDK4/6 and aromatase inhibitors: a correlative analysis from the PACE phase II randomized trial

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Background: DNADX, a novel machine learning-based approach, utilizes DNA copy-number aberration (CNA) data from plasma ctDNA to identify clinically relevant phenotypic tumor features and classify breast cancer into 5 groups (Nat Comm 2023). This study evaluates the ability of DNADX to predict prognosis and treatment benefit in advanced ER+/HER2- breast cancer after progression on CDK4/6 and aromatase inhibition.

Methods: DNADX was centrally evaluated in available baseline tumor plasma from the PACE trial (NCT03147287), a multicenter phase 2 clinical trial that randomized 220 patients (pts) with HR+/HER2- advanced breast cancer after progression on AI and CDK4/6 inhibitor to receive fulvestrant alone (F), F and palbociclib (F+P), or F+P and avelumab (F+P+A) in a 1:2:1 ratio. Shallow whole genome sequencing was performed on ctDNA, identifying 5 DNA-based groups through unsupervised analysis of 150 breast cancer signatures (Luminal-high, Proliferative, Basal-related, CNA-flat, and a group with tumor fraction [TF] < 3% [TF-low]). The primary objective was to evaluate the association of DNADX subtypes with progression-free survival (PFS). Secondary objectives included assessing the association of DNADX subtypes and TF with treatment benefit from palbociclib or avelumab. Uni- and multivariable Cox regression models were used.

Results: DNADX was evaluated in baseline plasma samples from 149 pts (67.7%). PFS across arms in this subset was similar to the original study population. DNADX in the pooled population identified 39 (26.2%) cases with TF-low, 4 (2.7%) with CNA-flat, 61 (41.0%) with Luminal-high disease, 30 (20.1%) with Proliferative disease, and 15 (10.0%) with Basal-related disease. The DNADX 5-group classification at baseline was significantly associated with PFS in both univariate (p=0.0298) and multivariable (p=0.012) analyses, adjusted by treatment arm and age. Compared to pts with TF-low, those with Luminal-high disease had inferior PFS (adjusted hazard ratio=1.96, 95% CI 1.19-3.24, p=0.008). The addition of palbociclib to F+/-A significantly improved PFS in the DNADX Luminal-high group (hazard ratio [HR] 0.45, 95% CI 0.22-0.92, p=0.029; interaction test p=0.081). The

addition of avelumab to F+/-P showed a nonsignificant trend towards PFS benefit in the DNADX TF-low group (HR 0.49, 95% CI 0.18-1.34, p=0.164; interaction test p=0.667). Similarly, DNADX plasma TF above the median was associated with a significant PFS benefit from the addition of palbociclib (HR 0.37, 95% CI 0.19-0.74, p=0.004; interaction p=0.025), whereas DNADX plasma TF below the median was associated with a nonsignificant trend towards PFS benefit from the addition of avelumab (HR 0.54, 95% CI 0.28-1.02, p=0.0576; interaction p=0.283).

Conclusions: In the PACE trial population, the liquid biopsy-based DNADX assay reveals substantial biological heterogeneity after progression on CDK4/6 and AI which impacts prognosis in advanced ER+/HER2- breast cancer. With further validation, DNADX may help identify a patient population that may derive benefit from continuation of CDK4/6 inhibition after progression.

PS2-10: Integrating ctDNA and Tumor Fraction Features for Deciphering Molecular Response and Resistance Mechanism to Endocrine Therapy and CDK4/6 Inhibition in Advanced HR-positive Metastatic Breast Cancer

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Introduction: Hormone receptor (HR)-positive breast cancer is the most common type of breast cancer and is frequently diagnosed among women worldwide. Treatments involving CDK4/6 inhibitors and endocrine therapies, such as aromatase inhibitors (AIs), have significantly improved patient outcomes. However, the risk of disease progression persists. This study employs a circulating tumor DNA (ctDNA) Next-Generation Sequencing (NGS) assay to profile gene variations in patients with advanced HR-positive breast cancer, aiming to identify gene biomarkers relevant to treatment.
Methods: In this retrospective study, 136 patients with advanced HR-positive breast cancer were recruited, and plasma samples were collected from these patients prior to first-line treatments. Of the cohort, 31 patients received CDK4/6 inhibitors with endocrine therapies, 21 received endocrine monotherapies, and 84 received chemotherapies. Among those treated with endocrine therapies, 36 were treated with AI combined with Fulvestrant. The study used PredicineCARE, a targeted NGS liquid biopsy assay, to detect somatic alterations in ctDNA from plasma. The median follow-up period until disease progression was 10 months, ranging from 1 to 60 months.
Results:
Among the 136 patients, the ctDNA assay identified 247 somatic mutations and 311 gene copy number variations. The most frequently mutated genes (prevalence ≥10%) were PIK3CA (37%), TP53 (35%), ATM (19%), FGFR1 (18%), RB1 (12%), ERBB2 (11%), PTEN (10%), ESR1 (10%), and GNAS (10%). Patients with variations in ATM (mPFS 7 vs. 11 months, p<0.001), TP53 (mPFS 7 vs. 12 months, p=0.002), and PTEN (mPFS 7 vs. 11 months, p=0.036) had significantly poorer median progression-free survival (mPFS).

Similar trends were seen for FGFR1 (mPFS 8 vs. 11 months, p=0.055) and PIK3CA (mPFS 8.5 vs. 12 months, p=0.068), though not statistically significant.
Among the 31 patients treated with CDK4/6 inhibitors, significant PFS benefits were observed (mPFS 22 vs. 8.5 months, p<0.001). Within this group, patients with ATM variations had significantly poorer PFS (mPFS 15 vs. 34 months, p<0.001), while other gene variations showed no significant prognostic value. The 36 patients treated with AI therapies showed no overall significant PFS benefit. However, within this group, those with ESR1 variations had significantly poorer PFS (mPFS 2.5 vs. 8 months, p=0.004).
Additionally, tumor fraction (TF) levels identified by the ctDNA assay showed significant prognostic value across various therapies. The median TF was 9.4%, ranging from 0.5% to 88.2%. Using a 1% cut-off, patients with higher tumor fractions had significantly poorer PFS (mPFS 9.4 vs. 15.7 months, p=0.002).
Conclusion: The sensitive ctDNA NGS assay effectively identified gene variations and tumor fraction levels as prognostic markers in advanced HR-positive breast cancer, demonstrating significant implications for personalized treatment.

PS3-01: Tislelizumab plus sitravatinib and nab-paclitaxel in patients with untreated locally recurrent or metastatic triple negative breast cancer (TNBC): updated efficacy and safety results

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Background: The PD-(L)1 inhibitor-chemotherapy combination has established efficacy as first-line treatment for PD-L1 positive metastatic TNBC, but novel treatment regimens are still needed to improve the clinical outcomes for the whole population of TNBC in the first-line setting. Emerging evidence indicates that the combination of anti-angiogenic therapies and PD-(L)1 blockade may act synergistically, thereby potentiating enhanced antitumor activity. Sitravatinib (Sitra) is a spectrum-selective tyrosine kinase inhibitor that could potently inhibit split kinase receptors and TAM receptors. SPARK study was a multi-cohort, two-stage design, phase II trial (NCT04734262) to evaluate the efficacy and safety of tislelizumab (Tisle) plus Sitra, with or without nab-paclitaxel (nab-P), for locally recurrent or metastatic TNBC. We previously reported that the triplet combination of Tisle, Sitra and nab-P (cohort C) yielded encouraging anti-tumor activity in the first-line treatment setting (Lei Fan, et al. Cancer Res (2024) 84 (9_Supplement): PO1-06-12). Here, we report the updated results of this cohort.
Methods: Eligible patients with untreated locally recurrent or metastatic TNBC were enrolled in the Tisle+Sitra+nab-P cohort to receive Tisle (200 mg, iv, day 1, Q3W) plus Sitra (70 mg, po, qd) and nab-P (100mg/m2, iv, days 1 and 8, Q3W) until disease progression or intolerable toxicity. The primary endpoint was ORR. Secondary endpoints were DCR, PFS, DOR, 1-year OS rate and safety/tolerability. Based on Simon's two-stage design, > 9

responders were required in stage 1 (n=18) to continue the study, and ＞19 responders were needed by the end of study (N=35) to demonstrate superiority with Tisle+Sitra+nab-P (assumed to be around 65%) to a historical control of 46% (1-sided alpha of 0.1, power of 80%). The stage 1 criterion was met to complete the full enrollment.
Results: Between September 9, 2022, and June 2, 2023, a total of 37 patients were enrolled, with a median age of 49 years. 15 (40.5%) patients had CD8+ disease defined as CD8 IHC staining ≥10%, 20 (54.1%) had CD8- disease, and the CD8 status was unavailable in 2 patients. After a median follow-up of 14.7 months (range: 2.8-20.1) (data cut-off: May 31, 2024), the Tisle+Sitra+nab-P cohort met its primary endpoint with 26 out of the first 35 efficacy-evaluable patients achieving objective response per RECIST v1.1. In the total of 37 efficacy-evaluable patients, the confirmed ORR was 75.7% (28/37; 95% CI: 58.8%-88.2%), including 7 CRs and 21 PRs. DCR was 97.3% (95% CI: 85.8%-99.9%). As of the data cut-off date, the median PFS was 10.3 months (95% CI 7.9-14.0). The median PFS was 12.9 months in patients with CD8+ disease, and 8.7 months in patients with CD8- disease. The median OS was not reached; 1-year OS rate was 90.5% (95% CI: 73.3%-96.9%). TRAEs occurred in 36 (97.3%) patients, of which 15 (40.5%) experienced grade ≥3 TRAEs. SAEs were reported in 11 (29.7%) patients. No new safety concerns were detected.
Conclusions: The triplet combination of Tisle, Sitra, and nab-P demonstrated clinically meaningful ORR and PFS with acceptable safety profile as first-line treatment for patients with untreated locally recurrent or metastatic TNBC. Notably, patients with CD8+ expression achieved impressive PFS, indicating that CD8 status might be a biomarker for efficacy prediction. Continued follow-up is being conducted to assess long-term survival and safety.

PS3-02: Induction of Cisplatin/abraxane/pembrolizumab followed by pembrolizumab ± Olaparib Maintenance in triple-negative Metastatic breast cancer patients (COMPLEMENT) – A Randomized, Open-label, Phase II Study

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Background: Triple negative breast cancer (TNBC) is a hard-to-treat disease requiring continuous administration of drugs, necessitating further exploration of optimal maintenance strategy. KEYNOTE-355 has proved that pembrolizumab (pembro) plus chemotherapy (chemo) significantly improved PFS than chemo alone as first-line treatment in PD-L1 positive with a combined positive score (CPS) ≥10. The PARP inhibitor olaparib is established as maintenance therapy for platinum-sensitive populations regardless of BRCA status in the setting of ovarian cancer. Moreover, both preclinical and clinical data supported olaparib and pembro have a synergistic effect, suggesting that the doublet might be a promising maintenance strategy. COMPLEMENT study is a randomized, open-label, phase II study, seeking to investigate if olaparib plus pembro will maintain the clinical benefit achieved after induction therapy with cisplatin and nab-paclitaxel (AP) regimen plus

pembro in CPS≥1 mTNBC patients (pts).
Methods: Eligible pts with locally advanced, recurrent or metastatic PD-L1 CPS ≥1 TNBC that had not been treated with chemo for the advanced disease received induction therapy of pembro (200 mg day, Q3W) + AP regimen (nab-paclitaxel 125 mg/m2 on day 1, 8 and cisplatin 75 mg/m2 on day 1, Q3W) for 4-6 cycles. Pts who have achieved CR, PR, SD based on the RECIST 1.1 criteria were eligible for maintenance period and were randomized to receive either pembro (200mg Q3W) plus olaparib (300mg po bid) group or pembro mono group until disease progression or withdrawal of consent. Pts were stratified by response status (CR or PR vs SD), genomic tumor status (BRCAm vs BRCAwt) and visceral metastases (yes vs no). The primary end point was PFS. And the key second endpoints were ORR, OS and safety signals.
Results: At the May 31, 2024 data cutoff, 52 pts have been enrolled. 20 pts (38%) were PD-L1 CPS ≥10 while 32 pts (62%) were PD-L1 CPS <10. 4 pts (8%) carried germline BRCA 1/2 mutation. 41 pts (78.8%) had TNBC at initial diagnose while 11 pts (21.2%) did not but conversed to TNBC in the recurrent lesions. At the induction stage, the overall response rate was 86.5%, the median depth of response was 60% and the median time to response was 1.5 months. A total of 29 pts (28 PR and 1CR) were randomly assigned to pembro mono group (n=15) or combo group (n=14) for maintenance treatment. 9 pts were not randomized due to disease progression and 14 pts were still at the induction stage. In pts with a follow-up time more than 12 months, the 12-month PFS rate was 72.7% (8/11). Preliminary results showed pts with conversion to TNBC obtained less clinical benefit from this maintenance strategy than pts without conversion (6.6 vs. 12.3 months). Immune-related AEs were reported in 11 (21.2%) pts, of whom 5 (9.6%) pts experienced hypothyroidism, 2 (3.8%) pts experienced pneumonitis, 2 (3.8%) pts experienced hepatitis, 1 (2%) pt experienced adrenal insufficiency and hypophysis, 1 (2%) pt experienced nephritis, and 1 pt (2%) experienced severe skin reaction. Treatment-related AEs were reported in all the pts, while grade ≥3 treatment-related AE occurred in 32 pts (61.5%) including neutrophil count decreased (52%), thrombocytopenia (4%), anemia (13%) and ALT/AST increased (8%).
Conclusions: Preliminary data indicated that induction therapy with pembro + AP was a highly potent regimen in the first-line treatment of TNBC with no new safety signal, and pts with conversion to TNBC seemed to benefit less from this regimen. More efficacy and safety data between two groups will be shared in the future.

PS3-03: Vaccination with MUC-1-targeting tecemotide improves Survival of patients receiving neo-adjuvant chemotherapy for early breast cancer: Results from the Prospective Randomized ABCSG 34 Trial

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Background: The synthetic lipopeptide tecemotide (liposomal BLP25, Stimuvax®) is an investigational cancer vaccine designed to induce a cellular immune response to cancer cells that express the glycoprotein MUC1. Aberrant MUC1 is widely over-expressed in malignancies such as lung, breast, and colorectal cancer. The aim of the prospective randomized two-arm multicenter phase-II ABCSG 34 trial was to investigate the efficacy and safety of tecemotide in 400 patients with early BC when given concomitantly with neoadjuvant systemic Standard-of-Care (SoC) chemotherapy or neo-endocrine therapy. The study was accompanied by an extensive translational program which included immune response parameters such as MUC1 and PD-L1 expression, TILs, monocyte and ctDNA.
Patients and Methods:
400 patients with HER2- early BC were randomized 1:1 to receive neo-adjuvant SoC systemic therapy with or without 12 concomitant tecemotide vaccinations. Postmenopausal women with luminal A tumors received letrozole 2.5mg od for 24 weeks as SoC. TNBC patients and patients with luminal B tumors, in whom chemotherapy was considered to be SoC, received 4 x epirubicin/cyclophosphamide and 4 x docetaxel q3w. MUC1 protein expression on tumor cells was analyzed by immunohistochemistry. Invasive disease-free survival (IDFS), distant recurrence free survival (DRFS) and overall survival (OS) were analyzed with Cox regression models.
Results:
Seven years after surgery, follow-up (FU) data were collected retrospectively and available for 289 women. Of these, 236 had received chemotherapy, while 53 had received neo-endocrine letrozole. 141 had been randomized to receive concomitant tecemotide, while 148 had been randomized to SoC only. Patient and tumor characteristics in the FU group were highly representative of the overall study population, and clinical-pathological characteristics in the two randomization arms were well balanced.
After a 7 year FU, patients who had received SoC + tecemotide had an IDFS of 69.1% vs 60.5% in the SoC alone group (HR 0.75, 95% CI 0.51–1.10, p=0.141). DRFS of patients who had received SoC + tecemotide was 80.8% vs 64.7% (HR 0.53, 95% CI 0.34-0.83, p=0.005); OS in the SoC + tecemotide was 83.0% vs 68.2% (HR for OS 0.53, 95% CI 0.33-0.85, p=0.008). Participants who had received chemotherapy + tecemotide had a particularly favorable outcome with an IDFS of 71.3% vs 59.8% (HR 0.69, 95% CI 0.45–1.05, p=0.084), a DRFS of 81.9% vs 65.0% (HR 0.50, 95% CI 0.31–0.83, p=0.007), and an OS of 83.6% vs 67.8% (HR 0.51, 95% CI 0.30–0.88, p=0.016). Sensitivity analysis adjusting for T-stage, Nodal status, KI67, cancer type, and age at randomization were consistent with the primary results and confirmed the robustness of the analysis.
MUC1 protein data were available for 278 pts at baseline and 199 pts at surgery. MUC1 was expressed in 97.9% of baseline samples and in 98.5% of the surgical samples in which pCR was not achieved. Expression was high (i.e. detected in >80% of tumor cells) in 72.1% and 74.5%, respectively. While MUC1 expression levels were neither prognostic nor associated with pCR, tecemotide-treated patients with high MUC1 protein expression in their surgical sample showed a particularly good outcome with an improved IDFS (HR 0.57, 95% CI 0.34–0.98), DRFS (HR 0.38, 95% CI 0.20-0.72), and OS (HR 0.35, 0.17–0.71), when compared to SoC alone. Subgroup analysis and additional biomarker analyses will be presented at the

meeting.
Conclusions: Tecemotide, when given concomitantly with neoadjuvant SoC systemic therapy, profoundly improved IDFS, DRFS and OS in a follow-up analysis of this prospective Phase II trial. The improved long-term outcome was particularly evident in patients whose tumors expressed high levels of MUC1. While long-term outcome is an exploratory objective, this is the first study in which a therapeutic cancer vaccine has resulted in a statistically significant and substantial long-term survival benefit in breast cancer patients.

PS3-04: Overall Survival Results of Bria-IMT Allogenic Whole Cell-Based Cancer Vaccine

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Background: Despite advances in metastatic breast cancer (MBC), patients encounter challenges from resistance or intolerance. Bria-IMT is a combination immunotherapy using an allogeneic whole-cell cancer vaccine (SV-BR-1-GM) combined with checkpoint inhibitors (CPIs). Irradiated SV-BR-1-GM breast cancer cells, engineered to secrete GM-CSF, present tumor-associated antigens on HLA-I and HLA-II molecules, directly stimulating anti-tumor immunity. The addition of anti-PD1 CPI further potentiates the activity of SV-BR-1-GM to overcome the immune-suppressive tumor microenvironment. Methods This phase I/II study evaluated the Bria-IMT regimen consisting of low-dose cyclophosphamide (Day –2) followed by SV-BR-1-GM and CPI (pembrolizumab or retifanlimab), then low-dose local peg-interferon α, all given every 3 wks. In the phase II study, patients were randomized 1:1 to start CPI immediately at cycle 1 or delayed start at cycle 2. There were 2 formulations of SV-BR-1-GM with or without IFNγ incubation. Cancer-associated macrophage-like cells (CAMLs), circulating tumor cells (CTCs), and PD-L1 expression scores on these cells were monitored as biomarkers. A Candida skin test assessed anergy status before cycle 1, while a delayed-type hypersensitivity (DTH) skin test was done at each cycle with a small SV-BR-1-GM dose before the full dose. Results A total of 54 patients were enrolled with 22 patients in phase I and 32 in phase II. 11 patients were treated with pembrolizumab and 44 patients with retifanlimab (1 patient received pembrolizumab and later retifanlimab). Median age was 61 (38-81) years; median prior regimen was 6 (2-13). 39(72%) patients were ER+/PR+/HER2-; 3(6%) HER2+, and 12 (22%) triple-negative (TNBC). The Bria-IMT regimen was well tolerated, with no drop-outs attributable to Bria-IMT. The majority of adverse events (67%) were grade 1-2, with few (13%) cases > grade 3, including pulmonary embolism, dehydration, and increased lipase. In the entire cohort, the median overall survival (OS) was 9.9 (1.8-28.5) months. Patients who received the SV-BR-1-GM phase 3 formulation (without IFNγ incubation) (n=37) had a significant increase in both PFS (3.6 vs 2.6 months, P 0.01) and OS (13.4 vs. 6.9 months, P 0.04), an objective response rate (ORR) of 9.5% and a clinical benefit rate (CBR) was 55%. Among randomized patients receiving phase 3 formulation (n=21) median OS not yet reached. In ER+/PR+/HER2-, OS was 13.3

(1.8-28.5) months, HER2+ 11.9 (7.2-11.9) months, and TNBC 8.5 (2.1-15.6) months. There was a significant difference in PFS but not OS among patients who received immediate vs. delayed start of CPI (PFS 3.6 vs. 2.6 months, P 0.02; OS 9.9 vs. 11.9 months, P 0.45). Among 36 patients with post-dose CAML/CTC data, patients with post-dose CTC count < 5 had a significantly improved OS compared with a CTC count > 5 (13.4 vs. 5.5 months, P 0.01). Patients with positive DTH had significantly improved PFS (3.5 vs. 2.4 months, P 0.001) and OS (11.9 vs. 5.5 months, P 0.002).

Conclusions: In heavily pretreated MBC patients, the Bria-IMT regimen demonstrated promising results across all subtypes of breast cancer with favorable safety profiles. There was no significant difference in OS among patients treated with immediate vs. delayed CPI. However, superior outcomes were observed in patients receiving SV-BR-1-GM without IFNγ incubation. This formulation has advanced into the ongoing clinical trials. Patients with post-dose CTC count < 5, and positive DTH had significantly improved outcomes. Based on these promising findings, the randomized phase III trial comparing the BRIA-IMT regimen to the treating physician's choice is currently ongoing (NCT06072612). Trial information: NCT03328026.

PS3-05: Evaluation of the safety and efficacy of ivonescimab in combination with chemotherapy as first-line (1L) treatment for triple-negative breast cancer (TNBC)

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Background: Locally advanced unresectable or metastatic TNBC is highly aggressive and has a poor prognosis. Ivonescimab, a tetravalent bispecific antibody targeting PD-1 and VEGF, has the potential to produce synergistic anti-tumor effects through both pathways via cooperative binding. This study aimed to evaluate the safety and efficacy of ivonescimab in combination with chemotherapy in locally advanced unresectable or metastatic TNBC.
Methods: This was an open-label, multicenter phase II study in patients (pts) with locally advanced unresectable or metastatic TNBC. Pts received ivonescimab at 20 mg/kg Q2W and paclitaxel at 90 mg/m2 or nab-paclitaxel at 100 mg/m2 on the 1st, 8th, and 15th day of each four-week treatment cycle. The primary endpoints were safety and objective response rate (ORR) by RECIST1.1. The secondary endpoints included duration of response (DoR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS).
Results: As of May 31, 2024, a total of 30 pts with locally advanced unresectable or metastatic TNBC were enrolled. The median age was 54.0 years (range, 35.4-73.3), 53.3% of pts had ECOG of 1, 80% of pts had a PD-L1 combined positive score (CPS) <10, and 60% of pts had previously received taxane-based neoadjuvant or adjuvant therapy. The median follow-up was 10.12 months (range: 2.66+, 15.77). A total of 53.3% (16/30) of pts experienced at least one ≥Grade 3 treatment-related adverse event (TRAE), but none led to treatment discontinuation or deaths. The most common ≥Grade 3 TRAEs with a frequency

≥10% included neutrophil count decreased (16.7%) and white blood cell count decreased (16.7%). Twenty-nine pts had at least one post-baseline tumor assessment; the ORR by investigator assessment was 72.4% (21/29) and the DCR was 100% (29/29). The ORRs of PD-L1 CPS ≥10 and PD-L1 CPS <10 were 83.3% (5/6) and 69.6% (16/23), respectively. The median PFS was 9.3 month (95% CI: 6.24, NE), the 9-month PFS rate was 56.6% (95% CI: 32.2, 75.1). In the PD-L1 CPS ≥10 population, the median PFS was not reached (95% CI: 5.36, NE), the 9-month PFS rate was 60% (95% CI: 12.6, 88.2). In the PD-L1 CPS <10 population, the median PFS was 9.3 month (95% CI: 5.55, NE), the 9-month PFS rate was 52.6% (95% CI: 22.5, 75.8). The median DoR was 7.49 month (95% CI:3.91, NE). In the PD-L1 CPS ≥10 population, the median DoR was not reached (95% CI: 3.58, NE). In the PD-L1 CPS <10 population, the median DoR was 7.49 month (95% CI: 3.91, NE). The median OS was not yet mature.
Conclusion: Ivonescimab in combination with chemotherapy showed promising anti-tumor activity and acceptable tolerable safety as 1L treatment of TNBC. Further trials to confirm the results are warranted.

PS3-06: A prospective phase 2 study on efficacy and safety of AK105, anlotinib combined with nab-paclitaxel (nab-P) as a first-line therapy in patients(pts) with advanced triple-negative breast cancer (TNBC)

Tao Sun, Liang Zhang, Xiujie Cui, Li Man, Zhaohui Li, Li Cai, Fangyuan Dong, Ying E, Yujun Jiang, Hui Cao, Yangyang Duan, Huan Li, Xiaorui Li, Zhichao Gao, Lei Jiang, Cui Jiang, Yufeng Jia, Dan Lv

Background: PD-1/PD-L1 inhibitor plus chemotherapy have shown tolerability and significant clinical benefits in pts with advanced TNBC. Antiangiogenic agent could remodel tumor blood vessels and increase the response to immune-checkpoint inhibitors (ICIs). AK105, an anti-PD-1 antibody, can effectively prevents PD-1 from binding to PD-L1 and PD-L2, and avoid immune evasion of tumor cells. Anlotinib is a novel antiangiogenic, multi-target tyrosine kinase inhibitor which inhibits VEGFR, FGFR, PDGFR, c-KIT, c-RET and MET. This investigator initiated trial (IIT) (NCT05244993) aims to investigate the efficacy and safety of AK105, anlotinib combined with nab-P as a first-line therapy in pts with advanced TNBC.
Methods: In this multicenter, prospective, single arm, phase 2 study, eligible pts were female aged 18-75 years, with ECOG PS 0-1, who had locally advanced or recurrent/metastatic triple-negative (estrogen receptor-, progesterone receptor- and HER2-) breast cancer. Eligible pts were treated with intravenous AK105 (200 mg on day 1), oral anlotinib (12 mg once daily on days 1-14) and intravenous nab-P (125 mg/m2 on days 1 and 8) . The triplet combination regimen repeated every 21 days until disease progression, death or intolerable toxicity. The primary endpoint is overall response rate (ORR), and the secondary endpoints are disease control rate (DCR), progression-free survival (PFS), overall survival (OS)and safety.
Results: From July 2022 to March 2024, 33 patients have been enrolled in this study.

Median follow-up was 6.54 months (95% CI 2.68-11.43). Of all the patients whose efficacy could be evaluated (26/33), 1(3.85%) patient achieved complete response (CR); 20(76.92%), 4(15.38%) and 1(3.85%) patients got partial response (PR), stable disease (SD) and progression disease (PD), respectively. ORR was 80.77 % (95% CI 60.65-93.45) and DCR was 96.15% (95% CI 80.36-99.90). The overall median PFS was 11.14 months (95% CI 8.34-18.04) , the median OS have not reached. The 6-month OS rate and 12-month OS were 95.83% (95% CI 73.92-99.40) and 80.90% (95% CI 50.05-93.72), respectively. The most common adverse events (AEs) were grade 1 or 2. The grade 3 AEs (incidence ≥ 10%) included were neutropenia (33.33%), leukopenia (12.12%) and elevated AST (12.12%). There were two case of grade 4 neutropenia and one case of grade 4 hypertriglyceridemia. One death (from hepatitis) was considered by the investigators to be related to the trial regimen.
Conclusion: The combination of AK105, anlotinib and nab-P showed better treatment response and tolerable toxicity in the treatment of first-line patients with TNBC. Further studies enrolling more patients are still needed.

PS3-07: SHR-A1811 as Neoadjuvant Treatment in Patients with HR-Positive, HER2-low Breast Cancer: The first-stage results from an open-label, single-arm, two-stage, phase II clinical trial

Zhenzhen Liu, Jianghua Qiao, Chengzheng Wang, Xianfu Sun, Min Yan, Lianfang Li, Xiuchun Chen, Jiujun Zhu, Jianfei Wang

Background: Patients with Hormone receptor (HR)-positive, human epidermal growth factor receptor 2(HER2)-negative breast cancer exhibit lower rates of achieving pathologic complete response (pCR) to neoadjuvant chemotherapy or endocrine therapy, with pCR rates of 10% and 5% respectively. HER2-low breast cancers are enriched in HR-positive breast cancers, but HER2-low patients had significantly lower pCR than those with HER2-0. The anti-HER2 ADC drug T-Dxd has demonstrated anti-tumor activity in HR+/HER2-low advanced breast cancer, based on the DESTINY-Breast 06 study. SHR-A1811, a novel anti-HER2 ADC, has also shown activity in HER2-low advanced breast cancer. Here we conducted this clinical trial to investigate the efficacy and safety of SHR-A1811 as a neoadjuvant treatment in patients with HR+/HER2-low breast cancer.
Methods: This was an open-label, single-arm, phase II clinical trial based on Simon's two-stage design. A total of 66 subjects were required for enrollment, with 35 subjects included in the first stage. If 18 or more patients having response, the trial would proceed to the second stage, enrolling an additional 31 subjects. Main inclusion criteria include: aged 18-70 years, treatment-naïve, histologically confirmed invasive breast cancer, cT2-3/N0-2M0, HR+/HER2-low and Ki67>14%. Eligible patients received SHR-A1811 (6.4 mg/kg IV q21 days) for eight cycles. The primary endpoint was objective response rate (ORR). Secondary endpoints included safety, residual cancer burden (RCB) 0-1 and pCR.
Results: As of June 17, 2024, 34 out of 35 patients in the first stage successfully received the study regimen and finished the operation on schedule. Of all patients, 74.3% (26/35) had

node-positive disease, 85.7% (30/35) with stage IIA/IIB, 14.3% (5/35) with stage IIIA at baseline. 74.3% (26/35) patients had baseline HER2 expression of IHC 2+, 25.7% (9/35) were IHC 1+. Our primary endpoint of the first stage was met, with 74.3% (26/35) of patients achieving an ORR, including 26 partial responses and 9 stable diseases. None of the patients achieved pCR. Treatment-related adverse events (TRAEs) were reported in all 35 patients, and the incidence of grade ≥3 TRAEs was 65.7% (23/35), the most common grade ≥3 TRAEs including neutropenia (48.6%,17/35), leukopenia (28.6%,10/35), anemia (22.9%, 8/35), diarrhea and platelet count decreased (11.4%, 4/35). Primary G-CSF prophylaxis was administered to 13 patients, and no grade 3 or higher neutropenia was reported. No interstitial lung disease (ILD) and treatment-related deaths were reported. Conclusions: SHR-A1811 has demonstrated promising anti-tumor activity and an acceptable safety profile as neoadjuvant treatment for patients with HR+/HER2-low breast cancer. The second stage is ongoing, Clinical trial information: NCT05911958.

PS3-08: Interim Overall Survival of Patients with Locally Advanced or Metastatic Triple-Negative Breast Cancer treated with First Line PM8002/BNT327 in Combination with Nab-Paclitaxel in Phase Ib/II Study

Jiong Wu, Jian Zhang, Zhongsheng Tong, Qingyuan Zhang, Yongsheng Wang, Qiao Cheng, Xin Chen, Zhihua Li, Yongmei Yin, Yiqun Du, Yanchun Meng

Background: PD-L1 and VEGF play important roles in immune evasion and tumoral angiogenesis, promoting cancer growth and metastasis. PM8002/BNT327 is an investigational bispecific antibody targeting PD-L1 and VEGF-A, in development for the treatment of solid tumors. We conducted a Phase Ib/II study of PM8002/BNT327 in combination with nab-paclitaxel in pts with locally advanced or metastatic triple-negative breast cancer (LA/mTNBC) in China. We provide updated results including interim overall survival (OS) since initial reports at SABCS 2023 and ESMO 2024.
Methods: 42 Pts with previously untreated LA/mTNBC were enrolled till 11 Apr 2023 to assess the safety and efficacy of PM8002/BNT327 in combination with nab-paclitaxel. All patients received PM8002 at 20 mg/kg (Q2W) and nab-paclitaxel at 100 mg/m2 on the 1st, 8th, and 15th day of each 28-day cycle until unacceptable toxicity or disease progression were observed. Efficacy was assessed every 8 weeks. Primary objectives were safety per CTCAE 5.0 and objective response rate (ORR) per RECIST v1.1 by investigator assessment, with progression free survival (PFS) and overall survival (OS) as secondary objectives.
Results: As of 13th Sep 2024, with a median follow-up time of 18.1 months (95% CI 16.9, 19.7), median duration of treatment was 10.0 months (range 2.0-22.0) with 9/42 patients still on treatment. The confirmed ORR (cORR) was 73.8% with a disease control rate (DCR) of 95.2%. The median time to response (TTR) was 1.9 months (95% CI 1.8, 2.0), the median duration of response (DOR) was 11.7 months (95% CI 7.2, 17.3). The matured median PFS was 13.5 months (95% CI 9.4, 18.1) for the ITT population. The median OS was not reached, while the matured 12-month OS rate was 80.8% (95% CI 65,3, 89.9), the matured 15-month OS rate was 78.1% (95% CI 62.1, 88.0) and the nearly matured 18-month OS rate was

72.2% (95% CI 55.2, 83.7). 38 pts had available PD-L1 expression results tested with E1L3N assay. cORR was 76.9% in 13 pts with PD-L1 combined positive score (CPS) < 1 and 72.0% in 25 pts with PD-L1 CPS ≥1. All 9 pts with PD-L1 CPS ≥10 achieved PR. All pts experienced treatment-related adverse events (TRAEs), 59.5% were Grade 3 or 4, no Grade 5 TRAEs were observed. The most common (≥30%) TRAEs included neutropenia, leukocytopenia, anemia, proteinuria, alopecia, hypertriglyceridaemia, hypercholesterolaemia, epistaxis and asthenia. 31.0% of pts experienced immune-related adverse events (irAEs), 9.5% were Grade 3, no Grade 4 or 5 irAEs were observed. The most common irAEs included hyperthyroidism, hypothyroidism and rash. The most common AEs typically associated with VEGF inhibition were hypertension (23.8%) and proteinuria (64.3%) which were mostly Grade 1 or 2.
Conclusions: In pts with LA/mTNBC, first-line therapy with PM8002/BNT327 combined with nab-paclitaxel showed clinically meaningful survival outcomes and antitumor activity regardless of PD-L1 status, together with a manageable safety profile. No new safety signals were observed beyond those typically described for anti-PD-1/PD-L1 and anti-VEGF therapies and nab-paclitaxel. A randomized controlled Phase III clinical trial (NCT06419621) of first-line treatment of TNBC in China and a global Phase II trial (NCT06449222) are ongoing in TNBC.
Acknowledgements: We would like to thank the pts and their families for their contribution to this trial. This trial was sponsored by Biotheus Inc. PM8002/BNT327 is jointly developed by Biotheus and BioNTech
Trial Registration: ClinicalTrials.gov: NCT05918133.
Ethics Approval: This study was approved by the Ethics Committee for Drug Clinical Trials, Fudan University Shanghai Cancer Center.

PS4-01: Predictor of benefit from dose-dense paclitaxel chemotherapy for patients with hormone receptor-positive HER2-negative breast cancer. A GEICAM/9906 sub-study

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Background: SETER/PR index is a genomic test designed to measure endocrine transcriptional activity that is related to estrogen receptor (ER) and progesterone receptor (PR), but not proliferation. Higher values of SETER/PR index indicate greater endocrine transcriptional activity in the cancer. It was previously reported that levels of SETER/PR index below 0.75 predicted benefit from dose-dense (q2-week), versus conventional (q3-week) anthracycline-paclitaxel chemotherapy for patients (pts) with hormone receptor-positive (HR+) lymph node-positive breast cancer (BC) in the CALGB 9741 trial (Metzger et

al, 2022, ASCO). Since that was the first report of a test that could predict benefit from a dose-dense taxane-based chemotherapy in HR+ BC, we sought to independently test its predictive validity. Therefore, we tested the SETER/PR assay at this predefined cutoff point in pts with HR+/HER2- lymph node-positive BC from the phase III GEICAM/9906 trial to predict benefit from weekly paclitaxel treatments after three cycles of q3-week anthracycline chemotherapy (FEC+P) compared to three additional anthracycline treatments (FEC).

Methods

We conducted a blinded independent validation study in 647 HR+/HER2- tumor RNA samples from GEICAM/9906 BC pts (51.9% of the trial's cohort). GEICAM translational laboratory sent the de-identified tumor RNA samples to the MDACC laboratory for blinded testing with results returned to the GEICAM statistician. Predefined cutoff point was SETER/PR index <0.75. Clinical endpoints were distant recurrence-free interval (DRFI) and overall survival (OS, secondary endpoint). Multivariable Cox proportional hazards models including SETER/PR index, treatment arm and their interaction term were used to calculate hazard ratios with 95% confidence interval (HR, 95%CI) and two-sided 0.05 as predefined level of significance.

Results

Of 647 HR+/HER2- tumor samples, 567 (87.6%) passed quality control for the SETER/PR assay, with 279 from the FEC+P and 288 from the FEC treatment arm. SETER/PR index was below 0.75 in 92/567 tumors (16.2%), 44 (47.8%) in the FEC+P arm and 48 (52.2%) in the FEC treatment arm. Overall, there were 149 distant recurrence events and 144 deaths. There was significant interaction between SETER/PR status and treatment arm on DRFI (P=0.046), but not OS (P=0.352). For patients with SETER/PR below 0.75, treatment with FEC+P significantly improved DRFI (HR 0.46; 95%CI, 0.22–0.95, P=0.035), even when adjusted for menopausal status and ≤3 versus ≥4 nodes involved (HR 0.48; 95%CI, 0.24–1.00, P=0.049), but there was no difference between treatments benefit when SETER/PR index was ≥0.75 (HR 1.02; 95%CI, 0.70–1.47, P=0.931).

Conclusion

In this GEICAM/9906 trial retrospective analysis, patients with node-positive HR+/HER2- BC that has low endocrine transcriptional activity (SETER/PR index <0.75) benefited from inclusion of weekly paclitaxel in their adjuvant chemotherapy. This result provides independent confirmation that SETER/PR index predicts benefit from dose-dense paclitaxel-containing chemotherapy.

Clinical trial information: NCT00129922.

PS4-02: Patient-derived Xenografts (PDX) Allow Deconvolution of Combination Chemotherapy Response

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Introduction: Triple-negative breast cancer (TNBC) patients frequently receive combination chemotherapy treatment, including most recently taxane/platinum combinations. However, treatment is not biomarker-guided. As such, it is not known which patient will respond to one or the other agent, or indeed which patients actually require the combination for the most effective treatment. We hypothesized that optimization of chemotherapy may be possible if molecular mechanisms and biomarkers underlying response to individual treatments can be identified. We evaluated this hypothesis in a preclinical trial using a cohort of 50 patient-derived xenograft (PDX) models of TNBC treated with either single-agent docetaxel or carboplatin, or their combination.
Methods: 50 TNBC PDXs were evaluated for response to four weekly treatments with either single agent docetaxel (20 mg/kg), or carboplatin (50 mg/kg); 42 of these were also treated with their combination. Multi-omics profiling (genomics, transcriptomics, proteomics) was conducted before treatment. Gene level associations by treatment type were used to construct consensus gene sets by integrating our data with external TNBC PDX and patient cohorts which were then used to build XGBoost models to predict treatment-specific responses. Pathway analysis was performed using signed -log10 p-values from gene level results as input for Gene Set Enrichment Analysis.
Results: Direct comparison of responses to carboplatin, docetaxel, and their combination showed that combination treatment was largely ineffective at generating enhanced responses over the best single agent. Only 13% of the 42 PDX showed enhanced responses in combination, with a comparable percentage (12%) showing antagonism between docetaxel and carboplatin, a phenomenon observed previously in vitro.
Proteogenomic profiles revealed distinct genes associated with responses to each agent and their combination, suggesting different molecular mechanisms underlying each treatment response. A substantial number of genes linked to single-agent and combination treatments were validated in multiple independent PDX and patient cohorts receiving platinum and taxane-containing neoadjuvant therapy, confirming the clinical relevance of our PDX panel. Chemotherapy-specific predictors for pathological complete response (pCR)/CR achieved AUROCs of 0.79, 0.67, and 0.70 for platinum, taxane, and combination treatments, respectively. The single-agent platinum model was the most effective in predicting platinum response, with a similar trend for taxane and platinum+taxane predictors. These findings reinforce the observation that distinct gene sets are linked to responses to different chemotherapy treatments. Since the predictors used treatment-associated genes found in both PDX and clinical samples, these results suggest biomarker combinations for translation and clinical development to select TNBC tumors that may respond to specific regimens. In PDXs responsive to any treatment, several basal cytokeratins were among the top upregulated proteins compared to PDXs resistant to all treatments. KRT5 was validated by IHC in PDX tumors, achieving an AUROC of 0.83 for predicting responsiveness, indicating its potential as a future chemoresponse marker in TNBC. PDXs refractory to all treatment arms

had higher levels of mitochondrial and cancer stem-cell-related pathways. Treatment with romidepsin, an HDAC inhibitor that targets these pathways and increases DNA damage, enhanced carboplatin response in a chemoresistant PDX model with high abundance of HDAC proteins.
Conclusion: Proteogenomic characterization identifies molecular mechanisms and putative biomarkers for stratifying TNBC tumors for single or combination chemotherapy treatments, suggests targeted therapies to augment chemotherapy response, and provides a valuable resource for researchers and clinicians.

PS4-03: Calcium Channel Blockers Enhance Efficacy of TROP2-ADC in Overcoming Resistance in Advanced Triple-Negative Breast Cancer

Jieer Luo, Qun Lin, Yu Shi, Zhuxi Duan, Jinpeng Luo, Xiaolin Fang, Chang Gong

The TROP2-directed antibody-drug conjugate (TROP2-ADC) has been approved for treating advanced triple-negative breast cancer (TNBC) that has progressed after chemotherapy. Recent findings from the ASCENT trial indicated that sacituzumab govitecan (SG), compared with standard chemotherapy, significantly prolongs median progression-free survival and overall survival in patients with advanced or refractory TNBC, reducing the risk of disease progression by 59% and the risk of death by 52%. However, approximately 20% of patients exhibit primary resistance to SG, experiencing disease progression within 3 months. Therefore, exploring the molecular mechanisms underlying TROP2-ADC resistance is crucial for reversing drug resistance and improving the prognosis of patients with advanced TNBC.
In this study, we retrospectively observed that advanced TNBC patients with hypertension who were co-treated with SG and nifedipine displayed better survival times compared to advanced TNBC patients without hypertension who were treated with SG alone. Based on these findings, we constructed TNBC patient-derived xenografts (PDXs) that were either sensitive or insensitive to TROP2-ADC. We found that combining calcium channel blockers (CCBs) with TROP2-ADC significantly improved anti-tumor efficacy and maintained a manageable safety profile in TROP2-ADC insensitive models, both in vivo and in vitro. High-throughput sequencing of TNBC patients treated with TROP2-ADC revealed that pathways related to vesicle trafficking, calcium ion binding, and cell membrane processes were highly enriched in resistant patients. Mechanistically, excess intracellular calcium activated PKC and PACSIN2, leading to increased caveolae-mediated endocytosis. This resulted in defective plasma membrane localization of TROP2, contributing to TROP2-ADC resistance. In conclusion, our results provide insight into the potential mechanisms of TROP2-ADC resistance and suggest that combining CCBs with TROP2-ADC may offer a new therapeutic strategy for patients resistant to TROP2-ADC.

PS4-04: MammaPrint® and BluePrint® predict pathological response to neoadjuvant chemotherapy in patients with HR+HER2- early-stage breast cancer enrolled in FLEX

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Background: For patients with hormone receptor-positive (HR+), HER2-negative early-stage breast cancer (EBC), pathological Complete Response (pCR) rates to neoadjuvant chemotherapy (NCT) are low. However, NBRST and I-SPY2 trials report that MammaPrint risk of distant recurrence and BluePrint molecular subtyping signatures can more accurately identify which HR+HER2- EBC patients are likely to achieve pCR. Here, MammaPrint and BluePrint as biomarkers for predicting pCR and residual cancer burden (RCB) class, was evaluated among patients with HR+HER2- tumors enrolled in FLEX.
Methods: Patients with HR+HER2- tumors treated with NCT with available pCR and/or RCB data enrolled in the ongoing prospective, observational FLEX Trial (NCT03053193), were included in this study (N = 457). Tumors were characterized as MammaPrint UltraLow (UL), Low (LR), High 1 (H1) or High 2 (H2) Risk. BluePrint subtypes were classified as either Luminal A, Luminal B, HER2, or Basal. pCR and Pathological Response (PR= pCR + minimal residual cancer burden [RCB-I]) were the study endpoints. Differences in clinical characteristics and response rates were evaluated by Chi-Squared test or two-sided proportional Z-test. The association between MammaPrint, BluePrint, and PR was assessed using multivariate logistic regression and was adjusted for menopausal status, T stage, and N stage.
Results: MammaPrint classified 2% as UL, 8% as LR, 56% as H1, and 34% as H2. Nearly 60% of patients in this study had clinical lymph node (LN) positive disease. Higher tumor stage and grade were significantly more likely to be associated with higher MammaPrint Risk. Age, menopausal status, race, and LN status were comparable among MammaPrint groups. All UL and LR tumors were Luminal A, most H1 tumors were Luminal B (98%), while 45% of H2 tumors were Luminal B and 54% were Basal (1% were HER2). pCR rates were significantly higher in H2 Basal (39.8%), followed by H2 Luminal B (15.7%), H1 Luminal B (7.4%), and lowest in UL and LR, Luminal A (4.5%) ($p<0.001$). Among H2 Luminal B tumors, LN negative tumors had a pCR rate of 21.0% whereas LN positive tumors had a pCR rate of 12.5%. Rates of RCB-I were significantly higher among H2 tumors (26.9%) than H1 tumors (7.3%), or UL and LR tumors (0%) ($p=0.026$). Among H2 tumors, PR rates measured higher than rates of pCR alone in Basal tumors (43.4% vs 39.8%) and more prominently in Luminal B tumors (21.4% vs. 15.7%). Multivariate analysis revealed that MammaPrint H2 was significantly associated with likelihood of PR (OR=12.6, 95% CI 2.43 – 23.28, $p=0.016$), whereas menopausal status, T stage, and N stage were not associated with PR.
Conclusion: MammaPrint and BluePrint predict chemosensitivity to NCT in HR+HER2- EBC. Patients with MammaPrint H2 tumors, including Luminal B and Basal subtypes, are more likely to achieve pCR or PR to NCT, compared to UL, LR, and H1 groups. Additionally, pCR

rates trended lower among LN positive disease, possibly due to the increased difficulty of clearing disease in LNs with systemic therapy. We observed very high rates of RCB-I in H2 Luminal B tumors. These findings should be confirmed in other studies. The very high rates of PR observed in this study suggest that MammaPrint H2 identifies a subset of patients with HR+HER2- disease that benefit from NCT, which may enable surgical downstaging efforts and translate to improvements in outcomes.

PS4-06: HER2DX ERBB2 mRNA Score in First-Line Metastatic HER2-Positive Breast Cancer Treated with Docetaxel, Trastuzumab and Pertuzumab: Correlative Analysis from CLEOPATRA Phase III Trial

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Introduction: In first-line HER2-positive (HER2+) metastatic breast cancer (MBC), the standard regimen of taxane-trastuzumab-pertuzumab (THP) faces increasing competition from new therapies. This shift highlights the need for precise biomarkers to guide treatment decisions. Two previous real-world evidence studies in HER2+ MBC have shown that, compared to HER2DX ERBB2-low/med groups, the HER2DX ERBB2-high group is associated with better progression-free survival (PFS) and overall survival (OS) following THP in the first-line setting (Bayona et al. ESMO Breast 2024) and following T-DM1 monotherapy in the second-line setting or beyond (Brasó-Maristany et al. JNCI 2022). Here, for the first time, we evaluated the ability of the HER2DX ERBB2 mRNA score to predict long-term outcomes in the pivotal trial that established THP in the first-line setting.
Methods: We analyzed available formalin-fixed paraffin-embedded (FFPE) tumor RNA from participants in the CLEOPATRA phase III trial (Baselga et al., NEJM 2012; Swain et al., Lancet 2020; NCT00567190) who were randomized to THP (docetaxel, trastuzumab, and pertuzumab). Standardized HER2DX assay was conducted at Reveal Genomics' central lab (Barcelona, Spain) blinded from clinical data. The HER2DX ERBB2 score, evaluated both as a continuous variable (1 to 99) and as pre-defined groups (low, medium, and high), was correlated with overall response rate (ORR), PFS, and OS in an exploratory analysis using logistic regression and Cox proportional hazards models. The significance level for all statistical analyses was set at a two-sided alpha of 0.05.
Results: In this CLEOPATRA subpopulation, representing 53% of the original cohort (214 out of 402 patients randomized to THP), the median PFS was 18.7 months (95% confidence interval [CI]: 16.2-22.8) and the median OS was 59.5 months (95% CI: 49.3-83.5). These survival outcomes are similar to the original trial cohort. The assay success rate was 96.8% (214/221). The HER2DX ERBB2-low, -medium, and -high groups distribution was 13.5% (n=29), 8.9% (n=19), and 77.6% (n=166), respectively. The HER2DX ERBB2 score, analyzed as a continuous variable, demonstrated an association with PFS (hazard ratio [HR] per 10-unit increment=0.90; 95% CI: 0.83-0.97) and OS (HR per 10-unit increment=0.91; 95% CI:

0.83-1.00), after adjusting for IHC status (IHC 0+, 1+, 2+ versus 3+). Patients with ERBB2-high tumors showed superior median PFS (20.3 months, 95% CI: 18.2-26.4) compared to those with ERBB2-low (10.4 months, 95% CI: 8.4-12.7) and ERBB2-medium (18.7 months, 95% CI: 16.6-36.9) disease. Similarly, a better median OS was observed in patients with ERBB2-high tumors (72.7 months, 95% CI: 51.8-102.1) compared to those with ERBB2-low (40.3 months, 95% CI: 28.8-59.5) and ERBB2-medium (56.5 months, 95% CI: 37.1-59.5) disease. The ERBB2 score, as a continuous variable, was not associated with the ORR (odds ratio per 10-unit increment=1.11, 95% CI: 0.95-1.31).
Conclusion: The HER2DX ERBB2 mRNA score is strongly associated with long-term survival outcomes in metastatic HER2+ breast cancer treated with first-line THP. Notably, HER2DX identified patients with ERBB2-low disease, as the subgroup with the poorest outcomes in both PFS and OS. Further analyses will be presented at the conference.

PS4-07: Residual disease after HER2 inhibition is driven by a primary tumor subpopulation expressing an ERBB2-low associated transcriptional program

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Background: In HER2+ breast cancer (BC), residual disease (RD) after neoadjuvant HER2 inhibition is associated with increased risk of recurrence. However, the genetic and non-genetic resistance mechanisms underlying RD remain incompletely characterized. Here we used single nuclei (sn) RNA and DNA sequencing to profile genetic and transcriptional changes in growing, residual, and recurrent tumors from an inducible mouse tumor model of HER2+ breast cancer. Methods: We used mammary tumors / tumor bed (n=2-3/time point) from female MMTV-rtTA; TetO-ERBB2 mice at 9 weeks DoxOn (HER2on), 7 days DoxOff (HER2off) and 12-21 weeks Doxoff (recurrent). Samples were snap frozen and single nuclei isolation was performed. snRNAseq was performed using 10X 5' v2 kits for library preparation and sequencing. snDNAseq was performed using a custom amplicon panel targeting the mouse genome and human ERBB2 locus via the MissionBio Tapestri platform. Results: For snRNAseq analysis, tumor cell clusters were identified by Epcam and ERBB2 gene expression. Tumor cells were further subclustered into 18 unique clusters that separated by ERBB2 expression. Seven subclusters of HER2on tumor cells were characterized by low ERRB2 expression: 1.38 vs 2.94 (median normalized log counts) in HER2on-ERBB2high cells. Top 5 differentially expressed genes (DEG) per condition (log2 fold change >1.5, adjusted p value < 0.05) were: HER2on: Erbb2, Csn3, Lcn2, Csn2, Wfdc18; HER2on-ERBB2high: Csn3, Igfbp5, Csn2, Lcn2, Trf; HER2on-ERBB2low: Lars2, Gm47283, Cmss1, Gm42418, Neat1; HER2off: Lars2, Hexb, Cmss1, Gphn, Gm42418; Recurrent: Capza2, Vim, Lgals1, Crabp1, S100a6. HER2on-ERBB2low cells were more closely related in transcriptional space to HER2off residual tumor cells than HER2on-ERBB2high tumor cells. Trajectory analysis suggested that HER2off RD evolved from HER2on-ERRB2low cells and formed the basis of recurrent disease. To determine clonal evolution over time, we used

common top 10 DEGs between HER2on vs HER2off vs HER2low snRNAseq data and intersected that set with significantly enriched single nucleotide variants (SNVs) from our snDNAseq data. We identified 16 clones containing at least 1 SNV at >1% frequency across all samples. We observed clonal persistence, relative enrichment and extinguishment between HER2on, HER2off and recurrent samples but without clonal dominance.
Conclusion: Time-series analysis of genetic and transcriptional evolution in a mouse model of HER2+ BC RD suggests that a tumor cell subpopulation characterized by an ERBB2low-associated transcriptional program drives RD after HER2 inhibition. Further functional analysis will be presented at the conference.

PS4-08: The tumor heterogeneous overexpressed CD155 promotes treatment resistance in HER2-positive breast cancer through immune microenvironment modulation

Yi Zhang, Bingqiu Xiu, Qi Zhang, Jiong Wu

Purpose: HER2-positive breast cancer is characterized by high malignancy and frequent recurrence. The combination of immunotherapy with HER2-targeted therapy necessitates a deeper understanding of the immuno-oncology of HER2 breast cancer. CD155, an immune checkpoint highly expressed on tumor cells, promotes tumor growth and interacts with its inhibitory ligand TIGIT to form an immunosuppressive microenvironment. This study investigated the tumor heterogeneous overexpressed CD155 promotes treatment resistance in HER2-positive breast cancer.
Methods: We collected 10 untreated human HER2-positive breast cancer scRNA-seq datasets to explore the expression pattern of CD155 and used GSEA and GSVA to characterize epithelial cells expressing high levels of CD155. In addition, 3 paird HER2+ scRNA-seq samples before and after neoadjuvant therapy were also incluced. Based on bulk RNA-seq of HER2 breast cancer from TCGA, we conducted gene co-expression analysis and WGCNA to define the expression network of CD155. To uncover the relationship between CD155 and HER2-targeted therapy resistance, we collected patient tissue microarrays and paired pre/post-treatment samples for IHC. In vitro and in vivo studies were conducted to verify the molecular mechanism of CD155 expression in HER2 breast cancer.
Results: Through scRNA-seq from patient samples, we found a strong negative correlation between CD155 and ERBB2 expression in breast cancer cells, with heterogeneous expression further verified in our multiplex IHC of patient samples. To further verify the CD155 high cells are enriched after anti-HER2 treatment, we use neoadjuvant cohorts to evaluate these markers through IHCs and found the upregulation of CD155 post treatment. In addition, based on the TCGA database, CD155 correlates with a worse prognosis only in the HER2-enriched subtype. The relevance between CD155 and worse prognosis was demonstrated in our 200-patient tissue microarray IHC. In scRNA-seq, clusters bearing high expression of CD155 exhibited more vigorous metabolic features. In the cell lines SKBR3

and HCC1954, knockdown CD155 showed a decline in proliferation and increased sensitivity to HER2-targeted therapy. Moreover, Cibersort analysis revealed that CD155 is negatively correlated with the infiltration of activated NK cells in the tumor microenvironment, a major force of the ADCC effect induced by trastuzumab.
Conclusion: CD155 plays a critical role in HER2 heterogeneity and mediates immune evasion. Blocking CD155 could be a potent treatment option to overcome resistance to HER2-targeted therapy while preventing NK cell exhaustion and eliciting robust anti-tumor immunity.

PS4-09: Spatial Omics Analysis Uncovers Microenvironmental Remodeling and Immune Dynamics in T-DXd Resistant Metastatic Breast Cancer

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Trastuzumab deruxtecan (T-DXd) is the standard of care for HER2+ or HER2 low metastatic breast cancer (mBC). However, approximately 20% of patients exhibit resistance to T-DXd therapy. As the development of more antibody-drug conjugate (ADC) therapies progresses, understanding ADC resistance mechanisms is critical. Our study aims to elucidate the etiology of ADC resistance, such as characterizing molecular changes and identifying novel biomarkers in tumor-immune interactions between T-DXd responsive (R) and non-responsive (NR) patients with mBC.
We employed Nanostring's GeoMX spatial proteomics and transcriptomics profiling technology to analyze 29 metastatic tissue sections (15R, 14NR) from heavily pre-treated patients with HER2+/low mBC, prior to T-DXd treatment. For each section, 6-8 circular regions of interest (ROIs) with a diameter of 300μm were chosen by researchers blinded to T-DXd R vs. NR outcomes and HER2 scores. ROIs were selected to capture spatially diverse tumor-dense and tumor-sparse regions. Immune markers, stromal markers, and HER2 expression levels were quantified for each ROI.
Consistent immune and stromal features related to T-DXd resistance were identified across various metastatic sites, including brain, bone, liver, lymph node, chest wall, and lung. NR tumors were characterized by relative immunosuppression and a disorganized immune response, with consistent upregulation of fibronectin and, surprisingly, granzyme B. Local immune response significantly varied based on HER2 expression. Organ-specific characteristics were also observed:
Bone metastases showed an increased correlation between various immune markers and fibronectin in NR, suggesting that fibronectin may modulate immune infiltration and promote a supportive microenvironment for tumor growth.
In contrast, chest wall metastases showed loss of correlation between various immune markers and smooth muscle actin in NR. This suggests that a remodeled, less dense smooth muscle environment might reduce physical barriers, potentially allowing better immune cell infiltration and activity in responsive tumors.

Furthermore, a subset of these patient samples was selected for spatial whole transcriptomic analysis to gain deeper insights.

PS4-10: TNBC-DX Genomic Test Predicts High pCR in Triple-Negative Breast Cancer with sTILs ≥30% Treated with Neoadjuvant Docetaxel-Carboplatin with/without Pembrolizumab

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Background: Stromal tumor-infiltrating lymphocytes (sTILs) and the novel 15-gene TNBC-DX test, which integrates immune, proliferation, tumor size, and nodal status markers, are predictors of pathologic complete response (pCR) in triple-negative breast cancer (TNBC) treated with neoadjuvant chemotherapy (NACT), with or without pembrolizumab. This study aimed to evaluate the complementary role of TNBC-DX pCR score and sTILs in predicting pCR in patients receiving NACT ± pembrolizumab.
Methods: sTIL quantification and TNBC-DX genomic testing were performed on pretreatment tumor samples from 401 stage I-III TNBC patients undergoing NACT in two clinical trials: MMJ-CAR-2014-01 (NCT01560663; n=292), where patients received 6 cycles of carboplatin plus docetaxel (TCb), and NeoPACT (NCT03639948; n=109), where patients received 6 cycles of TCb + pembrolizumab (TCb+P). sTILs were centrally evaluated. Pre-established cut-offs defined TNBC-DX pCR-low, -medium, and -high groups. Logistic regression models assessed the association of TNBC-DX and sTILs with pCR.
Results: In the TCb-treated group, the pCR rate was 49%, with both TNBC-DX pCR score and sTILs associated with pCR (OR per 1-unit increase=1.20, p<0.001 for TNBC-DX; and OR per 1-unit increase=1.01, p<0.001 for sTILs). In the TCb+P group, the pCR rate was 58%, with both TNBC-DX pCR score and sTILs as predictors (OR=1.46, p=0.001 for TNBC-DX; OR=1.27, p=0.001 for sTILs). The correlation between TNBC-DX pCR score and sTILs was weak (r=0.26-0.32). The distribution of TNBC-DX pCR-low, medium, and high groups was approximately one-third for each category across both trials. For TCb, pCR rates in TNBC-DX high, medium, and low groups were 66%, 58%, and 25%, respectively (OR=5.81 for high vs. low; p<0.001). For TCb+P, pCR rates in these groups were 78%, 66%, and 33%, respectively (OR=7.25 for high vs. low; p<0.001). In the sTILs ≥30% and 5-29% subgroups, TNBC-DX was significantly associated with pCR. In the sTILs ≥30% subgroup, the pCR rates for TNBC-DX high, medium, and low were 70%, 65%, and 29%, respectively, for TCb (OR=5.80; p=0.010) and 91%, 79%, and 33%, respectively, for TCb+P (OR=21.0; p<0.001). In the sTILs 5-29% subgroup, the pCR rates for TNBC-DX high, medium, and low were 67%, 60%, and 26% for TCb (OR=5.83; p<0.001) and 64%, 56%, and 14% for TCb+P (OR=21.0; p=0.003). In the sTILs <5% subgroup, the pCR rates for TNBC-DX high, medium, and low were 43%, 44%, and 24% for TCb (OR=2.40; p=0.300) and 33%, 29%, and 40% for TCb+P (OR=0.75; p=0.826). Bivariate analyses showed that both sTILs and TNBC-DX were

associated with pCR with TCb+P treatment (sTILs OR=1.23, p=0.005; TNBC-DX OR=1.36, p=0.006), while only TNBC-DX was associated with pCR with TCb treatment (sTILs OR=1.01, p=0.143; TNBC-DX OR=1.18, p<0.001). Overall, tumors with sTILs ≥5% and TNBC-DX pCR med/high scores represented 55-57% of the study population.
Conclusions: The TNBC-DX genomic test effectively predicts pCR to neoadjuvant TCb ± pembrolizumab in TNBC. Notably, the TNBC-DX pCR-high group identifies patients with high sTILs who have a pCR rate exceeding 90% when treated with TCb+P. This study confirms that gene expression and sTILs provide distinct and potentially complementary information for pCR prediction in TNBC.

PS5-01: The association between pembolizumab and risk of venous thromboembolism in patients with breast cancer

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Background: Patients with cancer are at an increased risk of venous thromboembolism (VTE) due to the underlying cancer biology or chemotherapeutic treatment. The use of immune checkpoint inhibitors (ICIs) further increases the risk of thrombosis. Pembrolizumab has recently been approved for breast cancer treatment, but data on its impact on VTE are limited. We aimed to investigate the incidence and risk of VTE associated with pembrolizumab use in breast cancer patients.
Methods: We performed a retrospective, propensity score-matched cohort study using the TriNetX Analytics Network database, which includes de-identified electronic health records from over 70 healthcare organizations and 101 million individuals. We included adult female breast cancer patients treated with pembrolizumab from November 2020 to June 2023. We excluded patients who received endocrine or HER2-targeted therapies. We further excluded patients with a prior history of VTE, atrial fibrillation, and those who received prior therapeutic anticoagulation. The comparator cohort comprised patients who received pembrolizumab and chemotherapy while the control cohort comprised patients who received chemotherapy only. The primary outcome was VTE, which was defined as a composite of pulmonary embolism (PE) and deep venous thrombosis (DVT). The secondary outcomes included individual components of PE and DVT. All outcomes were captured within 1 year following the start of ICI therapy.
Results: We identified 11,620 eligible patients, of whom 1,832 received pembrolizumab and chemotherapy, and 9,788 received chemotherapy only. After propensity score matching, 1,322 patients in each cohort were well balanced for demographics, breast cancer-directed therapy, underlying comorbidities, and medication use. The mean age for the pembrolizumab-chemotherapy and chemotherapy-only cohorts were 55.5 ± 13.8 and 55.6 ± 13.9 years, respectively. Both groups received similar cancer treatments, including mastectomy (both 6.4%) and radiation treatment (7.6% vs. 6.6%). The chemotherapy regimen between the two groups were similarly distributed, including taxanes (71.7% vs. 71.9%), platinum compounds (67.9% vs. 69.0%), anthracyclines (21.6% vs. 23.9%), and

cyclophosphamide (21.3% vs. 23.3%). Over a median follow-up of 12 months, the incidence of VTE was higher in the pembrolizumab-chemotherapy cohort (4.6 events per 100 patient-years) compared to the chemotherapy-only cohort (2.6 events per 100 patient-years). Patients who received pembrolizumab and chemotherapy had a 72% higher risk of VTE (Hazard ratio (HR), 1.72 [95% CI: 1.13-2.60]) than those who received chemotherapy only. In terms of individual VTE outcomes, the incidence of PE and DVT was higher in the pembrolizumab-chemotherapy cohort (2.7 and 2.4 events per 100 patient-years) compared to the chemotherapy-only cohort (1.3 and 1.7 events per 100 patient-years). Patients who received pembrolizumab and chemotherapy had a higher propensity to develop PE (HR, 2.10 [95% CI: 1.17-3.71]) and a higher trend towards DVT (HR, 1.41 [95% CI: 0.83-2.40]) than those who received chemotherapy only.
Conclusion: The use of pembrolizumab is associated with a higher risk of VTE among breast cancer patients. Further studies are needed to stratify patients at higher risk of developing ICI-associated VTE in this population.

PS5-02: The incidence and risk of cardiovascular events associated with pembrolizumab in patients with breast cancer

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Background: Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment. However, ICIs have been linked to serious immune-related cardiovascular adverse events. Pembrolizumab has recently been approved for breast cancer treatment, but data on its impact on immune-related cardiovascular adverse events are limited. We aimed to investigate the incidence and risk of cardiovascular events associated with pembrolizumab use in breast cancer patients.
Methods: We conducted a retrospective, propensity score-matched cohort study using the TriNetX Analytics Network database, which includes de-identified electronic health records from over 70 healthcare organizations and 101 million individuals. We included adult female breast cancer patients treated with pembrolizumab from November 2020 to May 2023. Patients who received endocrine or HER2-targeted therapies were excluded. We compared patients who received pembrolizumab and chemotherapy with those who received chemotherapy alone. The primary outcome was major adverse cardiovascular events (MACE), including myocarditis, pericarditis, myocardial infarction, heart failure, ischemic stroke, and cardiac arrest. Secondary outcomes included individual MACE components and other cardiovascular events such as atrial fibrillation and conduction disorders. All outcomes were captured within one year following the start of ICI therapy.
Results: We identified 9,913 eligible patients, of whom 1,834 received pembrolizumab and chemotherapy, and 8,079 received chemotherapy alone. After propensity score matching, 1,294 patients in each cohort were well balanced for demographics, cancer therapy, comorbidities, and cardiovascular medication use. The mean age for the pembrolizumab-chemotherapy and chemotherapy-only cohorts were 54.7 ± 13.4 and 54.7 ± 13.2 years,

respectively. The background prevalence for hypertension (24.4% vs 23.8%) and hyperlipidemia (19.3% vs 18.0%) were similar between the two groups. Both groups received similar cancer treatments, including mastectomy (5.0% vs. 5.3%), radiation treatment (6.3% vs. 6.2%), taxanes (73.6% vs. 72.5%), platinum compounds (70.4% vs. 69.7%), anthracyclines (22.2% vs. 23.7%), and cyclophosphamide (21.6% vs. 23.2%). Over a median follow-up of 12 months, the incidence of MACE was higher in the pembrolizumab-chemotherapy cohort (9.9 events per 100 patient-years) compared to the chemotherapy-only cohort (4.5 events per 100 patient-years). The pembrolizumab-chemotherapy cohort had an approximately 2.2-fold higher risk of MACE (Hazard ratio (HR), 2.18 [95% CI: 1.60-2.98]). There were five cases of myocarditis in the pembrolizumab-chemotherapy cohort, while no cases were detected in the chemotherapy-only cohort. Furthermore, pembrolizumab was associated with a significantly increased risk of heart failure (HR, 3.58 [95% CI: 1.89-6.78]), atrial fibrillation/flutter (HR, 3.78 [95% CI: 1.82-7.87]), conduction disorders (HR, 2.81 [95% CI: 1.37-5.76]), and cardiac arrest (HR, 4.80 [95% CI: 1.05-21.90]). Patients on pembrolizumab also showed a higher tendency to develop pericarditis (HR, 1.80 [95% CI: 0.96-3.37]) and myocardial infarction (HR, 1.64 [95% CI: 0.75-3.58]). Conclusion: The use of pembrolizumab is associated with more than a two-fold increased risk of MACE among breast cancer patients. Further studies are needed to optimize the detection and management of ICI-associated cardiovascular events in this population.

PS5-03: Incidence and Risk Factors of Immune-Related Adverse Events in Early-Stage Breast Cancer Patients: Findings from a Multi-Institutional Study

Alexis LeVee, Saya Jacob, Samantha Fisch, Carolyne Face, Madhuri Chengappa, Saliha Chaudhry, Nikita Baclig, Andrew Soliman, Nora Ruel, Megan Wong, Karen Tsai, Irene Kang, Laura Huppert, Laura Quintal, Michelle Melisko, Melanie Majure, Jo Chien, Melissa Lechner, Dame Idossa, Anne Blaes, Kelly McCann, Hope S. Rugo, Joanne Mortimer

Introduction: With the addition of immune checkpoint inhibitors (ICIs) to the neoadjuvant/adjuvant treatment regimen in early-stage breast cancer, an increasing number of patients (pts) with breast cancer are developing immune-related adverse events (irAE). Identifying risk factors and biomarkers predictive of irAE in pts with early breast cancer is critical to prevent and mitigate irAE and improve ICI outcomes. The aim of this study is to report the incidence and identify predictive biomarkers of irAE in a large cohort of pts with early-stage breast cancer.
Methods: We identified pts with stage I-III breast cancer who received ICI at four academic institutions between 2014-2024. Pts were excluded if currently receiving ICI. Charts were reviewed for any grade irAE during or after ICI therapy. The chi-square and Wilcoxon rank-sum tests were used to compare baseline and treatment characteristics. Uni- and multivariate logistic regression was performed to determine factors predictive of severe (grade ≥3) irAE including age at ICI treatment, menopausal status, breast cancer subtype, race/ethnicity, BMI, menopausal status, smoking history, co-morbidities, and baseline

laboratory values.
Results: Of the 423 pts with early breast cancer included in the analysis, 307 (72.6%) pts developed any grade irAE and 80 (18.9%) pts developed ≥1 grade 3-4 irAE. No deaths (grade 5) due to irAE occurred. Of the 307 pts that developed any grade irAE, 180 (58.6%) pts developed 1 irAE, 97 (31.6%) developed 2 irAE, and 30 (9.8%) developed 3+ irAE. Of the 80 pts that developed severe irAE, 73 (91.3%) pts developed 1 severe irAE, and 7 (8.8%) developed 2 severe irAE. Among the 437 any grade irAE, the most common were thyroiditis (24.3%), rash (23.0%), colitis (14.5%), hepatitis (10.6%), and adrenal insufficiency/hypophysitis (7.6%). Among the 87 severe irAE, the most common were adrenal insufficiency/hypophysitis (20.7%), rash (19.5%), hepatitis (12.6%), colitis (11.5%), and autoimmune diabetes mellitus (8.0%). Patients with irAE received fewer cycles of ICI compared to patients without irAE, with a median number of ICI cycles of 7 (IQR 4-15) and 11 (4-17), respectively (p=0.006). Similarly, patients with severe irAE also received fewer ICI cycles compared to patients without severe irAE, with a median number of ICI cycles of 4 (IQR 3-9) and 9 (4-17), respectively (p<0.0001). Multivariate logistic regression demonstrated that older age (age ≥50y vs. age <50y) [OR 1.69; 95% CI, 1.00-2.86; p=0.05] and the presence of CKD (OR 4.93; 95% CI, 1.59-15.3; p=0.006) were predictive of severe irAE.
Conclusion: In this cohort of pts with early-stage breast cancer treated with ICI, the incidence of any and severe irAE was 72.6% and 18.9%, respectively. Pts were more likely to develop severe irAE if they were older and had CKD. Further studies to identify risk factors and biomarkers of irAE in pts with early breast cancer are needed to prevent irAE and reduce the associated long-term complications.

PS5-04: Anti-RANKL bone resorptive therapy increases immune-related adverse events (irAE) in breast cancer patients (pts) treated with pembrolizumab

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Introduction: Immune-related adverse events (irAE) occur in approximately 33.5% patients (pts) with early TNBC and 26.5% of pts with advanced TNBC treated with pembrolizumab plus chemotherapy. Severe irAE (grade 3+) occur in approximately 5.3-12.9% of pts, which often lead to serious symptoms resulting in hospitalization, treatment discontinuation, organ dysfunction, and/or death. Bone-modifying agents (BMAs) are frequently used in early breast cancer to treat osteoporosis and to decrease disease recurrence in postmenopausal women, while advanced breast cancer pts with bone metastases are treated with BMAs to reduce skeletal-related events. Preclinical studies demonstrate that anti-RANKL therapy can have immunomodulatory effects through dysregulation of T cell negative selection and disruption of Treg function. We recently showed that pre-treatment with anti-RANKL therapy prior to receiving an immune checkpoint inhibitor (ICI) significantly increased the rate of endocrine irAE in a mouse model. Here, we report the

incidence of severe irAE in a cohort of breast cancer pts treated with pembrolizumab in combination with BMAs.
Methods: We identified female pts with breast cancer who started treatment with pembrolizumab between December 2017 to January 2024. Pts were categorized according to BMA received: zoledronic acid (ZA), denosumab (Dmab), or none (no BMA). BMA therapy was considered received within a dose interval prior to ICI (12 months for ZA; 6 months for Dmab), during ICI therapy, or within 1 month of last ICI cycle. Pts who received both ZA and Dmab were excluded. Severe irAE data according to CTCAE v5.0 were collected. Clinical characteristics and outcomes were compared using chi-square or Kruskal-Wallis tests among pts who received ZA, Dmab, and none.
Results: Our cohort consisted of 430 female breast cancer pts treated with pembrolizumab with 56 (13%) pts who received Dmab, 31 (7.2%) who received ZA, and 343 (79.8%) who received no BMA. A total of 148 (34.4%) pts had early-stage breast cancer, while 282 (65.6%) had late-stage disease. The median number of pembrolizumab cycles received was 8 (IQR 4-15), and the median duration of ICI therapy was 6.9 months (IQR 2.8-12.2). After a median follow-up of 19.4 months (95% confidence interval, 17.5-20.8), the incidence of severe irAE was 54/430 (12.6%) overall. Severe irAE occurred in 12/56 (21.4%) pts who received Dmab plus pembrolizumab, compared to 3/31 (9.7%) pts who received ZA plus pembrolizumab and 39/343 (11.4%) pts who received pembrolizumab alone. The median time from pembrolizumab initiation to onset of irAE was 2.3 months (IQR 1.1-6.9), which was not significantly different among the three groups (p=0.8). Of the 54 pts who developed severe irAE, the most common irAE included hepatitis (24%), pneumonitis (16.7%), colitis (13.0%), rash (13.0%), and hypophysitis (7.5%).
Conclusion: In breast cancer pts treated with pembrolizumab, Dmab had the highest rates of severe irAE compared to those who received ZA and those who received neither BMA. This study is consistent with higher rates of irAE seen in melanoma and lung cancer pts and in preclinical studies that combined ICI and anti-RANKL therapy. Given the expanding indication of ICIs in breast cancer treatment, further studies are needed to validate these results and to identify mechanisms to optimize the antitumor response while mitigating irAE.

PS5-05: Late-onset immune toxicity incidence & risk factors in breast cancer: a multi-institutional study

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Background: Immune checkpoint inhibitors (ICI) have improved outcomes in patients (pts) with breast cancer. Late-onset immune-related adverse events (irAE) have been observed. Prior analyses of late-onset irAE have focused on pts with non-breast cancers as well as breast cancer within a single institution. However, the incidence of late onset irAE in larger

groups of pts with breast cancer is unknown.

Methods: We identified pts with stage I-IV breast cancer receiving ICI at four institutions between 2014-2024. Delayed irAE were defined as occurring >90 days after ICI start, post-treatment irAE were defined as occurring >60 days after ICI discontinuation, & early toxicity was defined as an on-treatment irAE occurring <90 days after ICI start. Events were categorized as irAE if provider documentation indicated that the toxicity was related to ICI. Logistic regression was performed to determine predictors of delayed & post-treatment irAE.

Results: 700 pts with breast cancer, 424 metastatic & 276 early stage, 539 (80%) with triple negative disease, underwent treatment with ICI of which 434 (62%) developed irAE at any time point, 240 (34%) developed delayed irAE (177 early stage (ES), 62 metastatic), 60 (9%) had post-treatment irAE (46 ES, 14 metastatic) & 257 (37%) had an early toxicity. 67 pts (28%) with delayed & 20 pts (33%) with post-treatment also had early irAE. All pts with a post-treatment toxicity also had a delayed toxicity.[HR1] [JS2] The median number of ICI cycles for delayed & post-treatment groups was 10 & 5. Of the pts with early toxicity, 91 (35%) discontinued ICI < 90 days after start. In the delayed & post-treatment groups respectively, 200 (83%) & 49 (82%) pts had triple negative disease (similar to the overall population at 539 (80%)). Median time to onset of delayed toxicity & post-treatment toxicity was 205 days from start of ICI (range 90-1961) & 116 days from end of ICI (60-1144), respectively. The most frequent toxicities in both groups was thyroiditis (93; 39% & 18; 30% in the delayed & post-treatment groups), colitis (41; 17% & 11; 18%), dermatitis (46; 19% & 5; 8%), & adrenal insufficiency/hypophysitis (36; 15% & 13; 22%). The most common grade ≥3 irAE was adrenal insufficiency/hypophysitis (17; 7% & 7; 12%)[HR3] [SJ4] . Pts with metastatic disease were less likely to develop delayed toxicity (Odds ratio {OR} 0.48, p=0.0002) & post-treatment toxicity (OR 0.40, p=0.0008). Logistic regression analyses of potential risk factors one at a time, adjusted for metastatic disease & treatment site, demonstrated that pts were more likely to develop delayed toxicity with increasing cycles of ICI (OR 1.08, p<0.0001) & less likely to develop delayed toxicity if they had early toxicity (OR 0.44, p<0.0001) & HR+ disease (OR 0.58, p=0.0217). The lower likelihood of developing delayed toxicity after an early toxicity remained true even after adjusting for those patients that discontinued ICI due to early toxicity (OR 0.53, p=0.0008). When multiple factors including baseline labs, BMI, age, subtype, early toxicity & number of cycles are evaluated for their adjusted effects after accounting for the effects of the rest of factors & metastatic disease & treatment site, pts were more likely to develop delayed toxicity if they had increased baseline eosinophil count (OR 3.46, p=0.0484) or increased number of ICI cycles (OR 1.10, p<0.0001 in metastatic setting, OR 1.04, p=0.0269 in early-stage setting) & less likely if they experienced early toxicity (OR 0.46, p=0.0001).

Conclusions: Immune toxicities occurred in 62% of patients with breast cancer treated with ICI. Delayed & post-treatment toxicity is common, occurring in 34% & 9% of pts in this dataset respectively, in particular thyroiditis. Early-stage disease, higher baseline eosinophil

count, higher number of cycles of ICI & lack of early toxicity were risk factors for development of delayed toxicity.

PS5-06: Osteonecrosis of the jaw (ONJ) in patients with metastatic breast cancer treated with denosumab in a randomized phase III trial comparing 4 vs. 12 weekly administration (REDUSE, SAKK 96/12)

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Background: While reducing skeletal-related events in patients with bone metastases, bone-modifying agents such as bisphosphonates and denosumab have adverse events. One major adverse event of these agents is osteonecrosis of the jaw (ONJ), which often significantly impacts quality of life. The risk of ONJ increases with treatment duration and can reach cumulative rates of up to 10%. Here we report ONJ rates in a randomized phase III non-inferiority trial investigating the optimal dose schedule of denosumab (DN).
Methods: Patients with bone metastases from breast cancer (mBC) were randomized 1:1 to receive DN every 4 weeks (q4w) (arm A, standard schedule) versus every 12 weeks (q12w) (arm B, experimental schedule) after a 3-month induction phase with q4w therapy for both arms. Incidence of ONJ is a secondary endpoint of the study, while the primary endpoint is time to first symptomatic skeletal event (SSE). An oral inspection at baseline as well as before each application of DN was mandatory. In patients with risk factors for ONJ, a prophylactic dentist visit was recommended. Data from patients who received at least one dose of DN and who were randomized at least one year before data cut-off (December 11, 2023) were included in this interim safety analysis. Since the differentiation between ONJ and tooth abscess (the term according to CTCAE v5.0 is tooth infection) can be difficult, we report these two outcomes separately as well as combined.
Results: 725 patients from 50 centers with a median follow-up time of 3.4 years were evaluated for ONJ. The median number of administered DN-doses was 25 doses in arm A and 11 doses in arm B, the treatment duration was 108 weeks in arm A versus 99 weeks in arm B. During the 3-month induction phase (in which both arms had the same q4w DN-schedule) 1/725 (0.1%) patients experienced an ONJ. After this phase, when the schedules of DN begin to differ 27/333 (8.1%) patients in arm A and 17/328 (5.2%) in arm B experienced an ONJ. For tooth infections (tooth abscess) the numbers during the induction phase were 3/725 (0.4%), then after induction 30/333 (9.0%) for arm A and 15/328 (4.6%) for arm B. For the combined endpoint ONJ and/or tooth infection (tooth abscess) the numbers during the induction phase were 4/725 (0.5%), after induction 50/333 (15.0%) for arm A and 29/328 (8.8%) for arm B.Time to first ONJ differs with an advantage for the 3-month arm (HR 0.66; 95% CI 0.36 - 1.2). For the combined endpoint time to first ONJ and/or tooth infection the difference is even more pronounced (HR 0.63; 95% CI 0.4 - 0.98).

The median time to either event has not been reached for both endpoints.
Conclusions: The observed ONJ rate of 8.1% in the standard arm is in line with the literature for patients who received DN q4w for more than two years (6.0% to 9.8%). Administration of DN q12w reduces the risk of ONJ and/or tooth infections substantially. This risk reduction is clinically relevant with an absolute difference of 6.2% for ONJ and/or tooth infection after a median follow-up of 3.4 years. This suggests that DN given q12w has a more favorable long-term safety profile in terms of ONJ and/or tooth infection compared to DN q4w.Efficacy data for time to first symptomatic skeletal event (SSE) (the primary endpoint of this trial) is not yet mature and will be reported later.
Clinical trial information: NCT02051218Sponsor: SAKK (Swiss Group for Clinical Cancer Research)Funding: Santesuisse; Swiss State Secretariat for Education, Research and Innovation SERI; Helsana; CSS; Sutter-Söttner Stiftung and Swiss Cancer Foundation

PS5-07: Financial difficulty over time in young adults with breast cancer

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Introduction: Although young adults (YA) aged 18-39 represent the minority of breast cancer diagnoses, they are particularly vulnerable to financial hardship. Factors contributing to sustained financial hardship are incompletely understood. Arm morbidity, one such understudied factor and key source of expense, may be particularly salient for YAs given that a high proportion of this demographic presents with aggressive tumor subtypes requiring comprehensive axillary management (a known risk factor for treatment-related lymphatic injury). In this study, we leverage a multi-institutional prospective cohort of YAs to identify patterns of financial hardship over time and characterize factors associated with discrete trajectories hypothesizing that treatment-related arm morbidity would be among the factors predicting long-term financial difficulty.

Methods: This analysis utilized data from women ≤ 40 years with newly diagnosed stage 0 to III breast cancer enrolled in The Young Women's Breast Cancer Study (YWS), a multi-institutional prospective cohort study enrolling from 2006 and 2016 at Dana-Farber Cancer Institute and 12 other academic and community hospitals in the United States and Canada. Patient, disease, and treatment information was obtained from surveys serially collected through 10 years post-diagnosis. Arm morbidity was assessed by asking patients about the degree to which they experienced upper extremity swelling and/or functional limitations using two Likert-scale response items (range: 0-4). Medical record review was used to gather supplemental clinical data. The primary outcome of interest, perceived financial difficulty, was assessed serially using a single Likert-scale response item (range: 0-4) from the CAncer Rehabilitation Evaluation System (CARES) scale. Group-based trajectory modeling classified patterns of financial difficulty from baseline through 10 years post-diagnosis. Multinomial logistic regression identified patient, disease, and treatment

characteristics associated with each trajectory.

Results: 1008 (78%) of 1297 participants were included. Median age at diagnosis was 36 years (IQR 33-39). The majority of individuals were non-Hispanic (95%), White (88%), college graduates (83%), partnered at baseline (76%), parous (64%), and without comorbidities at enrollment (90%). Patients' tumors were primarily stage I-II (86%), ER/PR-positive (75%), and HER2-negative (68%). Patients were more frequently treated with mastectomy than breast conservation ($p<0.001$). Receipt of radiation (62%), chemotherapy (75%), and endocrine therapy (63%) were common. 72% (N=727) of patients reported arm symptoms within 2 years of surgery. Three distinct financial trajectories emerged: 54% had low financial difficulty (Trajectory 1), 30% had mild difficulty that improved (Trajectory 2), and 17% had moderate/severe difficulty peaking several years after diagnosis before improving (Trajectory 3). BMI ≥ 25, undergoing bilateral mastectomy, Hispanic ethnicity, being unemployed at both baseline and 1 year, and arm symptoms were predictive of Trajectory 2 and/or 3 (more financial difficulty). Having a college degree or being partnered were predictive of Trajectory 1 (low financial difficulty). CONCLUSION: This study of YAs with breast cancer identified a subset of patients who experienced a high degree of financial difficulty that persisted into early survivorship before it improved. Targeted interventions to mitigate financial toxicity, including those focused on modifiable factors such as arm symptoms and employability/return to work after cancer, are needed.

PS5-08: Effects of Cryotherapy on Objective and Subjective Symptoms of Taxane Induced Neuropathy in Patients with Early Breast Cancer: A National, Multicenter, Prospective, Randomized, Controlled Trial.

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Background: Due to well-proven survival benefit taxane-based chemotherapy regimens are first-line treatment in both the adjuvant and neoadjuvant setting of early-stage breast cancer. Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent and disabling side effect from taxane treatment. CIPN is experienced in up to 64% of cancer patients receiving docetaxel and up to 81% receiving paclitaxel. Treatment options are limited. So far, no evidence-based strategy exists for the prevention of CIPN.
We conducted a randomized controlled trial to evaluate the efficacy and safety of cryotherapy as a prevention strategy for CIPN.

Method: Patients with early-stage breast cancer starting treatment with docetaxel or paclitaxel between January 2021 and July 2023 at four oncology departments in Denmark were eligible. Patients were randomized (1:1) to receive either cryotherapy or usual care. Cryotherapy was applied as frozen gloves and socks on all four extremities from 15 min before taxane infusion until 15 min post-infusion at every cycle. Primary endpoint was

incidence of peripheral neuropathy at End of Treatment (EoT); CIPN was assessed using the Total Neuropathy Score (TNS) 3 weeks after completion of the last cycle of docetaxel or paclitaxel and compared to baseline. A TNS score ≥ 2 was considered an event. Secondary end points included level of experienced peripheral neuropathy using Common Terminology Criteria for Adverse Event (CTCAE), patient-reported outcomes (EORTC Quality of Life Questionnaire EORTC (QLQ)-CIPN20) and EORTC (QLQ)-C30) and Quantitative sensory testing (QST).

Results: A total of 268 patients were randomized (cryotherapy 133 patients; usual care 135 patients), of which 51 dropped out before or during taxane treatment, leaving 217 patients for the final analyzes. The cryotherapy group N=123 and the usual care group N=94. There was no significant difference in CIPN assessed with TNS between the cryotherapy and usual care group 3 weeks after taxane treatment ($p = 1.0$). Nor did we find a significant difference in the incidence of CTCAE grade ≥ 2 sensory ($p = 0.2$) or motor peripheral neuropathy ($p = 0.2$). However, when using EORTC QLQ CIPN20 subscales the analyses showed that patients in the cryotherapy group experienced reduced tingling in hands($p=0.0005$) and feet ($p=0.04$), reduced numbness in hands($p=0.02$) and feet ($p=0.00002$) and less trouble opening a jar or bottle due to loss of strength in hands ($p=0.002$) compared with the usual care group. Concerning patient reported QoL or global health status no difference was found ($p=0.5$). QST revealed reduced tactile disturbance development($p=0.006$) but no difference in thermosensory disturbance($p=0.433$) in the cryotherapy arm. No difference in mean dose, dose reductions or premature cessation of the chemotherapy was observed. Data revealed a compliance with cryotherapy of 67% and no serious side effects were associated with cryotherapy.

Conclusions: This is so far the largest randomized controlled trial (n=217) evaluating the preventive effect of cryotherapy on taxane induced CIPN. No significant difference in incidence of CIPN at EoT was seen. However, an important benefit of cryotherapy was observed as patients reported significantly fewer sensory symptoms. Furthermore, a better result from monofilament testing of tactile sensitivity was seen in the cryotherapy treated group. Cryotherapy did not influence the administered dose of the cytotoxic drug. The benefits of cryotherapy regarding sensory symptoms will be further investigated in the present study as long-term follow-up are planned.
Clinical Trial Registration: ClinicalTrials.gov: NCT05928429

PS5-09: Feasibility of an Interactive Care Plan for Self-Management of Toxicity Symptoms and Surveillance for Non-Metastatic Disease in Breast Cancer Survivors

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Purpose: To assess the feasibility of an app-based interactive care plan (ICP), a self-management and surveillance tool, in breast cancer (BC) survivors, after completion of active medical treatments.

Methods: A single arm feasibility trial of patients who recently completed active BC treatments was conducted. ICP tasks were delivered for 12 months, and included: (1) daily "Be active at least 20 minutes today" activity reminders; (2) monthly assessments of four treatment-related toxicity symptoms: fatigue, insomnia, sexual dysfunction, and hot flashes (Likert scale 0-10); (3) monthly surveillance questionnaires assessing for symptoms of recurrence; and (4) quarterly quality of life (QOL) PROMIS 29 s (scored on a T-score metric with 50 being the mean of a general US adult reference population, 10 is the standard deviation and higher scores denote more of the tested outcome). On-demand education was available for the four self-reported toxicity symptoms. Positive answers to recurrence assessments and high anxiety and depression subscale scores of the PROMIS questionnaires escalated through decision trees embedded in the ICP to nurses who contacted the patient. The primary outcome was feasibility defined as 75% ICP tasks completion rate and <1 escalation/participant. Secondary outcomes were changes in toxicity symptom burden and in QOL. PROMIS scores were assessed between baseline and 6-months using paired t-tests tests.

Results: 101 patients were enrolled between August 2020 and May 2022, and the intervention was completed on May 2023. Three withdrew during the study period. Mean participant age was 56 years (range 30-78) and 97% were white. Of the daily "Be active" reminders, a median of 26.6% were checked (53.3% at month 1 decreasing to 6.7% at month 12). Similarly, 53.8% of the monthly toxicity and surveillance symptoms questionnaires were answered (84.7% at baseline to 6.1% at month 12) and 40.0% of the quarterly QOL questionnaires were answered (66.3% at baseline and 18.4% at month 12). The care team received 251 messages over the 1 year intervention, of which 90 (0.9/participant) were escalations related to symptoms worrisome for recurrence (68) and to anxiety or depression (22). The remainder 161 messages were related to restarting the ICP after dismissal from a hospital admission (52) and the need to discontinue the ICP at the completion of the intervention (106).
Among the treatment-related symptoms assessed at baseline, hot flashes was most frequently endorsed (48.2%), followed by fatigue (34.9%), insomnia (22.9%), and sexual dysfunction (13.3%). At baseline, 34.9% of participants noted treatment-related fatigue (average intensity of 4.6 on a 0-10 point scale), and this endorsement was near 30% for the first few months before dropping gradually. Among those reporting fatigue at baseline, 54% of them requested additional information to help better understand and manage the symptom. Similar trends were observed for the other treatment related symptoms.
At baseline, the PROMIS T-scores were generally near the population average of 50. Comparing baseline to month 6 among 24 participants with complete data at both time points, the average physical function T-score increased from 47.9 to 50.5 ($p=0.03$),

depression T-score decreased from 49.3 to 46.0 (p=0.006), and social roles/activities T-score increased from 53.7 to 56.3 (p=0.02).

Conclusion: Implementation of an app-based ICP in BC survivors was feasible although engagement decreased over time. The impact of escalation messages to the care team was minimal, therefore clinical implementation of the ICP is acceptable. Findings were difficult to compare over time due to a drop in response rate for the monthly surveys. Further improvement of the ICP with patient input is needed, to increase engagement and interest.

PS6-01: The PARP inhibitor rucaparib is a strong radiosensitizer in women with residual triple-negative breast cancer treated concurrently with adjuvant radiotherapy

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Purpose/objectives: Women with triple-negative breast cancer (TNBC) who have residual disease after neoadjuvant chemotherapy (NAC) have worse outcomes, including higher local-regional failure. ~50% of TNBCs have a high rate of homologous recombination repair defects (HRD). HRD cells are uniquely sensitive to PARP inhibitors (PARPi) through synthetic lethality. Radiation therapy (RT) also induces DNA lesions requiring PARP for repair. We hypothesized that the combination of RT and PARPi could improve outcomes. Materials/Methods: From June 2018 - June 2022, we enrolled 30 evaluable patients to a phase 1 trial of concomitant rucaparib with adjuvant RT using a TITE-CRM design with a target DLT rate of 30%. RT was delivered to 50 Gy in 25 fractions with a mandatory 10 Gy boost in lumpectomy cases (optional in mastectomy). Oral rucaparib was started at a dose of 400 mg bid, with 2 escalations to the target dose of 600 mg bid and one de-escalation to 300 mg bid. The drug was taken concurrently during RT and then for 4-5 weeks after RT for a total of 10 weeks. Non-hematologic DLTs included moist desquamation exceeding 25 cm2, delay of radiotherapy exceeding 5 business days, or any other grade 3 non-hematologic toxicity by CTCAE v4.03. The primary objective was to identify the maximum tolerated dose (MTD) of concurrent rucaparib and RT. Plasma was collected at baseline (pre-RT), during- (week 3), and post-RT (week 10), to evaluate circulating tumor DNA (ctDNA) using a personalized assay targeting multiple tumor-specific variants (RaDaR). Results: Median age of enrolled patients was 50 (range 31-75). Median clinical tumor size before NAC was 5 cm (range 1.3-8) and 19 patients were clinically node positive. Fourteen women had lumpectomy while the remainder had mastectomies, of which all but 5 had reconstructions. Two patients were enrolled at 400 mg bid of which one had a DLT (skin). Five patients were enrolled at a dose of 300 mg bid of which 3 had a DLT (2 skin and one neutropenia). Based on our observed DLTs, we amended the study to introduce lower dose levels: 200 mg bid, 300 mg qd and 200 mg qd. Three patients were enrolled at the

lowest dose of 200 mg qd without DLT. Three additional patients were enrolled at 300 mg qd with one DLT (skin). The dose was escalated to 200 mg bid in two patients, with one DLT (neutropenia). The dose was again reduced to 200 mg qd for 3 patients without DLT. The final 12 patients were treated at a dose of 300 mg qd, with 2 DLTs observed (skin), and this was declared to be MTD. Two patients progressed during the study period. With a median follow-up of 31 months, there were 2 local-regional recurrences (LRR, 1 isolated) and 13 distant metastases. 6/14 lumpectomy patients had fair or poor physician rated cosmesis (43%). For the 26 patients with at least one plasma collection for ctDNA, 22 had available tumor tissue for sequencing and 19 had a successful custom ctDNA panel created and used on plasma samples from at least one time point. 18 had a baseline result, 13 had on-RT, and 9 had at least one post-RT result at any time. Four of the 9 patients with a post-RT sample had ctDNA detected, and all four recurred. Lead times were 48, 91, 207, and 427 days. The other 5 patients were ctDNA negative and did not recur, indicating 100% accuracy. Of note, ctDNA detection at any post-surgery timepoint, regardless of timing relative to RT, was associated with recurrence (7/7 patients recurred). Two of 12 patients without ctDNA detected developed a clinical relapse, though sampling was limited in one.
Conclusions: When combined with adjuvant breast/chest wall radiotherapy, the MTD of rucaparib was 300 mg daily, corresponding to one-fourth of the approved monotherapy dose. The majority of DLTs seen were skin desquamation events (6/8 DLTs). We conclude that rucaparib is a very potent radiosensitizer, possibly leading to improved LRR, but poorer cosmesis in BCT patients. ctDNA detection associated with ultimate recurrence, but numbers were small and validation is required.

PS6-02: Association of VMAT versus 3D-CRT Radiotherapy Treatment Technique with Acute Toxicity of Regional Nodal Irradiation: A Secondary Analysis of the SAPHIRe Phase III Randomized Clinical Trial

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Background: Regional nodal irradiation (RNI) improves breast cancer survival but is associated with treatment-related toxicity. Volumetric Modulated Arc Therapy (VMAT)/Intensity Modulated Radiation Therapy (IMRT) treatment technique has been shown in other disease sites to improve dose homogeneity while reducing side effects compared to 3-Dimensional Conformal Radiation Therapy (3D-CRT). To evaluate the association of radiotherapy (RT) treatment technique with acute toxicity for patients receiving RNI, we performed a secondary analysis of the Shortening Adjuvant Photon Irradiation to Reduce Edema (SAPHIRe) trial, a Phase III trial evaluating conventional (CFx) vs. hypofractionation (HFx). We hypothesized that VMAT technique would be associated with reduced acute toxicity compared to 3D-CRT.

Methods: Patients with clinical or pathologic T0-3 N0-2a/3a invasive breast cancer dispositioned to receive comprehensive RNI were randomized to CFx vs. HFx (50Gy/25Fx or 40.05Gy/15Fx). Nodal target volumes included the axilla, infraclavicular and supraclavicular nodal basins, and internal mammary chain. Acute RT-related toxicity was graded utilizing the NCI CTCAE v4.0 scale at the end of RT. Associations between treatment technique with clinicopathologic and treatment variables, dosimetric data, and toxicity endpoints were determined using the Fisher's Exact, Mann-Whitney U, and Kruskal-Wallis tests. Univariate analysis and multivariable binomial logistic regression were performed to calculate adjusted odds ratios (OR) for factors associated with Grade 2+ toxicity at the end of RT.
Results: A total of 645 patients with available RT variables and end of RT toxicity assessments were enrolled from 2017-2024 (median follow-up, 20 months [IQR, 7-35]). Patients treated with VMAT technique were balanced across randomization arm (CFx vs HFx) as well as clinicopathologic and treatment variables but had significantly higher body mass index (BMI) (30 [25-34] vs. 28 [24-33], p=0.004) and were more likely to undergo plastic surgery reconstruction (40% vs. 21%, p<0.001).
Patients treated with VMAT technique experienced significantly reduced Grade 2+ toxicity at the end of RT treatment compared to 3D-CRT (38% vs. 51%, p=0.002), including Grade 2+ dermatitis (32% vs. 47%, p<0.001), Grade 1+ fatigue (50% vs. 60%, P=0.03), Grade 1+ pruritus (40% vs. 49%, p=0.02), and Grade 1+ breast edema (0.4% vs. 3.9%, p=0.02). VMAT technique was associated with significantly reduced volume of the body receiving ≥105% (V105%) of the prescription dose (72cc vs. 351cc), V107% (2cc vs. 186cc), and V110% (0cc vs. 77cc; all p<0.001), as well as the maximum percentage dose (Dmax) to the nodes (106% vs 120%, p<0.001). 3D-CRT technique was associated with significantly increased dose to the ipsilateral lung (V20Gy [CFx]/V16Gy [HFx]>35% = 12% vs. 1%, p<0.001) as well as mean heart dose (MHD>4Gy [CFx]/3.2Gy [HFx] = 6% vs. 1%, p=0.007). V105% to the body and Dmax to the nodes were significantly associated with increased rates of acute dermatitis (p=0.004 and p=0.01, respectively) and breast edema (p=0.02 and p=0.005, respectively) while V107% was associated with significantly increased fatigue (p=0.02). On multivariable analysis, increased BMI (OR [95% CI]=1.04 [1.00-1.07], p=0.03) was significantly associated with increased rates of Grade 2+ toxicity at the end of RT while hypofractionation (OR=0.28 [0.19-0.42], p<0.001), VMAT treatment technique (OR=0.38 [0.21-0.68], p=0.001), and absence of boost (OR=0.38 [0.15-0.87], p=0.03) were associated with significantly decreased rates of Grade 2+ toxicity at the End of RT.
Conclusion: In this secondary analysis of a prospective randomized clinical trial, patients treated with RNI utilizing VMAT technique compared with 3D-CRT experienced significantly decreased rates of acute treatment-related toxicity, including any Grade 2+ toxicity, in the setting of improved dose homogeneity.

PS6-03: Post-Mastectomy Proton Therapy Imparts Increased Risk of Capsular Contracture in Reconstructed Breast Cancer Patients

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Background: The rising utilization of post-mastectomy proton therapy (PMPT) in the setting of breast reconstruction has raised concerns about increased complication rates. There is some limited data indicating PMPT may increase the risk of capsular contracture (CC) when compared with photon therapy. We examined the capsular contracture rate in the largest number of PMPT patients in the literature to date. This study aims to evaluate capsular contracture rates following PMPT and assess the relationship between CC and patient demographics and treatment characteristics.
Methods: An IRB approved retrospective study was conducted on breast cancer patients who underwent two stage tissue expander/implant (TE/I) or direct-to-implant (DTI) breast reconstruction and received PMPT between January 2018 and January 2023 at the Miami Cancer Institute, Baptist Health South Florida. Descriptive statistics were used to analyze patient demographics and treatment characteristics. Binary logistic regression was employed to assess the impact of patient and treatment factors on CC development. The Kaplan-Meier method was utilized to evaluate time to CC development.
Results: The study cohort comprised 89 patients with 89 reconstructed breasts. The median age was 50 years (24-78). Patient characteristics included 67.4% Hispanic, 10.1% African American, and 68.5% left sided breast cancer. 56.2% underwent total mastectomy, 32.6% had nipple sparing mastectomy, and 11.2% had skin sparing mastectomy. 59.5% of patients had TE at time of PMPT and 40.5% had permanent implant. Tumor characteristics were 89.9% invasive ductal carcinoma, 64% hormone receptor (HR) positive HER2 negative, 15.7% HR positive HER2 positive, 11.2% HR negative HER2 positive, and 9% triple negative. The majority of patients (88.8%) received 50.4 Gy/28 fractions of PMPT (45-56 Gy/25-28 fractions), and 62.9% received neoadjuvant chemotherapy. CC was observed in 51.7% of patients (46 patients), with a mean time to development of 15 months (0.4-152) post-PMPT. Of these 46 patients, 34 underwent surgical intervention, although all were advised to do so. Acute toxicity was observed in 81% of patients, with 62.9% developed grade 2 dermatitis and 5.6% developed grade 3 dermatitis. Logistic regression analysis revealed that DTI significantly increased CC rates compared to TE (OR 3.5, 95% confidence interval 1.4-8.5, p=0.007). No other patient factors were significantly associated with CC development. The mean follow-up was 36 months. At the time of last follow-up, 95.5% of patients were alive and 3.7% have experienced locoregional tumor recurrence.
Conclusion: This study demonstrates that breast cancer patients who underwent TE or DTI and received PMPT had an increased risk of CC when compared with photons in the existing literature (50% vs 20%). DTI was associated with a significantly higher risk of CC compared to TE. In the context of PMPT, careful consideration of breast reconstruction modalities and patient discussion is crucial with regard to complication rates. We plan to perform a matched case control study to compare proton vs photon PMRT to further elucidate the CC rate difference. We expect prospective clinical trials to illustrate the increased risk of CC.

PS6-04: Eliminating breast surgery for invasive, hormone-positive breast cancers with an exceptional response to endocrine therapy and ablative radiotherapy: a single-arm, phase 2 trial

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Objective(s): To define pathologic response rates to endocrine therapy and ablative radiotherapy, with omission of breast surgery, for early-stage, hormone receptor (HR)+ breast cancer in a prospective, phase II trial (NCT02945579). Methods: Twenty eligible patients with HR+, HER2-, clinical stage I, unicentric, non-lobular breast cancers with no lymphovascular space invasion, Oncotype ≤25 and age ≥50 were accrued to an IRB approved-protocol. Enrolled patients received three months of endocrine therapy followed by restaging ultrasound and ablative radiotherapy, 37.5Gy/5 fractions every other day. MR LINAC was used when feasible. After radiotherapy, patients continued on endocrine therapy and underwent percutaneous vacuum-assisted, image-guided core biopsy (VAIGCB) of the tumor 6-12 months following radiation, with a minimum of 12 9G cores. Near complete response (nCR) was defined as Miller-Payne 4 and pCR as 5. Patients with a pathologic complete response (pCR) were followed every 6 months with imaging; those without a pCR were recommended for standard-of-care surgery. Miller-Payne score was evaluated on core biopsy and surgical specimens. Co-primary endpoints are pCR on VAIGCB and tumor control at 3 years. We report here the former co-primary endpoint of pCR along with the 95% credible interval (CI). Results: 19 of 20 (95%) of patients underwent VAIGCB; 1 declined and elected continued observation. Of the 19 biopsies, 10 (52.6%) demonstrated pCR (Miller-Payne 5), 7 (36.8%) nCR (Miller-Payne 4) and 2 (10.5%) Miller-Payne 3. Of patients who had a VAIGCB 6 months after radiotherapy, 5/11 (45.4%, 95% CI 18.9%-71.5%) had pCR; of those with VAIGCB 12 months after RT, 5/8 (62.5%, 95% CI 27.4%-86.6%) had pCR. 7/9 patients with residual disease (Miller-Payne <5) underwent surgery, one of whom had pCR in the surgical specimen, consistent with complete removal at VAIGCB. There were no postoperative complications. Two patients with nCR declined surgery: one underwent cryoablation and one continued endocrine therapy and underwent repeat biopsy 4 months later with pCR. In total, 17/19 pts who underwent VAIGCB (89.5%) had pCR or nCR. The 1 patient who declined VAIGCB has no residual disease on imaging 2 years after RT. Median follow-up time for all patients who did not have surgery is 26 (range 18 to 38 mo months), with none (0/12) experiencing progression or recurrence. Conclusion: This is the first study to demonstrate a high rate of VAIGCB pCR and nCR following endocrine therapy and ablative radiotherapy for early stage, HR+, HER2- breast cancers. This may be an appealing approach for patients with breast cancer interested in non-surgical approaches to definitively treat their tumors and highlights the efficacy for non-surgical candidates.

PS6-05: First report of clinicopathologic characteristics and surgical outcomes of patients in the Avoid axillary Sentinel Lymph node biopsy After Neoadjuvant chemotherapy (ASLAN) trial (KBCSG-28)

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Background: With advances in neoadjuvant systemic therapy (NST), response to treatment and pathologic complete response (pCR) rates have increased considerably, producing de-escalation of surgery strategies. Multiple trials are ongoing to demonstrate the oncologic safety of omitting breast or axillary surgery. The ASLAN trial (NCT04993625) is a prospective, multicenter, single-arm non-inferiority trial with a target accrual of 178 patients that aims to demonstrate the oncologic safety of omitting axillary surgery in patients with a pathologic complete response in the breast after NST for cT1-3, cN0-1 triple-negative, human epidermal growth factor 2 (HER2)-positive, or low-estrogen receptor (ER) breast cancer. Patients with excellent response to NST on physical examination and radiologic imaging (≤2cm mass on mammogram, breast ultrasound, and MRI or ≤4cm non-mass enhancement on MRI) who are planning to undergo breast-conserving surgery (BCS) were screened for eligibility. After BCS without axillary surgery, patients with a pCR defined as no residual invasive cancer were enrolled in the trial and avoided axillary surgery. Those with residual invasive cancer received additional sentinel lymph node biopsy (SLNB) and/or axillary lymph node dissection (ALND) and were registered into a prospective registry. We aimed to investigate the clinicopathologic characteristics and surgical outcomes of the patients in the ASLAN trial.
Methods: The ASLAN trial screened 254 patients who met the inclusion criteria from September 2021 to December 2023. A total of 245 patients who received BCS were included for analysis. Clinicopathologic variables, including the pCR status of the breast and axillary lymph nodes (LNs), were analyzed.
Results: Most patients had cT2 (217/245, 88.6%) and cN0 (189/245, 77.1%) disease before NST. Among 56 (22.9%) cN1 patients, a fine-needle aspiration was performed on the suspicious LNs in 37 (66.1%) and 24 (42.9%) had LN metastasis. 130 (53.1%) were TNBC, 113 (46.1%) were HER2-positive, and 2 (0.8%) were low-ER. After BCS, a breast pCR was confirmed in 182 (74.3%) patients, two of whom were dropped from enrollment due to refusal of radiation therapy or lost to follow-up resulting in 180 patients with ongoing follow-up. Among 63 (25.7%) patients with a breast non-pCR, SLNB only was performed in 60, SLNB followed by ALND in one, and no axillary surgery in two (patients refused). 95.1% (58/61) of patients who received axillary surgery had no LN metastasis, and two had micrometastasis in one LN. Patients with a non-pCR had more ER-positive disease (p=0.002), lower Ki67 (p=0.025), larger post-NST size on ultrasound (p=0.006), and no difference in the proportion of cN1 patients (p=0.824).
Conclusion: The ASLAN trial completed screening of 245 patients by performing BCS of the breast and enrolled 182 patients who were confirmed to have a pCR on BCS and were omitted axillary surgery. Axillary surgery on cN0-1 patients with excellent radiologic response to NST had LN metastasis in less than 5%. This trial will be the first prospective

trial to determine the oncologic safety of avoiding axillary surgery in exceptional responders to NST. Data lock is expected in December 2028.

PS6-06:Upstage of N-Stage by Diagnostic Axillary Lymph Node Dissection in Patients w/ Isolated Tumor Cells or Micrometastases in Sentinel/Target Lymph Node after Neoadjuvant Chemotherapy - Results from the Prospective Multicenter AXSANA / EUBREAST 3 Study

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Introduction: The role of completion axillary lymph node dissection (cALND) in patients with low volume residual disease in the sentinel (SLN) or target lymph node (TLN) after neoadjuvant chemotherapy (NACT) is a matter of controversial debate. While increasing evidence suggests no therapeutic benefit from cALND, additional lymph node surgery is also performed for diagnostic purposes to identify patients for post-neoadjuvant systemic treatment or regional radiotherapy.
Material and Methods: We analyzed the conversion rate of patients with isolated tumor cells (ITCs) or micrometastases in the SLN or TLN to a higher nodal (N)-stage by cALND from the prospective multicenter AXSANA trial (NCT04373655), which compares different axillary surgical staging procedures (ALND, SLN biopsy (SLNB) and targeted axillary dissection (TAD)) after NACT in patients with initially positive axillary lymph nodes.
Results: 5,328 patients from 291 study sites in 26 countries were included in the AXSANA study between June 2020 and March 3rd, 2024. Of these, 2,193 had completed surgery including SLNB or TAD at the time of analysis. All datasets were monitored. 51 out of 2,193 patients (2.3%) were ypN0(i+)(sln/tln) stage and 153 out of 2,193 (7.0%) were ypN1(mi)(sln/tln). cALND was performed in 16/51 patients (31.4 %) with isolated tumor cells (ITC) and 71/153 (46.4 %) women with micrometastases in the SLN or TLN.
Among 16 patients with a ypN0(i+)(sn/tln) stage who underwent cALND, 3 (18.8 %) had further non-SLN/TLN involvement including 2 with additional ITCs and 1 with macrometastatic lymph node involvement. 8 out of 16 patients (50%) achieved complete response in the breast (breast-pCR). The only macrometastasis was found in a patient with a non-pCR in the breast. Overall, 1 out of 16 patients with ypN0(i+)(sln/tln) stage was upgraded to ypN1 (6.25 %) by cALND. Among patients with a breast-pCR, no patients' N-stage increased to a higher stage.

Among 71 patients with a ypN1(mi)(sln/tln) stage who underwent cALND, 28 women (39.4 %) had further non-SLN/TLN involvement, 1 (1.4 %) had additional ITCs, 17 micrometastatic (23.9 %) and 10 (14.1 %) macrometastatic non-SLN/TLN disease. 18 out of 71 patients in this subgroup (25.4 %) achieved breast-pCR. Of these, 7 patients had additional micro- and 1 patient macrometastatic non-SLN/TLN involvement (38.9 % and 5.6 % respectively). One patient converted to a ypN1-stage (5.6 %) and no patient to a ypN2/3-stage. Among 53 patients without breast-pCR, 10 women had further micrometastatic and 9 patients additional macrometastatic involvement (18.9 % and 17.0 %). Overall, 9 out of 53 patients (17 %) with a ypN1(mi)(sln/tln)-status and non-pCR in the breast were upgraded to a higher ypN-stage (3 to ypN1, 5 to ypN2, and 1 to ypN3) (22.6 %, 15.1 %, and 1.9 %, respectively).
Conclusion: An upgrade of nodal stage in ypN0(i+)(sln/tln) patients is a rare event. cALND for diagnostic purposes is not justified in this group and should be discouraged, especially in patients with a breast-pCR.
Also for patients with a ypN1(mi)(sln/tln)-stage, an upgrade to a higher N-stage is also rare if breast-pCR is achieved. In patients with non-pCR in the breast, cALND is associated with a higher risk for an upgrade of the nodal stage, so that diagnostic cALND may be indicated in selected patients with potential therapeutic implications. Provided that further research confirms no therapeutic benefit from cALND, additional axillary surgery for diagnostic purposes can be omitted in most patients with initially positive lymph nodes and a low tumor burden in the SLN or TLN after NACT.

PS6-07: Clinical and patient reported outcomes in women offered oncoplastic breast conserving surgery as an alternative to mastectomy: 12-month results of the UK ANTHEM multicentre prospective cohort study

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Background: Oncoplastic breast conserving surgery (OPBCS) may be a better option than mastectomy for many women, but high-quality comparative evidence is lacking. The UK ANTHEM study (ISRCTN18238549) aimed to explore the clinical and patient-reported outcomes (PROs) in a multicentre cohort of women offered OPBCS as an alternative to mastectomy +/- immediate breast reconstruction (IBR).
Methods: Women aged >18 with invasive breast cancer or DCIS who were offered OPBCS with either volume displacement (therapeutic mammaplasty, TM) or replacement (chest wall perforator flap, CWPF) techniques to avoid mastectomy were recruited prospectively. Demographic, operative, oncological and 3- and 12-month (m) complication data were collected. The rate of successful breast conservation and the numbers of additional procedures required to achieve clear margins were explored.
Participants completed the validated BREAST-Q at baseline, 3 and 12m post-operatively. Questionnaires were scored according to the developers' instructions and scores compared

across timepoints in each group.
Results: 362 women from 32 UK breast units participated in the study. Of these 294 (81.2%) had OPBCS as their first procedure with the remainder opting for mastectomy with (n=35) or without (n=33) IBR. Women undergoing IBR were significantly younger (p=0.005) with bilateral surgery performed more frequently in the TM and IBR groups (TM n=81, 38.0%; IBR n=9, 25.7% p<0.001).
Of the 255/294 patients opting for OPBCS in whom post-operative margin status was reported, 210/255 (82.4%) had clear margins following their initial operation. Of the remainder, 28 (62.2%) patients achieved clear margins after one additional procedure and 20% (n=10) with two or more additional operations. The overall rate of successful BCS was 95.7% with only 10 women (3.9%) having completion mastectomy +/- IBR. No differences were seen in the final histology, recommendation for adjuvant chemotherapy or time to receipt of adjuvant treatment between the groups. Radiotherapy was more frequently recommended after OPBCS (256/284, 90.1% vs 39/78, 50.0%, p<0.001).
A total of 96 (26.5%) women experienced a post-operative complication at 3 months of whom 22 (6.1%) had a major complication requiring readmission or re-operation. No difference in total complications was observed between groups, but major complications were higher after IBR (IBR=22, (25.0%), OPBCS n=12 (4.9%), mastectomy only n=0, IBR=22, p<0.001).
At least one BREAST-Q scale was completed by 329 (90.9%), 279 (77.1%) and 273 (75.4%) participants at baseline, 3m and 12m respectively. There were clinically meaningful and statistically significant increases in both the 'Satisfaction with Breasts' and 'Psychosocial Well-being' scores from baseline to 3m that were sustained at 12m in the TM group whereas 'Satisfaction with Breasts' decreased from baseline to 3m in the mastectomy only group with no improvement at 12m. Both OPBCS groups reported significant decreases in 'Physical Well-being-Chest' scores from baseline to 3m with further decreases in scores between 3 and 12m in the CWPF group. No other significant changes in any other BREAST-Q scale were seen in any patient group over the study period.
Conclusions: OPBCS successfully allowed over 95% of women to avoid mastectomy with lower major complication rates than IBR and improved patient-reported outcomes, especially in women having therapeutic mammaplasty. OPBCS should be offered as an alternative to mastectomy in all women in whom it is technically feasible.

PS6-08: Quality of life following total mastectomy, breast-conserving surgery, and immediate breast reconstruction in patients with breast cancer: A multicenter cross-sectional study

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Background: The development of various treatment modalities has led to improved prognosis for patients with breast cancer. The diversity of individual values and lifestyles must be considered when deciding on a surgical approach. However, there is a paucity of reports assessing postoperative health-related quality of life (HR-QOL) in these populations from multiple perspectives. Therefore, this study aimed to identify differences in the HR-QOL and the impact on postoperative life for each surgical procedure using patient-reported outcomes in Japanese patients with breast cancer.
Patients and Methods: This multi-institutional cross-sectional study included patients with primary breast cancer who underwent total mastectomy (MT), breast-conserving surgery (BCS), or immediate breast reconstruction (IBR) between August 2013 and July 2021. Data were collected using a questionnaire administered postoperatively between October 2022 and March 2024 to patients who provided consent. BREAST-Q was administered to investigate postoperative HR-QOL and satisfaction. An ad-hoc questionnaire was used to investigate the effects on postoperative life. The BREAST-Q scores and impacts of the three surgical procedures on postoperative life were compared. One-way ANOVA was used to compare the differences in BREAST-Q scores among these groups. Multiple regression analysis was performed to evaluate the relationship between BREAST-Q scores, clinical factors, and social backgrounds. The differences in the impact on postoperative life among these groups were compared using the chi-square test.
Results: The questionnaire response rate was 90.2% (577/640). The analysis included 194 patients with MT, 185 with BCS, and 194 with IBR, with mean ages of 58.1, 54.8, and 48.2 years, respectively ($p < 0.001$). The rates of clinical tumor size (T) ≥ 3 ($p < 0.001$), clinical nodal stage (N) ($p = 0.003$), axillary lymph node dissection ($p < 0.001$), and adjuvant chemotherapy ($p < 0.001$) were significantly higher in the MT group than in the BCS and IBR groups. Additionally, the frequency of Grade ≥ 3 complications was significantly higher in the IBR group than in the MT and BCS groups ($p < 0.001$). Of the 198 patients in the IBR group, implant reconstruction was performed in 72.2% of patients, nipple-sparing mastectomy in 39.9%, and one-stage reconstruction in 21.7%. Significant differences were observed in satisfaction with the breasts (SB), psychosocial well-being (PSW), and sexual well-being (SW) using the BREAST-Q among the groups. The SB scores for the MT, BCS, and IBR groups were 46.7, 66.7, and 60.5, respectively ($p < 0.001$). The PSW scores were 53.3 (MT), 62.9 (BCS), and 61.5 (IBR) ($p < 0.001$). The SW scores were 31.6 (MT), 44.5 (BCS), and 41.3 (IBR) ($p < 0.001$). Multiple regression analysis indicated that procedures (SB: MT vs. BCS or IBR, $p < 0.001$; PSW: MT vs. BCS, $p = 0.001$; MT vs. IBR, $p = 0.027$; SW: MT vs. BCS, $p = 0.005$; MT vs. IBR, $p = 0.041$) and age (SB, $p = 0.006$; PSW, $p < 0.001$; SW, $p = 0.016$) were significant factors affecting the patients' quality of life. When comparing the impact on postoperative life between procedures, the proportion of women who did not experience any negative impact on their postoperative lives was the highest in the BCS group ($p < 0.001$), whereas the proportion of women who developed clothing issues was the highest in the MT group.
Conclusions: Surgical procedure and age may have significant impacts on postoperative HR-QOL in women with breast cancer. Women with breast cancer who underwent BCS or IBR

had a significantly high aesthetic breast satisfaction and psychosocial and sexual well-being. Postoperative life was significantly less affected in patients who underwent BCS.

PS7-01: Efficacy of RLY-2608, a mutant-selective PI3Kα inhibitor in patients with PIK3CA-mutant HR+HER2- advanced breast cancer: ReDiscover trial

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Background Oncogenic PIK3CA mutations constitutively activate PI3Kα and drive approximately 40% of HR+HER2- breast cancer (BC); however, the toxicity (hyperglycemia, rash, diarrhea, stomatitis) of non-selective inhibitors (i) limits their tolerability and efficacy. RLY-2608 is the first oral, mutantselective, allosteric PI3Kαi designed to overcome these limitations. We report efficacy and safety of RLY-2608 + standard-dose fulvestrant (F) in pts with PIK3CA-mutant, HR+HER2- BC treated in the FIH study, ReDiscover (NCT05216432). Methods Previously treated adult pts with advanced HR+HER2- BC and PIK3CA mutation per local assessment were eligible. Key objectives were investigator-assessed efficacy per RECIST 1.1 and adverse events (AEs) per CTCAE v5.0. Safety was assessed in all pts, and efficacy in pts without detectable PTEN/AKT co-alterations treated at the RP2D. Results As of 24JUN24, 116 pts received RLY-2608 (100-1000 mg BID) + F. All pts received prior endocrine therapy and CDK4/6i with 51% having 2 prior systemic therapies for advanced disease including 57% with prior F/SERD and 22% with prior chemotherapy or antibody-drug conjugate. Treatment-related AEs (TRAEs) were generally low-grade, manageable and reversible, most commonly hyperglycemia (42% any grade; 2% Gr 3), nausea (40%; 1% Gr 3), creatinine increased (34%; 0 Gr 3), fatigue (32%; 7% Gr 3), and diarrhea (28%; 1% Gr 3). There were no grade 4/5 TRAEs. Sixty-two pts (30 kinase, 24 helical, 8 other) were treated at the 600 mg BID RP2D which provided exposure in the target therapeutic range and rapid decline in mutant PIK3CA ctDNA. Treatment was ongoing in 34/62 (55%) and discontinued in 28 (22 due to PD and 2 due to TRAE). Of 52 pts without PTEN/AKT alterations, mPFS was 9.2 months (95% CI 5.5-12.4), 18/26 (69.2%) evaluable for response had radiographic tumor reduction and 8 achieved an objective response (30.8%, 95% CI 14.3-51.8) with median time-to-response 8 weeks. Conclusion RLY-2608 demonstrates durable initial efficacy and favorable safety/tolerability across PIK3CA genotypes in heavily pretreated pts previously exposed to CDK4/6i with advanced PIK3CA-mutant HR+HER2- BC without concurrent PTEN/AKT alterations. These data validate RLY-2608 as the first allosteric pan-mutant selective PI3Kαi and warrant pivotal clinical development.

PS7-02: First-in-human results of STX-478, a mutant-selective PI3K alpha inhibitor, in HR+ breast cancer and advanced solid tumor patients

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Background: PI3Kα is commonly mutated in cancer, most frequently in breast and gynecologic cancers. PI3Kα inhibitors have shown clinical benefit in hormone receptor positive (HR+), HER2- breast cancer (BC) in Phase 3 studies, but are limited by toxicities from wild-type (WT) PI3Kα inhibition. STX-478 is an oral, allosteric, CNS-penetrant, mutant-selective PI3Kα inhibitor designed to improve efficacy while sparing WT toxicities. Preclinically, STX-478 alone and in combination with fulvestrant and CDK4/6 inhibitors led to deep and sustained tumor regression without causing metabolic abnormalities in both PIK3CA kinase and helical domain mutant BC xenografts. Here, we report the initial Phase 1 trial results of STX-478 monotherapy in BC and other solid tumors.

Methods: This first-in-human, Ph1/2 study is evaluating STX-478 alone or in combination with fulvestrant and fulvestrant + CDK4/6 inhibitors in patients with advanced PIK3CA-mutant solid tumors and/or HR+HER2- BC. Dose escalation occurred per 3+3 design followed by expansion. Patients were required to have a PIK3CA mutation, measurable disease and HbA1c less than 7%. Patients with pre-diabetes, or Type 2 diabetes controlled on medications (including insulin) and those intolerant to PI3K pathway inhibitors were permitted.

Results: As of June 21st, 2024, 61 patients (29 HR+/HER2- BC, 32 other solid tumors) were treated at STX-478 monotherapy doses of 20 mg to 160 mg daily. In patients with HR+/HER2- BC, median age was 64 (range 37-81) and median prior lines of therapy in the metastatic setting was 3 (range 1-7). All patients received prior endocrine therapy, 97% received a prior CDK4/6 inhibitor and 66% received at least one line of chemotherapy. Additionally, 48% of patients were pre-diabetic/diabetic and 41% had a prior PI3K pathway inhibitor. STX-478 was well-tolerated with a MTD of 100 mg daily. For all 61 patients (all tumor types), treatment-related adverse events (AEs) of 15% or higher and AEs of interest included fatigue (30%), hyperglycemia (23%), nausea (20%), diarrhea (15%), rash (10%) and neutropenia (0%). PI3Ka WT AEs (hyperglycemia, diarrhea, and rash) were all grade 1/2. No patient discontinued due to an AE. STX-478 exposure was dose proportional up to the MTD and reached steady state by day 15. At doses of 40 mg and higher, STX-478 achieved target coverage several fold higher than other PI3Ka inhibitors. In 22 HR+/HER2- BC patients with measurable disease, the confirmed/unconfirmed ORR was 23% with 68% of patients exhibiting radiographic tumor reductions. Objective responses were seen in both kinase and helical domain mutant tumors. Multiple responses deepened over time on therapy, including one patient with durable PR on therapy for over one

year. Responses have been observed in patients with other solid tumors with a similar ORR (19%) to that in patients with HR+/HER2- BC. PIK3CA-mutant variant allele frequency, as measured by ctDNA, markedly decreased on therapy in the majority of assessed patients. Conclusions: In heavily pre-treated patients, STX-478 was well tolerated, with infrequent Grade 1/2 PI3Ka WT-associated toxicities in a high-risk patient population including diabetic patients and those intolerant to PI3K pathway inhibitors. STX-478 was active in HR+ BC and other solid tumors, with a monotherapy ORR generally exceeding historical comparisons to other PI3K inhibitors. Efficacy was observed in both PIK3CA kinase and helical domain mutations, which comprise of 80-90% of all PI3Ka mutations. These data support STX-478 as a potential best-in-class mutant selective PI3Ka inhibitor. Enrollment is ongoing with combination cohorts with STX-478 + fulvestrant and STX-478 + fulvestrant + CDK4/6 inhibitors.

PS7-03: A first-in-human phase 1a/b trial of LOXO-783, a potent, highly mutant-selective, brain-penetrant, allosteric PI3Kα H1047R inhibitor in PIK3CA H1047R-mutant advanced breast cancer and other solid tumors: Results from the PIKASSO-01 study

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Background: Phosphoinositide 3-kinase alpha (PI3Kα) H1047R mutations occur in ~15% of breast cancers (BC). Approved treatments for PI3Kα mutant-driven BC include inhibitors of the PI3K/AKT pathway; these block signaling from mutant PI3Kα but also inhibit wild-type PI3Kα leading to dose-limiting toxicities (DLTs) that include hyperglycemia, rash, and GI side effects. Thus, there is a need for novel PI3Kα inhibitors with improved therapeutic indices. LOXO-783 is an oral, brain-penetrant allosteric PI3Kα inhibitor, highly selective for H1047R that induces tumor regressions in ER+, HER2- PI3Kα H1047R-mutant BC models without increasing plasma insulin or C-peptide. Here we present clinical data from PIKASSO-01, a global, first-in-human phase 1a/b trial of LOXO-783 as monotherapy and in combination with other anticancer therapies in patients (pts) with PIK3CA H1047R-mutant advanced BC (aBC) and other solid tumors (NCT05307705).
Methods: Phase 1a (ph1a) evaluated LOXO-783 dose escalation (mTPI-2 design) in pts with PIK3CA H1047R-mutant solid tumors (excluding colorectal cancers). Phase 1b (ph1b) included the evaluation of LOXO-783 plus (+) endocrine therapy (ET: fulvestrant [F] / aromatase inhibitor [AI]), or LOXO-783 + paclitaxel (P) in PI3Ki-naïve/intolerant pts with PIK3CA H1047R aBC. Key objectives included safety, tolerability, PK, and antitumor activity (objective response rate, ORR and clinical benefit rate, CBR per RECIST v1.1). Serial plasma samples were collected for ctDNA analysis and glucose metabolism markers.

Results: As of 14 June 2024, 149 pts were enrolled: 43 pts (35 aBC [30 HR+ HER2-, 5 TNBC], 8 other solid tumors) into ph1a and 106 pts with aBC (90 HR+ HER2-, 16 TNBC) into ph1b. Median age was 58 years. In ph1a, pts received monotherapy doses from 200 – 600 mg BID or 500 – 600 mg QD. Median prior regimens was 3 (1-5) including prior PI3K/AKT/mTORi (28%). DLTs occurred in 4 pts, 1 each at: 500 mg QD (grade [G] 3 fatigue), 600 mg QD (G3 ALT, AST, and G1 bilirubin elevation), 400 mg BID (G3 sepsis and enterocolitis), and 600 mg BID (G2 photosensitivity). In ph1b, pts received LOXO-783 (200 – 400 mg BID or 300 – 600 mg QD) in doublet therapy: + ET (n=86; 6 + AI; 80 + F) or + P (n=20). Median prior regimens for aBC was 2 (1-7).
Across all treated pts in the monotherapy or doublet therapy cohorts, diarrhea was frequently observed (84% any grade [G], 7% G ≥3) and was more common/severe (G ≥3) at higher doses (≥400 mg QD and BID dosing). Overall, 37% of pts who experienced diarrhea required a dose modification. Other frequent (any G/G ≥3) treatment-emergent AEs with LOXO-783 +/- ET included fatigue (32%/2%) and nausea (31%/2%); + P included neutrophil decreased (65%/45%), anemia (40%/20%), fatigue (35%/0%), and WBC decreased (30%/25%). Treatment-related AEs led to discontinuation of any study treatment in 4% and 10% of pts who received LOXO-783 +/- ET and + P respectively. No treatment-related hyperglycemia was observed.
Preliminary PK analyses showed dose-dependent and time-dependent nonlinear increases in LOXO-783 exposure across all doses (reaching H1047R IC90 from 200 mg BID + ET); tmax ~2 h and half-life ~19 h; and no combination drug-drug interactions.
Minimal activity was observed with LOXO-783 monotherapy (ORR/CBR in evaluable pts: 3%/17% [n=29]). In doublet therapy cohorts, ORR/CBR was 5%/19% + ET (n=77) and 19%/25% + P (n=16). Most (80%; 85/106) evaluable pts had a decrease (any) in PIK3CA H1047R variant allele frequency at C1D15 compared to baseline.
Conclusions: LOXO-783 demonstrated proof of concept of mutant selectivity by entirely sparing hyperglycemia in the clinic, but exhibited high rates of diarrhea, limiting ability to achieve the optimal preclinical dose of LOXO-783.

PS7-04: BBO-10203, a first-in-class, orally bioavailable, selective blocker of the PI3Kα:RAS interaction inhibits tumor growth alone and in combination with standard of care therapies in breast cancer models without inducing hyperglycemia

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Aberrant activation of the PI3Kα pathway is one of the most frequent oncogenic events across human cancers and leads to promotion of tumor cell growth, survival, glucose metabolism, and acute resistance to numerous standard of care cancer therapies. While small molecule inhibitors of the kinase activity of PI3Kα have been approved for the

treatment of HR+ HER2- breast cancer patients with PI3Kα mutations, a large medical need remains to increase their safety profile due to dose-limiting on-target hyperglycemia. This toxicity may limit target coverage, the number of eligible patients, and the duration of treatment which could result in suboptimal efficacy. An alternative novel strategy is to block RAS-mediated activation of PI3Kα, a signaling event prevalent mostly in malignant cells. Previous elegant preclinical studies have established that RAS activation of PI3Kα is important in tumor cells but may not be involved in normal cell types controlling glucose metabolism because insulin activation of PI3Kα does not depend on RAS. Here, we report on a novel first-in-class covalent small molecule designed to block the PI3Kα:RAS protein-protein interaction and inhibit RAS-mediated activation of the AKT pathway via PI3Kα without the resultant hyperglycemia associated with direct inhibition of PI3Kα kinase activity. BBO-10203 covalently and selectively binds PI3Kα on cysteine 242 in the RAS binding domain, which prevents the interaction of PI3Kα with KRAS, HRAS, and NRAS. BBO-10203 shows potent cellular target engagement with an IC50 of 1.4 nM and full target engagement achieved at 10 nM in ER+ HER2amp breast cancer BT-474 cells, which harbor a PIK3CAK111N mutation. BBO-10203 potently inhibits phosphorylated AKT (pAKT) across a diverse panel of 18 human breast cancer cell lines with amplification of HER2 or mutations in PI3Kα with a mean EC50 of 3.2 nM. Transcriptional and post-translational cellular changes driven by treatment with BBO-10203 are consistent with PI3Kα-specific inhibition. BBO-10203 displays excellent drug-like properties and oral bioavailability. Single dose treatment of BT-474 tumor bearing mice with increasing doses (10 to 100 mg/kg) of BBO-10203 results in dose and time dependent inhibition of pAKT. In the BT-474 xenograft model, BBO-10203 daily oral dosing of 100 mg/kg results in 88% tumor growth inhibition. Importantly, BBO-10203 does not induce hyperglycemia or hyperinsulinemia during an oral glucose tolerance test in fasted male C57BL/6 mice, demonstrating independence of insulin receptor signaling from RAS. Since activation of AKT provides acute resistance to multiple cancer therapies, BBO-10203 has the potential to enhance the long-term responses in combination with targeted and conventional anti-cancer agents in multiple settings. In vitro and in vivo studies show that BBO-10203 significantly enhances the anti-tumor activity of the HER2-targeted antibody trastuzumab in the BT-474 (ER+, HER2amp, and PIK3CAK111N) and MDA-MB-453 (ER-, HER2+, and PIK3CAH1047R) breast cancer models. In addition, BBO-10203 also significantly enhances the anti-tumor activity of the SERD fulvestrant or the CDK4/6 inhibitor palbociclib in the MCF7 (ER+, HER2-, and PIK3CAE545K) breast cancer model. All of these combinations induce tumor stasis or regression through direct effects on tumor cells and are well tolerated. In conclusion, BBO-10203 blocks RAS-mediated activation of PI3Kα and strongly inhibits pAKT signaling in tumor cells without affecting glucose metabolism. BBO-10203 has entered phase 1 clinical trials and may provide clinical benefit without the limiting toxicities that have restricted the use of PI3Kα inhibitors.

PS7-05: Impact of prior treatment, ESR1 mutational (ESR1m) landscape, and co-occurring PI3K pathway status on real-world (RW) elacestrant outcomes in patients (pts) with hormone receptor-positive (HR+)/HER2-negative advanced breast cancer (aBC)

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Background: Elacestrant is the first oral selective estrogen receptor degrader FDA-approved as endocrine therapy (ET) in pts with ESR1m HR+/HER2- aBC, based on results from the EMERALD trial which demonstrated improved progression free survival (PFS) compared to standard-of-care ET. Here, we used a large clinical-genomic database to assess RW use and the impact of prior treatment, the type/number of ESR1m, and co-occurring PI3K pathway alterations on pt outcomes.
Methods: Pts with HR+/HER2- aBC were identified via the GuardantINFORM database; pts with ESR1m detected on circulating tumor DNA (ctDNA) testing done within 6 months prior to elacestrant initiation and treated with elacestrant after FDA approval in January 2023 were included. Real-world time to treatment discontinuation (rwTTD) and time to next treatment (rwTTNT) were analyzed as proxies for PFS (measured in months). Pts were required to have >28 days of follow-up after the first elacestrant claim to be included in outcomes analysis. For rwTTD/rwTTND, non-adjusted Kaplan-Meier (KM) curves were generated with 95% confidence intervals (CI) and Cox-Regression hazard ratios (HR) adjusted for patient age, gender, year of ctDNA result and line of therapy were used. Oncogenic alterations in AKT1, PTEN, and PIK3CA were included as PI3K pathway alterations.
Results: A total of 1,015 aBC pts received elacestrant post-FDA approval, 772 of whom had a ctDNA test 6 months prior to treatment start, with a median age of 63 years. The most common ESR1m were D538G (69%), Y735S (51%), Y537N (20%), and E380Q (10%). 45% of pts had >1 ESR1m (2: 24%, 3: 10%, 4: 5%, 5+: 6%). Of 758 pts eligible for outcomes analysis, the overall rwTTD was 4.57 months (95% CI 4.03 - 5.4 months) and rwTTNT was 6.53 months (95% CI 5.60 - 8.10 months). There were no significant differences in rwTTD or rwTTNT based on specific ESR1m (D538G vs Y537S and Y537S vs other ESR1m), prior exposure to an aromatase inhibitor or fulvestrant (vs no prior exposure), or elacestrant line of therapy (2 vs 3 vs 4+). Pts with >4 ESR1m (but not 2-3 ESR1m) had significantly worse rwTTD compared to those with 1 ESR1m (3.73 mos vs 5.27 mos, p=0.01, HR 1.69; 95% CI 1.23 - 2.33); rwTTNT was numerically shorter but did not reach statistical significance (5.17 mos vs 7.67 mos, p=0.145, HR 1.47, 95% CI 1.03 - 2.11). Pts with oncogenic PI3K pathway alterations detected (210/758) had significantly worse rwTTD and rwTTNT compared to those without (rwTTD: 3.97 mos vs 5.27 mos, p<0.0001, HR 1.55, CI 95% 1.25-1.93; rwTTNT: 4.60 mos vs 7.57 mos, p=0.0003; HR 1.58, CI 95% 1.24-2.00).
Conclusions: Using a large clinical-genomic database, we demonstrate robust RW outcomes in a cohort of over 700 pts with ESR1m aBC treated with elacestrant. Pt treatment

outcomes, as measured via rwTTD and rwTTNT, exceeded the PFS reported in the EMERALD trial. This may be due to multiple factors impacting patient selection and restaging protocols, which can differ in prospective trials versus routine clinical care. Treatment outcomes did not significantly differ by specific ESR1m, prior ET exposure, or elacestrant line of therapy. Pts with >4 ESR1m had significantly worse rwTTD and numerically worse rwTTNT compared pts with 1 ESR1m, suggesting a possible relationship between complex ESR1m polyclonality and response to ET. Oncogenic PI3K pathway co-mutations had a negative prognostic impact on treatment duration. Given the availability of multiple targeted therapies for pts with aBC, including two drugs targeting the PI3K pathway, these results suggest that additional biomarker analysis may help elucidate optimal therapy selection, including combination therapy and drug sequencing, in the post-CDK4/6 inhibitor treatment landscape.

PS7-06: Elacestrant combinations in patients with estrogen receptor-positive (ER+), HER2-negative (HER2-) locally advanced or metastatic breast cancer (mBC): Update from ELEVATE, a phase 1b/2, open-label, umbrella study

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Background: Endocrine therapy (ET) + CDK4/6i is the mainstay in 1L ER+/HER2- mBC; however, ET resistance develops. Intrinsic resistance mechanisms include PI3K/AKT/mTOR or cell cycle pathway alterations; acquired resistance mechanisms include ESR1-mut during ET in mBC. In EMERALD, single-agent elacestrant significantly improved PFS vs standard of care (SOC) ET (ESR1-mut tumors: HR = 0.55; 95% CI, 0.39-0.77; P = 0.0005; all patients: HR = 0.70; 95% CI, 0.55-0.88; P = 0.0018) with manageable safety in patients with ER+/HER2- mBC previously treated with ET+CDK4/6i (Bidard 2022). In those with prior ET+CDK4/6i ≥12 months and ESR1-mut tumors, median PFS (mPFS) with elacestrant was 8.6 vs 1.9 months (HR = 0.41; 95% CI, 0.26-0.63) with SOC ET (Bardia 2022). To address various resistance mechanisms, ELEVATE (NCT05563220) evaluates elacestrant in combination with everolimus, alpelisib, capivasertib, ribociclib, palbociclib, or abemaciclib.
Methods: Eligible patients have ER+/HER2- mBC. Phase 1b objective is to identify the recommended phase 2 dose (RP2D) of each combination. ELECTRA (NCT05386108) evaluated the RP2D of elacestrant + abemaciclib. Phase 2 primary objective is to evaluate PFS in patients receiving elacestrant combined with each of the other study drugs at the RP2D. This analysis reports updated safety from the phase 1b portion for the following combinations: everolimus, alpelisib, ribociclib, and palbociclib. Preliminary phase 1b efficacy is reported for the elacestrant + everolimus combination; efficacy evaluation for additional combinations is ongoing.

Results: As of July 2024, 23 patients have been enrolled in the elacestrant (258-345 mg) + everolimus (5-10 mg) cohorts. The most common (≥30%) treatment-emergent AEs (TEAEs) were nausea (n=13, 57%; 4% Gr ≥3), stomatitis (n=12, 52%; 9% Gr ≥3), diarrhea (n=10, 43%; 9% Gr ≥3), and fatigue (n=10, 43%; 9% Gr ≥3). mPFS was not yet reached after a median 0.8 to 12.3 months of follow-up across cohorts. Preliminary efficacy in response evaluable patients (n=16) for the elacestrant + everolimus combination demonstrated a CBR at 24 weeks of 81% and ORR of 25%. The elacestrant (258 mg) + alpelisib (250-200 mg) cohort enrolled 9 patients. The most common TEAEs (≥30%) were nausea (n=8, 89%; 11% Gr ≥3), rash (n=4, 44%; 22% Gr ≥3), vomiting (n=4, 44%; 0 ≥G3), dry mouth, stomatitis, and dizziness (n=3, 33%; 0 Gr ≥3). Elacestrant (86-258 mg) + ribociclib (400-600 mg) cohorts enrolled 18 patients. The most common (≥30%) TEAE was neutropenia (n=7, 39%; 28% Gr ≥3). Elacestrant (258-345 mg) + palbociclib (100 mg) cohorts enrolled 12 patients. The most common (≥30%) TEAEs were neutropenia (n=5, 42%; 17% Gr ≥3) and nausea (n=4, 33%; 0 Gr ≥3). Phase 1b of the capivasertib combination is currently ongoing to determine the RP2D. Updated data will be presented.
Conclusion: The phase 1b combinations of elacestrant with either everolimus, ribociclib, or palbociclib demonstrated a manageable safety profile consistent with previously reported data for each compound. The elacestrant + everolimus combination shows favorable efficacy. The phase 1b combinations with ribociclib, palbociclib, or capivasertib are ongoing. The phase 1b combination with alpelisib is under evaluation. The elacestrant + everolimus and elacestrant 345 mg QD + abemaciclib 150 mg BID (RP2D determined in ELECTRA trial) combinations are currently enrolling patients in phase 2. Elacestrant has the potential to become the ET backbone with targeted therapies, to enable all-oral combinations as a treatment option that can replace fulvestrant-based combinations and delay chemotherapy or ADC-based regimens.

PS7-07: Elacestrant plus abemaciclib (abema) combination in patients (pts) with estrogen receptor-positive (ER+), HER2-negative (HER2-) advanced or metastatic breast cancer (mBC)

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Background: Endocrine therapy (ET) plus a CDK4/6 inhibitor (CDK4/6i) is the primary first-line treatment for ER+/HER2- mBC; however, resistance eventually develops. In the EMERALD trial, single-agent elacestrant significantly prolonged progression-free survival (PFS) vs standard-of-care (SOC) ET with manageable safety in pts with ER+/HER2-, mBC previously treated with ET+CDK4/6i (ESR1-mut tumors: HR = 0.55; 95% CI, 0.39-0.77; P = 0.0005; all pts: HR = 0.70; 95% CI, 0.55-0.88; P = 0.0018). In those with prior ET+CDK4/6i ≥12 months and ESR1-mut tumors, median PFS with elacestrant was 8.6 vs 1.9 months with

SOC ET. Combining elacestrant + abemaciclib (abema) may overcome additional resistance mechanisms, improve efficacy, and enable an all-oral treatment option that can replace fulvestrant-based combinations and delay chemotherapy or ADC-based regimens. Elacestrant + abemaciclib combination is being evaluated in the phase 1b/2 ELECTRA (NCT05386108) and ELEVATE (NCT05563220) trials. Here, we present a pooled analysis of efficacy and safety.
Methods: Eligible pts were treated for ER+/HER2- mBC in ELECTRA and ELEVATE trials. Pts must have previously received ET in mBC, including ≥1 line of ET, CDK4/6 inhibitors, or chemotherapy (ELECTRA only). Safety was evaluated in all patients who received elacestrant + abemaciclib. The efficacy evaluable population included pts who had measurable disease (ie, ≥1 target lesion) at baseline and ≥1 post-baseline RECIST assessment.
Results: As of July 2024, 55 pts have received elacestrant + abemaciclib, the majority with 1-2 lines of prior ET (91%), prior CDK4/6i (95%), and visceral metastases (66%); 25% received prior chemotherapy. The most common all-grade AEs (≥20%) were diarrhea (n=37, 67%; 2% Gr ≥3), nausea (n=34, 62%; 6% Gr ≥3), neutropenia (n=17, 31%; 27% Gr ≥3), vomiting (n=16, 29%; 2% Gr ≥3), fatigue (n=12, 22%; 2% Gr ≥3), anemia (n=11, 20%; 4% Gr ≥3), and decreased appetite (n=11, 20%; 0 Gr ≥3). No Grade 4 AEs were observed. In all efficacy-evaluable pts (n=27), mPFS was 8.6 mo. In the ESR1-mut population (n=11), mPFS was 8.7 mo. In ESR1-mut not detected (n=13), mPFS was 7.2 mo. In patients with only 1-2 lines of prior ET in mBC (n=23), mPFS was 8.7 mo. Median PFS according to dose level was 8.6 mo (elacestrant 258 mg QD + abema 100 mg BID, n=8), 7.5 mo (elacestrant 345 mg QD + abema 100 mg BID, n=7), and 8.7 mo (elacestrant 345 mg QD + abema 150 mg BID, n=12). Elacestrant 345 mg QD + abema 150 mg BID was determined as the recommended phase 2 dose (RP2D) for both ELECTRA and ELEVATE Phase 2 arms. Updated safety and efficacy will be reported.
Conclusion: The RP2D dose combination of elacestrant 345 mg QD + abemaciclib 150 mg BID shows favorable efficacy with a manageable and predictable safety profile. Elacestrant has the potential to become the ET backbone for combination regimens. The Phase 2 portions of both studies are currently enrolling.

PS7-08: Results from SERENA-1 Parts K/L: A Phase 1 study of the next-generation oral selective estrogen receptor degrader (SERD) camizestrant (AZD9833) in combination with ribociclib in women with ER-positive, HER2 negative advanced breast cancer

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Lindemann, Patricia Martin Romano, Alastair Mathewson, Christopher J Morrow, Andy Sykes, Ewa Warwick, Jincheng Yang, Bairu Zhang

Background: SERENA-1 (NCT03616587) is a Phase 1, multi-part, open-label study of camizestrant in women with ER+/HER2− advanced breast cancer. Study parts examining camizestrant as monotherapy and in combination with palbociclib, abemaciclib, and capivasertib have been presented previously. Here, we present the data from Parts K and L, which examined camizestrant in combination with ribociclib.
MethodsThe primary objective was to determine the safety and tolerability of camizestrant 75 mg once daily (QD) in combination with intermittent ribociclib 400 mg or 600 mg QD (21 days on, 7 days off). Objectives included assessment of the anti-tumor response and pharmacokinetics (PK). Patients were women with advanced breast cancer, and premenopausal women were required to receive ovarian function suppression. Prior treatment with ≤2 lines of chemotherapy in the advanced setting was permitted, with no limit on prior endocrine treatment lines; previous treatment with CDK4/6 inhibitors (CDK4/6i) and/or fulvestrant was also permitted.
Results: As of April 26, 2024, 58 patients (median age 57 (range [31–80]) were enrolled; 26 and 32 patients received camizestrant 75 mg in combination with ribociclib 400 or 600 mg, respectively. Overall, patients were heavily pre-treated in the advanced setting; 17/58 (29%) patients had received prior chemotherapy, 40/58 (69%) prior CDK4/6i, and 28/58 (48%) prior fulvestrant. 35/58 (60%) patients had visceral metastases.
Steady state ribociclib exposure in combination with camizestrant 75 mg was comparable to simulations from a published ribociclib monotherapy population PK (popPK) model. Exposure to camizestrant 75 mg in combination with ribociclib 400 or 600 mg is comparable to a popPK simulation exposure of monotherapy camizestrant 150 mg.
Visual effects and bradycardia, causally related to camizestrant (per investigator's opinion), were reported in 20/58 (35%) and 15/58 (26%) patients, respectively; all grade 1. The most common ribociclib-related adverse events (AEs) were neutropenia (24/58; 41%), nausea (15/58; 26%), and fatigue (14/58; 24%). 17/58 (29%) patients reported ribociclib-related AEs that led to ribociclib-only interruptions (most commonly neutropenia [n=13] and QT prolongation [n=3]); 6/58 (10%) patients reported AEs related to both camizestrant and ribociclib leading to interruption of both treatments (most commonly QT prolongation [n=2]). One patient discontinued both camizestrant 75 mg and ribociclib 600 mg due to grade 4 QTc prolongation, which resolved 7 days after cessation of dosing. There were no patients with AEs leading to camizestrant-only interruptions.
At Cycle 1, Day 1, 20/58 (34%) patients had a detectable ESR1 mutation (ESR1m) by ctDNA and an evaluable Cycle 2 Day 1 (C2D1) result; 85% of these patients experienced ESR1m reduction of >50% at C2D1, including 50% of patients where ESR1m was cleared.
While the median duration of exposure to camizestrant and ribociclib at this data cut was 4.5 months, median progression-free survival has not yet been reached, with 40% (23/58) experiencing a progression event to date. Updated data will be presented at the meeting.
Conclusion: Camizestrant 75 mg in combination with ribociclib 400 or 600 mg was well tolerated, consistent with data for each drug individually, and resulted in ESR1m ctDNA suppression in 85% of patients with ESR1m at baseline. This combination is included in the

ongoing Phase 3 SERENA-6 trial (NCT04964934) of camizestrant combined with CDK4/6i versus an aromatase inhibitor plus CDK4/6i in patients with detectable ESR1m during first-line therapy, which will further clarify the role of camizestrant in the treatment of patients with ER+/HER2− advanced breast cancer.

PS8-01: ZN-1041, a potential best-in-class BBB Penetrable HER2 Inhibitor, has high antitumor activity in patients with Breast Cancer with CNS Metastases

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Background: An estimated 50% of patients with HER2-positive metastatic breast cancer will develop brain metastases (BCBM). BCBM is associated with shortened overall survival and disruption of quality of life, representing an area of investigation for newer and more effective treatments. Previous studies, including Phase 1a and 1b, have demonstrated that ZN-1041, a HER2 TKI designed to penetrate the blood-brain barrier (BBB) with high selectivity and a broad safety margin, exhibits promising antitumor activity both as monotherapy and in combination with trastuzumab and capecitabine. This abstract presents the Phase 1c data to evaluate the safety and efficacy of the combination when treating HER2-positive BCBM and extracranial disease.
Methods: The phase 1 multicenter open-label study ZN-1041-101 (NCT04487236) includes dose escalation of ZN-1041 monotherapy (Phase 1a) in patients with HER2-positive solid tumors, with or without brain metastases, followed by dose escalation (Phase 1b) and expansion (Phase 1c) of ZN-1041 with capecitabine (1000 mg/m^2 bid for 14 days, followed by 7 days off) and trastuzumab (8 mg/kg loading dose, followed by a 6 mg/kg maintenance dose iv q3wks) in HER2-positive BCBM. The primary objective was to evaluate safety and tolerability and determine the RP2D for the subsequent study, while secondary objectives included pharmacokinetics and antitumor response rate (RR) per RECIST 1.1 and RANO-BM criteria.
Results: A total of 40 TKI-naïve, HER2-positive BCBM pts were enrolled in phase 1c with ZN-1041 1000 mg bid combined with capecitabine and trastuzumab, and all patients tolerated the treatment well. Grade 3 or higher treatment-related adverse events (>5%) included hyperbilirubinemia (20%), GGT increase (17.5%), white blood cell decrease (15%), ALT increase (5.7%), AST increase (7.5%), and weight decrease (7.5%), headache (7.5%). The incidence of diarrhea was 37.5 % for all grades, with no patients experiencing Grade 3 or higher diarrhea.
Among the 34 BCBM pts who met the tumor assessment criteria,t The median duration of follow-up was 13.9 months, the confirmed overall response rate (RR) was 79.4% (95% CI: 62.10–91.30), the intracranial RR was 75.0% (95% CI: 56.6–88.5), and the disease control rate (DCR) was 100%. Kaplan-Meier analysis estimated the median progression-free survival (PFS) at 14 months (95% CI: 9.13-17.94), with a median duration of response

(DOR) of 12.6 months (95% CI: 6.7-16.59). The 12-month PFS rate was 56.4% (95% CI: 37.76-71.45). Subgroup analysis of 32 patients with measurable brain lesions indicated benefit from the regimen, with a median PFS of 17.4 months (95% CI: 8.74-19.61) and a median duration of intracranial response (DOIR) of 14.7 months (95% CI: 6.51–NR). As of May 22nd, 2024, 10 patients were still in treatment, the longest duration of which being 22.8 months.
Conclusions: Encouraging preliminary efficacy and tolerability were observed for ZN-1041 either as monotherapy or combined with capecitabine and trastuzumab in TKI naïve, HER2+ BCBM patients. Further development of ZN-1041 combination therapies in a larger population is warranted, including the ongoing ZN-1041-101-US study which is evaluating the safety and efficacy of ZN-1041 in combination with trastuzumab deruxtecan and with trastuzumab and pertuzumab in a larger population across the US, EU, UK, Australia, and New Zealand.

PS8-02: A Phase 1 study Evaluating the Safety, Efficacy and Pharmacokinetics of TL938 in HER2-Positive Patients with Advance Solid Tumors

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Background: Patients with human epidermal growth factor receptor 2 positive (HER2+) breast cancer (BC) who develop brain metastasis (BM) often have a poor prognosis. The treatment options for HER2+ BCBM is limited due to the presence of the blood–brain barrier (BBB). TL938 is an oral HER2 inhibitor capable of crossing the BBB, demonstrated by a brain-to-plasma partition coefficient (Kp, uu,brain) of over 400% in rat. This suggests TL938 has significant potential for the treatment of HER2+ BCBM.
Methods: Cancer patients with advanced HER2+ solid tumors (overexpression, amplification, or mutation) confirmed by histology or cytology were treated with TL938. Seven dose levels of TL938 were investigated: 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, and 350 mg. TL938 was administered orally once daily. The study followed a 3 + 3 design. The primary endpoints were the maximum tolerated dose (MTD), recommended phase 2 dose (PR2D) and safety of TL938. Secondary endpoints included Investigator-assessed (RECIST v1.1) objective response rate (ORR), duration of response (DoR), disease control rate (DCR), progression free survival (PFS), and pharmacokinetics.
Results: As of the cutoff date of May 17, 2024, TL938 has been administered to 44 patients. No dose-limiting toxicities (DLTs) were observed, and the MTD was not reached.
The most common treatment related adverse events (TRAEs) (>20%) were diarrhea (75.0%), followed by increased blood creatine phosphokinase (38.6%), increased aspartate aminotransferase (36.4%), increased blood creatinine (31.8%), increased α-hydroxybutyrate dehydrogenase (27.3%), increased alanine aminotransferase (22.7%), proteinuria (22.7%), decreased lymphocyte count (22.7%), vomiting (22.7%), and increased blood creatine phosphokinase MB (20.5%). Grade 3 or higher TRAEs included

diarrhea (18.2%), increased blood creatine phosphokinase (11.4%), and upper abdominal pain (2.3%). Three subjects (6.8%) experienced dose reduction due to TRAEs, including diarrhea (2.3%) and increased blood creatine phosphokinase (4.5%), and three subjects (6.8%) had dose interruption due to TRAEs, increased blood creatine phosphokinase (4.5%), vomiting (2.3%), gastritis (2.3%), upper abdominal pain (2.3%), and gastroesophageal reflux disease (2.3%).
Among the 41 patients with tumor assessments, a total of 7 cancer types were represented, with the most common being BC (44%), lung cancer (22%), and salivary gland cancer (12%). Of the 18 BC patients who had tumor assessments at least once, a 50.0% overall response rate (ORR) and an 83.3% disease control rate (DCR) were observed. Notably, for the BC patients treated at a dose of 200 mg (N=10), an ORR of 70.0% and a DCR of 100.0% were achieved, including 1 patient who achieved a complete response.
TL938 demonstrated intracranial disease control in five (including one with BC) patients with brain metastasis, who had previously undergone surgery, chemotherapy, and HER2-antibody-drug conjugate therapy. Among these patients, two achieved significant regressions in intracranial target lesions with reductions of 60% and 41%, respectively.
After a single oral dose of TL938, the maximum plasma concentration (Cmax) was reached at a median time (Tmax) of 6-8 hours in most dosage groups. After 15 consecutive once-daily oral doses, minimal fluctuation of plasma concentrations around the average levels was observed across all dose cohorts, with fluctuation percentages below 30% for doses ranging from 100 to 250 mg. This indicates relatively stable exposure with repeated dosing.
Conclusions:
TL938 has demonstrated preliminary efficacy as a single agent therapy in HER2+ metastatic breast cancer patients with good tolerance. The most common TRAE observed was diarrhea, which is consistent with other similar tyrosine kinase inhibitors.

PS8-03: Exploratory biomarker analysis of Trastuzumab deruxtecan (T-DXd) vs Trastuzumab emtansine (T-DM1) efficacy in human epidermal growth factor receptor 2–positive (HER2+) metastatic breast cancer (mBC) in DESTINY-Breast03 (DB-03)

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Background: This is the first exploratory biomarker analysis from DB-03 (NCT03529110), which showed a 22-mo improvement in median progression-free survival (mPFS) with T-DXd vs T-DM1 in patients (pts) with HER2+ mBC previously treated with trastuzumab and taxane.
Methods: This analysis assessed the impact of genomic alterations (alt) on efficacy (objective response rate [ORR] and mPFS), at baseline (BL) and alt emerging at progression. Genomic alt from the GuardantINFINITY panel of >700 genes were examined in circulating tumor DNA (ctDNA) samples collected at BL from 204 pts and 217 pts, and at end of

treatment due to disease progression (DP) for 67 pts and 141 pts, treated with T-DXd and T-DM1, respectively. Data cutoff was July 25, 2022. Median follow-up was 28.4 mo and 26.5 mo in the T-DXd and T-DM1 arms, respectively.
Results: The DB-03 genomic landscape largely recapitulated that of HER2+ BC, with most frequent alt (single nucleotide variants, amplifications [amp], and indels) observed in TP53 (73%), HER2 (ERBB2; 61%), CDK12 (52%) and PIK3CA (48%).
HER2 genomic status
Detection rate of HER2 amp in ctDNA was relatively low: 56% (235/420) detected, including aneuploidy and focal HER2 amp. HER2 plasma copy number (CN; adjusted for ctDNA tumor fraction) was higher in the subgroup of pts with HER2 IHC3+ vs IHC2+ tumors. Efficacy was comparable in the T-DXd arm regardless of high/low median HER2 plasma CN at BL, whereas numerically shorter PFS and lower ORR were observed in the T-DM1 arm with lower HER2 plasma CN at BL.

T-DXd: HER2 CN high (n = 62 [30.4%]) vs CN low (n = 59 [28.9%]) vs not detected (ND; n = 83 [40.7%])

ORR, % (95% CI): 87.1 (76.1-94.3) vs 81.4 (69.1-90.3) vs 77.1 (66.6-85.6)
mPFS, mo (95% CI): 23.9 (18.0-not estimable [NE]) vs 21.1 (12.3-NE) vs 37.3 (26.2-NE)

T-DM1: HER2 CN high (n = 56 [25.8%]) vs CN low (n = 59 [27.2%]) vs ND (n = 102 [47.0%])

ORR, % (95% CI): 50.0 (36.3-63.7) vs 35.6 (23.6-49.1) vs 31.4 (22.5-41.3)
mPFS, mo (95% CI): 9.7 (6.8-25.7) vs 5.4 (3.0-6.8) vs 8.1 (4.4-10.9)

No difference in response based on BL HER2 activating mutation (mut) status was observed in either arm. Data to be presented.
PI3K pathway
T-DXd efficacy was comparable irrespective of PI3K pathway mut status. In contrast, a trend towards reduced efficacy was observed in pts with PI3K mut in the T-DM1 arm.

T-DXd: PI3K mut (n = 87 [42.6%]) vs ND (n = 117 [57.4%])

ORR, % (95% CI): 80.5 (70.6-88.2) vs 82.1 (73.9-88.5)
mPFS, mo (95% CI): 27.6 (15.0-NE) vs NE (22.1- NE)

T-DM1: PI3K mut (n = 87 [40.1%]) vs ND (n = 130 [59.9%])

ORR, % (95% CI): 33.3 (23.6-44.3) vs 40.0 (31.5-49.0)
mPFS, mo (95% CI): 4.4 (3.2-6.8) vs 9.7 (6.8-12.1)

Homologous recombination deficiency (HRD) and BRCA1/2 alt status
ORR was similar regardless of HRD status in the T-DXd arm and numerically lower in the HRD+ subgroup of the T-DM1 arm. mPFS tended to be shorter in both arms with HRD+ status.

T-DXd: HRD+ (n = 23 [11.3%]) vs ND (n = 181 [88.7%])

ORR, % (95% CI): 82.6 (61.2-95.0) vs 81.2 (74.8-86.6)
mPFS, mo (95% CI): 12.4 (6.8-NE) vs 37.3 (23.7-NE)

T-DM1: HRD+ (n = 27 [12.4%]) vs ND (n = 190 [87.6%])

ORR, % (95% CI): 14.8 (4.2-33.7) vs 40.5 (33.5-47.9)
PFS, mo (95% CI): 2.8 (1.4-4.3) vs 7.1 (5.7-9.7)
m
Similar trends were observed based on BRCA1/2 alt status. Data to be presented.
Conclusions
Genomic alt at BL were not significantly associated with response to T-DXd vs T-DM1. T-DXd maintained superior activity to T-DM1 regardless of the presence of detectable BL genomic alt. T-DXd was comparably active in pts with PI3K pathway mut whereas T-DM1 activity appeared lower. T-DXd outperformed T-DM1 regardless of HRD+/- or BRCA1/2 alt status, although HRD+ status and BRCA1/2 alt were poor prognosticators for PFS in both arms. Mutational analysis at DP will be presented.

PS8-04: Targeting clinically advanced breast cancer with conjugated and unconjugated HER2 antibodies: Does copy number matter?

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Background: HER2-targeted therapy is broadly used in advanced breast cancer (aBC). For HER2+ aBC (HER2 IHC 3+ or 2+/ISH+), 1L standard of care includes unconjugated HER2 antibodies trastuzumab and pertuzumab (HP) in combination with chemotherapy. In the 2L+ setting, antibody drug conjugate trastuzumab deruxtecan (T-DXd) can be used for HER2 low (IHC 1+, 2+/ISH-) patients with significant benefit seen in DESTINY Breast 04 relative to physicians' choice chemotherapy. For some targeted therapies, including MET inhibitor capmatinib, the magnitude of genomic copy number (CN) gains predict benefit. Here, we examined a real-world cohort of aBC patients (pts) treated with HER2 antibody therapies to determine if HER2 genomic CN ratio predicts outcomes.
Methods: This study used the US nationwide de-identified Flatiron Health (FH) and Foundation Medicine Inc. (FMI) clinico-genomic database including patient-level structured and unstructured retrospective longitudinal clinical and genomic data from approximately 280 cancer clinics (approximately 800 care sites) in the United States and included patients who underwent tissue comprehensive genomic profiling

(FoundationOne®/FoundationOne®CDx) after 2014. HER2 CN ratio was defined as ratio of modeled absolute CN to specimen ploidy.

Results: 121 pts with HER2+ aBC were treated with HP + chemotherapy in 1L and received genomic profiling before start of 2L. HER2 CN ratio was generally high (median HER2 CN ratio of 10; IQR 2.8-17.8). Pts with a HER2 CN ratio of >5 (equivalent to CN 10 in a diploid tumor) had significantly better TTD (median 8.4 v 5.3 mo, HR = 0.55, 95% CI 0.36-0.84, p = 0.006), and OS (median 76 v 31mo, HR = 0.33 95% CI 0.18-0.61, p <0.001) than pts with a HER2 CN ratio of ≤5, and comparable trends were observed for PFS (median 13v 9mo, HR = 0.81 (95% CI 0.52-1.26), p = 0.35). Similar benefit was seen for pts with a HER2 CN ratio of 5-10, 10-15, 15-20, and 20+ relative to HER2 ratio ≤5.
For 95 pts with HER2 low BC treated with T-DXd in the 2L or 3L setting, HER2 CN ratio was generally low (median ratio of 1, IQR 1-1) with a maximum HER2 CN ratio of 2.5 in the cohort. Across HER2 CN levels, outcomes were similar with the exception of pts with a HER2 CN ratio of ≤0.5 (11/95; 12%) who had significantly worse PFS (median 2.5 v 6.1 mo, HR = 0.37, 95% CI 0.17-0.79 p = 0.01) and OS (median 6.5 v 25.2 mo; HR =0.32, 95% CI 0.14-0.75; p=0.008) than pts with a HER2 CN ratio of >0.5, with similar trends for TTD (median 1.6 v 4.8 mo, HR = 0.52, 95% CI 0.26-1.06 p = 0.07).
Conclusions: In a cohort of real-world aBC pts treated with HER2 antibody therapies, HER2 CN ratio was significantly associated with clinical outcomes. For unconjugated antibodies, pts with a HER2 CN ratio of <5 had significantly shorter TTD, PFS and OS. Future work should explore whether these pts may benefit from therapy escalation (e.g. use of conjugated HER2 antibodies) and/or increased surveillance. For the HER2 conjugated antibody T-DXd, pts with HER2 low BC with a hemizygous or deep deletion in HER2 (HER2 CN ratio ≤0.5) had significantly worse outcomes, consistent with partial or complete target loss. These results suggest potential additional predictive value of an NGS-based HER2 CN quantitative biomarker that could be deployed in conjunction with the currently employed qualitative IHC status biomarker to identify pts for whom an alternative therapy may be more efficacious. For pts with a ratio of >0.5, benefit was seen across the range of HER2 CN ratios suggesting that even low levels of HER2 are enough for activity of T-DXd, consistent with the high payload:antibody ratio and bystander effect for T-DXd. Prospective or observational studies should be performed to validate these findings.

PS8-05: ACE-Breast-08: a phase I study of ARX788, a novel anti-HER2 antibody-drug conjugate, in patients with TKI pretreated HER2 positive advanced breast cancer

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Background: Anti-HER2 TKI-resistant, advanced breast cancer has a poor prognosis with limited treatment options. ARX788 is a next-generation antibody-drug conjugate (ADC) of HER2 monoclonal antibody site-specifically conjugated with a potent cytotoxic tubulin inhibitor (AS269). This phase Ⅱ study aimed to evaluate the efficacy and safety of ARX788 in patients with HER2 positive advanced breast cancer previously treated with trastuzumab and anti-HER2 tyrosine kinase inhibitor (TKI).
Methods: This multicenter, open and single-arm study was conducted in 13 centers in China. Eligible patients were aged between 18 and 75 years, had an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1, had HER2 positive (FISH+ or IHC3+) unresectable or metastatic breast cancer pretreated with trastuzumab and anti-HER2 TKI, and had at least one measurable lesion per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1). All patients were received ARX788 1.5mg/kg, administered by intravenous infusion every 3 weeks. Primary endpoint was independent review committee (IRC)-assessed confirmed objective response rate (cORR) per RECIST 1.1 in all patients.
Results: Between February 9, 2022 and April 30, 2024, totally 138 patients were enrolled and received ARX788 at lease one dose. At baseline there were 8.8% (12/138) of patients with brain metastasis and 76.8% (106/138) with visceral metastasis. The median prior lines of therapy in the metastatic setting were 3 (1, 9). In the intent-to-treat population, IRC-assessed cORR was 44.9% (95%CI: 36.5- 53.6) with a complete response (CR) seen in 3 patients and partial responses (PR) in 59 patients. cORR assessed by investigators was 42.0% (95%CI: 33.7-50.7) with 3 patients of CR and 55 patients of PR. With the median follow-up time of 14.78 months, the IRC-assessed progression-free survival (PFS) was 5.68 months (95%CI: 5.49-8.25), while investigators-assessed PFS was 6.90 months (95%CI: 5.45-7.89). The median duration of response was 13.86 months (95%CI: 6.87-NR). The median OS was not reached and 1-year OS rate was 81.3%. The incidence of treatment-emergent adverse events of any grade occurred in 99.3% (137/138) patients. The most common treatment-related adverse events (>30%) were aspartate aminotransferase increased (65.9%), dry eye (52.9%), alanine aminotransferase increased (44.9%), blurred vision (36.2%), interstitial lung disease (ILD, 35.5%), platelet count decreased (32.6%) and hypokalemia (31.2%). 10.1% of patients had adverse events requiring treatment discontinuation and 27.5% had grade ≥3 adverse events of special interest, mainly containing blurred vision (10.9%), ILD (5.8%), dry eye (7.2%) and keratitis (3.6%). 1 case diagnosed with ILD was reported of treatment-related death. Overall, adverse events were consistent with the known safety profile with low incidence of hematological and gastrointestinal toxicity in the previous studies.
Conclusions: ARX788 showed encouraging efficacy and manageable toxicities in patients with HER2-positive advanced breast cancer previously treated with trastuzumab and anti-HER2 TKI.
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Clinical trial information: CTR20213419.
Research Sponsor: NovoCodex Biopharmaceuticals Co.,Ltd.

PS8-06: A randomized, open-label phase III study comparing disitamab vedotin (an anti-HER2 monoclonal antibody-MMAE conjugate) with lapatinib plus capecitabine in patients with HER2-positive, advanced breast cancer with liver metastasis

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Background: The prognosis of patients (pts) with HER2-positive, advanced breast cancer with liver metastasis is poor and there is no standard therapy recommended for this pts population. RC48-C006 is a randomized, multicenter, phase II/III clinical trial to compare disitamab vedotin (DV) versus lapatinib+capecitabine (L+C) in HER2-positive locally advanced or metastatic breast cancer in phase II part and in HER2-positive advanced breast cancer with liver metastasis in phase III part (ClinicalTrials.gov Identifier: NCT03500380). Here, we first report the phase III data.
Methods: Key inclusion criteria were histologically/cytologically confirmed breast cancer with radiologically confirmed liver metastasis, HER2-positive (defined as IHC 3+ or FISH+), and prior treatment with trastuzumab and taxanes. Pts were randomized at 1:1 (stratification factors: prior lines of chemotherapy [≤1 or 2], and lung metastasis [yes or no]) to receive DV (2.0 mg/kg IV) every 2 weeks or lapatinib (1250 mg, orally daily) + capecitabine (2000 mg/m2, orally daily on days 1-14) every 3 weeks until occurrence of disease progression or unacceptable toxicity. Pts in L+C group were allowed to cross over to receive DV after disease progression. Tumor was assessed every 6 weeks (±7 days) as per RECIST v1.1. The primary endpoint was IRC (Independent Review Committee)-assessed progression-free survival (PFS); secondary endpoints included investigator-assessed PFS, OS, ORR, and DoR. The data cutoff date for this analysis was December 31, 2023.
Results: As of the cutoff date, 104 pts from 39 sites in China were randomly assigned (53 in DV group and 51 in L+C group). At a median follow-up of 15.38 months for DV and 19.35 months for L+C, DV significantly improved IRC-assessed PFS vs L+C (median: 9.86 vs 4.90 months; HR: 0.561 [95%CI: 0.350-0.897]; P=0.0143), consistent with investigators' assessment (HR: 0.621 [95% CI: 0.392-0.985], nominal P=0.0418). OS was immature with 25 events to data cutoff (median: not evaluable [NE] vs 25.92 months for DV vs L+C; HR: 0.563 [95% CI: 0.246-1.289]); after adjusting for crossover (21 pts) using the two-stage method, the HR was 0.410 (95% CI: 0.189-0.944). Per IRC, ORR was 58.5% vs 54.9%, and median DoR was 11.20 vs 6.97 months for DV and L+C groups, respectively. Among all treated pts, 22 (41.5%) of 53 in DV and 20 (40.0%) of 50 in L+C experienced grade ≥3 treatment-emergent adverse events (TEAEs), mostly (incidence ≥5%) neutrophil count decreased (18.9% vs 10.0%), gamma-glutamyltransferase increased (9.4% vs 0.0%), white blood cell count decreased (5.7% vs 4.0%), hypertriglyceridemia (5.7% vs 4.0%), hypokalemia (3.8% vs 8.0%), hand-foot syndrome (0.0% vs 10.0%), and diarrhea (0.0% vs

6.0%). No TEAEs led to death in either group.
Conclusion: DV is the first HER2-targeting ADC that demonstrated significant PFS benefit vs L+C and an acceptable safety profile in pts population with HER2-positive breast cancer with liver metastasis. It provides a potential new standard treatment option for these pts.
Funding: RemeGen Co., Ltd., Yantai, China.

PS8-07: Efficacy and Safety of GQ1005, a Promising HER2-ADC, in Patients with Metastatic HER2-Positive Breast Cancer

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Introduction: GQ1005 is a novel HER2 antibody-drug conjugate (ADC) designed using advanced Ligase-Dependent Conjugation (iLDC) technology. This ADC features a highly stable linker, an improved safety profile, and an expanded therapeutic window. Here, we present the latest findings from the ongoing phase I clinical trial of GQ1005, focusing on the cohort with HER2-positive metastatic breast cancer (MBC).(NCT06154343)

Methods: Participants aged 18 years and older with locally advanced or metastatic HER2-positive MBC (IHC 3+; IHC 2+ and FISH positive) were enrolled. These patients had previously received and progressed on standard therapies and had measurable disease per RECIST v1.1 criteria. The primary objectives were to assess the safety, tolerability, pharmacokinetics, and antitumor efficacy of GQ1005. The drug was administered intravenously at doses of 2, 4, 6, 7.2, and 8.4 mg/kg every three weeks until intolerable toxicity or disease progression was observed.

Results: As of May 10, 2024, 60 HER2-positive MBC patients (6 mg/kg n=3; 7.2 mg/kg n=57) were enrolled, with a median age of 53 years. These patients had undergone multiple previous treatments, with a median of four prior anti-tumor therapies (range 1-11). All participants had received chemotherapy, 93.3% had received trastuzumab, 86.7% had received HER2 TKIs (nearly all received pyrotinib), and 63.2% had received other HER2 ADCs (including T-DM1, FS-1502, A-166, etc.). No dose-limiting toxicities (DLTs) were observed, and the maximum tolerated dose (MTD) was not reached. Adverse effects were manageable, mainly involving gastrointestinal toxicity, bone marrow suppression, and elevated transaminases. Grade 3 or higher treatment-related adverse events (TRAEs) occurred in only 5 patients (8.3%), including leukopenia (3.3%), hypokalemia (1.7%), and anemia (1.7%). Treatment was temporarily suspended in 2 patients due to grade 1 interstitial lung disease (ILD). In the 7.2 mg/kg group, no subjects experienced TRAEs necessitating dose reduction, permanent discontinuation, trial withdrawal, or resulting in death.

Efficacy assessments were conducted in 49 subjects (6 mg/kg n=3, 7.2 mg/kg n=46). The overall response rate (ORR) was 65.3% (95% CI: 50.36-78.33), with a confirmed ORR of

51.0% (95% CI: 36.34-65.58). The disease control rate (DCR) was 95.9% (95% CI: 86.02-99.50), with a confirmed DCR of 75.5% (95% CI: 61.13-86.66). The median progression-free survival (PFS) was 8.87 months (95% CI: 5.55-NA), and the median duration of response (DoR) was 7.52 months (95% CI: 4.37-NA). The median time to response (TTR) was 1.41 months (95% CI: 1.35-2.73), and the median follow-up time for overall survival (OS) was 4.21 months, with median OS not yet reached. In the 7.2 mg/kg group, ORR was 63.0%, DCR was 95.7%, median PFS was 10.91 months (95% CI: 5.55-NA), and the 9-month PFS rate was 69.14% (95% CI: 41.82-85.54%). OS was not reached.
Conclusion: GQ1005 demonstrates robust anti-tumor activity and a favorable safety profile in patients with heavily pretreated HER2-positive MBC. Notably, GQ1005 exhibits significantly superior safety profiles, attributable to its higher linker stability and minimal payload shedding in circulation. These promising results warrant further investigation in randomized, controlled phase III studies. (sponsored by GeneQuantum Healthcare (Suzhou) Co., Ltd.)

PS8-08: Efficacy and safety of SHR-A1811, an anti-HER2 antibody-drug conjugate (ADC), in 391 heavily pretreated multiple solid tumors with HER2-expression or mutations: a global, multi-center, first-in-human, phase 1 study

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Background: SHR-A1811, an anti-HER2 ADC comprising trastuzumab, a cleavable linker, and the topoisomerase I inhibitor payload SHR169265, has shown substantial tumor response and a manageable safety profile in heavily treated multiple solid tumors with HER2 expression or mutations (Yao. et al., JCO, 2024). Here we present for the first time the progression-free survival (PFS) analysis results of SHR-A1811 and updated safety results, including an additional 1-year of follow-up and an expanded cohort from 307 to 391 patients.
Methods: Patients eligible for this study had HER2-expressing or mutated unresectable, advanced, or metastatic solid tumors and were refractory or intolerant to standard therapies. SHR-A1811 was administered intravenously at doses ranging from 1.0 to 8.0 mg/kg every 3 weeks. Primary endpoints included dose-limiting toxicity, safety, and the recommended phase 2 dose.
Results: Between Sep 7, 2020 and Jan 12, 2024, 391 patients received SHR-A1811 treatment, including 136 HER2-positive breast cancers, 110 HER2 low-expressing breast cancers, 42 biliary tract cancers, 39 urothelial carcinomas, 22 gynecological cancers, 14 colorectal cancers (CRC), 13 gastric or gastro-esophageal junction adenocarcinomas (GC/GEJ), 4 non-small cell lung cancers (NSCLC), and 11 other types of solid tumor. These

patients had undergone a median of 3 (IQR 2–5) prior treatment regimens for metastatic disease. Of these, 261 (66.8%) had an ECOG performance status of 1, 196 (50.1%) had liver metastasis, and 186 (47.6%) had lung metastasis. As of data cutoff (Feb 29, 2024), the median follow-up for HER2-positive breast cancer, HER2-low breast cancer, and non-breast tumor was 13.4, 9.5, and 6.3 months (mo), respectively. The adverse events remained consistent with previous findings in terms of frequency, severity, and specificity. No new safety signals were identified. Grade ≥3 treatment-related adverse events (TRAEs) were reported in 247 patients (63.2%) and 26 patients (6.6%) discontinued treatment due to TRAEs. Incidence of interstitial lung disease was limited, occurring in only 10 patients (2.6%), predominantly at grade 1–2. Of the patients whose tumor responses were evaluable, the confirmed objective response rate (ORR) was 79.1% (95% CI 71.2–85.6) in HER2-positive breast cancer, 62.0% (95% CI 52.2–71.2) in HER2-low breast cancer, and 40.0% (95% CI 31.5–49.0) in non-breast tumor. Responses were durable, with median duration of response (DoR) of 23.6 mo (95% CI 15.6–NE), 12.2 mo (95% CI 7.3–NE), and 15.2 mo (95% CI 9.9–20.9), respectively. Median PFS was 20.0 mo (95% CI 15.1–NE) in HER2-positive breast cancer, 11.0 mo (95% CI 8.2–13.7) in HER2-low breast cancer, and ranged 3.4–8.5 mo in various non-breast tumor types. In breast cancer patients with liver metastasis, the median DoR and PFS (HER2-positive: not reached for DoR, 20.0 mo for PFS; HER2-low: 10.8 mo for DoR, 10.9 mo for PFS) were consistent with the total breast cancer population. Similarly, for breast cancer patients with visceral metastasis, the median DoR and PFS (HER2-positive: 23.7 mo for DoR, 21.9 mo for PFS; HER2-low: 9.9 mo for DoR, 9.8 mo for PFS) also aligned with the overall breast cancer population. Additionally, patients with HER2-positive non-breast tumors showed a trend of better efficacy compared to the overall non-breast tumor cohort in terms of ORR (45.1%), DoR (median 15.2 mo), and PFS (median 7.9 mo).
Conclusions: This updated analysis reaffirms the manageable safety profile and promising efficacy of SHR-A1811 in heavily pretreated multiple solid tumors with HER2 expression or mutations. Pivotal study results are highly expected in HER2-positive breast cancer, HER2-low breast cancer, CRC, GC/GEJ, and NSCLC.

PS8-09: Zanidatamab in combination with evorpacept in HER2-positive and HER2-low metastatic breast cancer: Results from a phase 1b/2 study

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Background: Patients (pts) with HER2-expressing breast cancer (BC) are in need of novel therapies, including chemotherapy-free treatments and more options post-progression on available therapies, such as trastuzumab deruxtecan (T-DXd). Zanidatamab (zani) is a HER2-targeted bispecific antibody that binds two domains on HER2, leading to crosslinking of adjacent HER2 molecules and multiple mechanisms of action, including inhibition of

growth signaling, complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and antibody-dependent cellular phagocytosis (ADCP). Evorpacept (evo) is a high-affinity CD47-blocker with an inactive IgG Fc region designed to enhance ADCP. Here, we report results from a phase 1b/2 trial of zani + evo in advanced HER2-expressing cancers.

Methods: This two-part, open-label, multicenter study (NCT05027139) included pts with previously treated, inoperable, locally advanced, or metastatic (m) HER2-expressing (by local or central assessment) cancers. Part 1 evaluated safety/tolerability and established the recommended dose (RD) for Part 2. The primary objective in Part 2 was to evaluate the antitumor activity by cORR per RECIST 1.1 in HER2-positive (HER2+) mBC (cohort 1), HER2-low mBC (IHC1+ or IHC2+/ISH negative; cohort 2), and other HER2-overexpressing advanced tumors (cohort 3). DCR, DOR, PFS, OS, and safety were secondary endpoints. Prophylactic treatment for infusion-related reactions (IRRs) was mandatory. Based on the IRR rate in the first 25 pts enrolled, the protocol was amended to reverse the dosing order to zani followed by evo.

Results: As of March 27, 2024, enrollment was complete with a total of 52 pts; 44 at the RD (cohort 1, n=21; cohort 2, n=15; cohort 3, n=8). Median age (range) was 59 (34-81) yrs; 15 pts (29%) had a history of brain metastasis, and 15 pts (29%) had de novo metastatic disease. By central HER2 assessment, 9/21 (43%) pts in cohort 1 had HER2+ mBC and 14/15 (93%) pts in cohort 2 had HER2-low mBC. The median number of prior systemic regimens in the metastatic setting was 6 in cohort 1, 5 in cohort 2, and 3.5 in cohort 3. All pts in cohort 1 and 5 pts in cohort 2 had prior T-DXd. Median follow-up was 7 months, with 8 pts on treatment at data cutoff.

In Part 1, there were two dose-limiting toxicities, both grade 3 IRRs that resolved following treatment discontinuation. The Part 2 RD was zani 1200 mg (pts <70 kg) or 1600 mg (pts ≥70 kg) + evo 30 mg/kg IV Q2W.

Among all 52 pts, treatment-related (zani or evo) adverse events (TRAEs) of any grade occurring in ≥20% of pts were diarrhea (62%), fatigue (31%), nausea (27%), and IRRs (21%). Grade 3 TRAEs occurring in ≥2 pts were diarrhea (6%) and IRRs (4%). There were no grade 4 or 5 TRAEs. Three (6%) pts had serious TRAEs (dyspnea, GGT increase, and IRR; 1 pt each). TRAEs of special interest included 1 (2%) pt with a grade 2 LVEF decrease and 12 (23%) pts with IRRs, 11 of whom had the event before the dosing order was reversed. No non-infectious pulmonary toxicities occurred. TRAEs led to treatment discontinuation and dose reductions in 2 pts (4%) each.

Among response-evaluable pts treated at the RD, in cohort 1 (n=19) the cORR (95% CI) was 37% (16, 62) and the DCR (95% CI) was 74% (49, 91). The median DOR (range) was not reached (2-22 months). In the 9/19 pts in cohort 1 with centrally confirmed HER2+ mBC, the cORR (95% CI) was 56% (21, 86) and the DCR (95% CI) was 78% (40, 97). For cohort 2 (n=15), the cORR (95% CI) was 20% (4, 48) and the DCR (95% CI) was 40% (16, 68). The median DOR (range) was 6 (4-7) months. Additional efficacy results (including PFS) will be presented at the congress.

Conclusions: Zani + evo showed a manageable safety profile and promising antitumor

activity, particularly in pts with heavily pretreated HER2+ mBC, including prior exposure to T-DXd. Further development of this novel chemotherapy-free regimen is warranted.

PS9-01: Actionable Genomic Alterations in Localized Hormone Receptor Positive (HR+) Breast Cancer and Impact on Clinical Outcomes: Results from Comprehensive Whole Exome Sequencing (WES) and Tumor-Informed circulating tumor DNA (ctDNA) analysis.

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Background: The development of targeted therapies directed towards particular tumor genomic alterations has revolutionized the treatment of HR+ breast cancer in the metastatic setting, yet little is known about whether these agents have utility in the early-stage setting. Here, we conducted a comprehensive analysis using data from tumor-informed minimal residual disease (MRD) testing to identify actionable genomic alterations and ctDNA dynamics in localized HR+ breast cancer and evaluate the impact on clinical outcomes.
Methods: Actionable genomic alterations were analyzed in tumors from 1639 women in the United States with early-stage (stage I-III) HR+/HER2- breast cancer who had ctDNA testing via a clinically-validated, personalized, tumor-informed 16-plex PCR-NGS ctDNA assay (Signatera™, Natera, Inc.). Targetable alterations, including somatic mutations in PIK3CA, AKT, PTEN, BRCA1/2, ESR1, and ERBB2, were assessed via whole exome sequencing (WES) of tumor tissue performed for generation of patients' personalized plasma MRD assays. All tests were ordered in the real-world clinical setting. For a subset of patients, distant recurrence-free survival (DRFS) events (including distant relapse and date of either last follow up or death) were obtained by review of electronic medical records. DRFS was evaluated by the presence or absence of actionable genomic alterations as well as MRD status (ctDNA + vs -). Timing of MRD testing was divided into three discrete categories of 0-2 years, 2-5 years, and >5 years after primary breast cancer surgery.
Results: 1639 patients with early-stage HR+ HER2- breast cancer and tumor-informed ctDNA testing were included in the analytical dataset. Included patients had a median of 5 ctDNA tests performed (range: 1-33). The first ctDNA test was performed within 2 years after surgery for 900/1639 (54.9%) patients, between 2-5 years for 467/1639 (28.5%), and after 5 years for 272/1639 (16.6%) patients. 287/1639 (17.5%) had >1 positive ctDNA (ctDNA+) test. Of patients with ctDNA+, 127/287 (44.2%) had at least 1 targetable genomic alteration including PIK3CA (99/287, 34.5%), AKT (14/287, 4.9%), PTEN (9/287, 3.1%), BRCA1/2 (1/287, 0.3%), ESR1 (5/287, 1.7%), and ERBB2 (10/287, 3.5%). Of ctDNA+ patients with PIK3CA mutations, 32/99 (32.3%) first were ctDNA+ 0-2 years post-surgery, 34/99 (34.3%) between 2-5 years and 33/99 (33.3%) >5 years. DRFS data were available for 205 patients. In the patients with ctDNA+ 0-2 years after surgery, the median DRFS was

34.7 months vs not reached in the ctDNA- patients. For patients with ctDNA+ testing 0-2 years post-surgery, stratifying by PIK3CA status, those with PIK3CA-mutated tumors had a median DRFS of 23.67 months vs 34.72 months in PIK3CA WT (reference: ctDNA- patients; PIK3CA mutated/ctDNA+: HR=36.9; PIK3CA WT/ctDNA+: HR=16.3). The 5 year DRFS in patients with ctDNA+ was 25% vs 92% in ctDNA-. In patients with PIK3CA mutation and ctDNA+, the 5 year DRFS was 20% vs 29% in those with PIK3CA WT.
Conclusions: To our knowledge, this is the first analysis incorporating comprehensive WES data with tumor-informed ctDNA analysis to evaluate the impact of targetable genomic alterations on ctDNA detection and DRFS in early-stage HR+ breast cancer. Overall, 44% of patients with ctDNA+ had at least one targetable genomic alteration. Among patients with early ctDNA positivity, those with PIK3CA mutated tumors tended to have worse early DRFS than those with PIK3CA WT tumors. Overall, this analysis provides the landscape of actionable genomic alterations in localized HR+ breast cancer and guides future therapeutic interception trials to potentially improve outcomes for patients with high recurrence risk due to MRD.

PS9-02: Serial circulating tumor DNA (ctDNA) assessment to predict treatment response and identify genomic evolution in patients with metastatic breast cancer (mBC)

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Background: Next generation sequencing of ctDNA can provide a comprehensive assessment of a tumor's genomic landscape. ctDNA is non-invasive, enabling serial liquid biopsies to monitor for clinical response and resistance over time. The Individualized Molecular Analyses Guide Efforts in Breast Cancer (IMAGE) II study evaluated clinical utility of serial ctDNA assessment in patients (pts) with mBC.
Methods: IMAGE II (NCT02965755) is a prospective, multi-center trial for pts with mBC of any subtype planning to initiate a new line of therapy (LOT). Plasma samples from baseline, 1-2 weeks after next LOT initiation, first restaging, and progression underwent comprehensive genomic profiling (CGP) by Foundation Medicine. Baseline plasma CGP and clinical tumor tissue sequencing results were reviewed in real time by the Johns Hopkins Genetic Alteration In Tumors With Actionable Yields (GAITWAY) Tumor Board to provide treatment recommendations. ctDNA tumor fraction (TF) was calculated using FoundationOne®Liquid CDx. Maximum variant allele frequency (max VAF) was calculated by filtering out variants with VAF 40-60% or >90% and variants in genes commonly associated with clonal hematopoiesis (CH). We examined TF and maxVAF dynamics vs clinical response at first restaging as extracted from clinical documentation and dichotomized into stable/responding versus progressing. We also identified alterations that were emerging (not detected at baseline, detected subsequently), disappearing (present at baseline, not detected later), or persistent (present throughout). Statistical

significance was assessed by χ-squared analysis.
Results: Enrollment was 197 pts: 136 ER/PR+ and HER2-, 21 HER2+, and 40 ER/PR- and HER2-. Most (193) were women, and median age was 57 years (range 27-86). Median prior metastatic LOT was 2 (44 pts with 4+ prior LOT). TF was estimated for 84 samples at baseline and 107 at week 1-2. At time of first restaging, 44% of patients who had a clinical response had a decrease in TF at week 1-2 by 90% from baseline, compared to only 9% of patients who had clinical progression (p=0.028). MaxVAF was estimated for 178 baseline samples and106 at week 1-2. A decrease in maxVAF at week 1-2 by 90% compared to baseline was not associated with clinical response first restaging (23% vs. 12%, p=0.2715). A total of 5566 alterations were detected in 397 baseline and serial samples. Of the 958 alterations not classified as variants of unknown significance (non-VUS) detected in 122 patients with plasma from more than one time point, 408 (43%) were emerging, 165 (17%) were disappearing, 268 (28%) were persistent and 117 (12%) were intermittently detected. Genes most commonly identified as having non-VUS emerging mutations were DNMT3A (39 alterations), TP53 (26), BRCA2 (22), ATM (20), and PALB2 (18) whereas non-VUS disappearing mutations occurred inTP53 (31), ESR1 (23), PIK3CA (19), PALB2 (14), and BRCA2 (12). When restricting analysis to only oncogenic alterations in clinically actionable genes in breast cancer (AKT, BRCA1, BRCA2, ERBB2, ESR1, PIK3CA, PTEN), 33 pts (27%) had emerging mutations, most frequently in BRCA2 and ESR1, while 35 pts (27%) had disappearing mutations, most frequently in ESR1 and PIK3CA. Emerging mutations were most often first detected at progression, while disappearing mutations were most often lost at week 1-2.
Conclusions: Decrease in TF, but not maxVAF, at 1-2 weeks after starting next therapy is correlated with clinical response at time of restaging and can provide an early assessment of treatment response. Genomic evolution with both emerging and disappearing alterations, including in genes clinically actionable in breast cancer as well as genes likely associated with CH, was frequently identified by serial liquid biopsies, both at time of progression and while on treatment.

PS9-03: Circulating tumor DNA (ctDNA), dormant disseminated tumor cells (DTCs) and recurrence outcomes in breast cancer survivors on the SURMOUNT Study

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Background: Recurrence after early-stage breast cancer (BC) is a challenge, occurring in ~30% of patients (pts). Recurrences may arise from reactivation of disseminated tumor cells (DTCs) persisting in a dormant state after primary treatment. The presence of minimal residual disease (MRD) as bone marrow DTCs and/or circulating tumor DNA (ctDNA) in the

blood increases the risk of BC recurrence/death. It remains unclear which pts with DTCs will have these reactivate or develop detectable ctDNA before clinical relapse. We evaluated the association and temporal relationship of ctDNA with DTCs in a population of high-risk BC survivors, and the relationship of these markers with subsequent metastatic recurrence.
Methods: "PENN SURMOUNT" is a single center, prospective, longitudinal cohort study examining MRD biomarkers among pts within 5 years (y) of BC diagnosis who completed all curative treatment except endocrine therapy. Eligible pts must have had: 1) TNBC, or 2) HER2+ or HR+ BC with positive LN and/or residual disease after neoadjuvant therapy, or 3) HR+ BC with a 21-gene Recurrence score >25 and/or high risk Mammaprint. Pts had annual bone marrow aspirate (BMA) for DTCs by immunohistochemistry (using methods of Naume et al.). DTC+ pts went on therapeutic trial; DTC- pts had up to 5y of annual BMA and blood testing. ctDNA was retrospectively assessed using the RaDaR assay, which targets pt-specific somatic mutations identified by whole-exome sequencing (WES) of primary tumor tissue.
Results: Of 184 pts enrolled from 2016 – 2021, 121 had tissue available; 114/121 (94%) had successful WES. A total of 338 plasma samples from 96 pts (median 2 timepoints each, range 1-12) have been successfully tested by RaDaR to date. Overall, ctDNA was detected in 11 samples from 9/96 pts (9.3%) with a median eVAF of 0.009% (range 0.002-0.084%). Two pts were ctDNA+ at baseline (BL), and 7 became positive on surveillance. 87/96 (90.6%) were ctDNA- across all timepoints. 34/96 pts (35%) were DTC+, either at BL (n=24, 25%) or after (n=10, 10%). Considering all timepoints, concordance was 64%. Of 34 ever-DTC+ pts, 4 (12%) were ctDNA+ (of whom 3/4 recurred) and 30 remained ctDNA- (with 1/30 who recurred). Among the 62 pts who remained DTC-, 5 (8%) were ctDNA+ (with 5/5 who recurred), and 57 remained ctDNA- (of whom 5/57 recurred). All ctDNA positivity in DTC+ pts occurred at the time of or after DTC positivity. Over median follow-up (f/u) of 65 months (m), BC recurrence occurred in 14/96 pts (15%), with 2 locoregional-only and 12 distant +/- locoregional recurrences (involving the bone, liver, lung/pleura, and brain); 8/14 pts (57%) were ctDNA+ prior to relapse. 7/12 (58%) with distant recurrences were ctDNA+ prior to metastatic diagnosis, at a median lead time of 15 m (range 0 – 25). Overall, ctDNA+ pts experienced a median lead time from ctDNA positivity to recurrence of 13 m (range 0 – 25). Only 1 of 9 ctDNA+ pts has not recurred; this pt was DTC+ and went on therapeutic trial, without evidence of recurrence over 20 m f/u. 30/34 DTC+ pts (89%) who went on therapeutic trial have not had ctDNA detected during f/u and have not recurred. Overall, ctDNA status was significantly associated with relapse ($p<0.01$), with a PPV of 89% and NPV of 93%. Of the 24 BL DTC+ pts, 2 became ctDNA+ at subsequent timepoints, an average of 18 m after DTC assessment, and both relapsed (3 and 5 m from ctDNA detection, respectively).
Conclusions: In this surveillance study of high-risk BC pts, DTC+ pts were identified who subsequently developed detectable ctDNA and clinical relapse. Where there were discordant results, the timing of DTC and ctDNA positivity revealed a window of opportunity for intervention. A strategy combining both markers for surveillance and intervention to prevent metastatic disease may be of value.

PS9-04: Evaluating racial genomic differences in de novo metastatic breast cancer utilizing ctDNA: results from a large multi-center consortium

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Background: Prior studies have shown that Black patients (pts) are more likely to present with de novo metastatic breast cancer (mBC) compared to White pts and have worse survival outcomes. Our prior research demonstrated some somatic differences in circulating tumor DNA (ctDNA) mutation profiles between Black and White pts with mBC (Podany et al., SABCS 2023). However, there remains a research gap in exploring somatic differences between Black and White pts with de novo versus recurrent mBC.
Methods: This retrospective cohort study included pts with mBC who underwent genomic profiling using the commercially available Guardant360 ctDNA assay. All pts were treated at Washington University in St. Louis, Massachusetts General Hospital, or Northwestern University. Race was pt reported and ancestry data were not available. Clinical and pathologic data were obtained through electronic medical record review. We performed descriptive analysis of clinical variables and pathway variants in Black and White pts with de novo mBC, then performed univariate, multivariate, and multinomial analyses to evaluate single gene mutations and pathway variants within the entire cohort and the estrogen receptor positive, HER2 negative (ER+/HER2-) subpopulation. We then performed a logistic regression to determine clinical and genomic differences between Black pts with de novo mBC versus recurrent mBC.
Results: 274 of 1134 pts (24.2%) had de novo mBC in the overall cohort, 33 of whom self-identified as Black (12.0%) and 210 as White (76.6%). 26.2% of pts had ctDNA collection prior to any therapy, 24.0% after 1st line therapy, and the remaining pts after 2 or more lines of therapy. In the ER+/HER2- population, there were 193 pts (193/855, 22.6%) with de novo mBC, of whom 29 were Black (15.0%). ctDNA results from Black pts with de novo mBC showed a higher frequency of CCND1 copy number variants (cnv) (OR 4.08, CI 1.49-11.15, p=0.006) and GATA3 single nucleotide variants (snv) (OR 3.11, CI 1.10-8.78, p=0.032) on univariate analysis when compared to White pts with de novo mBC. The CCND1 cnv remained significant (OR 3.64, CI 1.30-10.17, p=0.014) on multivariate analysis, controlled for lines of therapy. Black pts with de novo mBC also had a higher frequency of mutations within the cell cycle pathway (OR 2.59, CI 1.15-5.83, p=0.022). On multivariate analysis within the ER+/HER2- cohort, Black pts with de novo mBC were more likely to have nodal metastases (OR 2.63, CI 1.07-6.44, p=0.035), GATA3 snv (OR 3.99, CI 1.23-12.95, p=0.021), CCND1 cnv (OR 3.91, CI 1.21-12.63, p=0.023), and cell cycle pathway mutations (OR 3.19, CI 1.17-8.71, p=0.023) when compared to White pts with ER+/HER2- de novo mBC. When compared to Black pts with recurrent mBC, Black pts with de novo mBC were significantly more likely to have cell cycle pathway mutations (OR 2.71, CI 1.03-7.13, p=0.044) and less likely to have lung metastases (OR 0.33, CI 0.12-0.90, p=0.030). We observed consistent results when performing multinomial analysis comparing de novo

versus recurrent mBC across Black and White pts.
Discussion: Our findings in this large multi-institutional clinical genomic ctDNA database demonstrate that GATA3 snv, CCND1 cnv, and cell cycle pathway alterations were more common in Black versus White pts with de novo ER+/HER2- mBC, all of which have been associated with more aggressive tumor biology. Moreover, in Black patients, cell cycle pathway alterations were more common in de novo versus recurrent mBC. These differences in tumor genomics may partially explain outcome inequities between Black and White pts, and we are further exploring these findings in the context of social determinants of health.

PS9-05: Somatic Structural Variation in Breast Cancer and its Application in Longitudinal Analysis of Circulating Tumor DNA in Early Breast Cancer

Mitchell Elliott, Karen Howarth, Sasha Main, Jesús Fuentes Antrás, Philippe Echelard, Aaron Dou, Eitan Amir, Michelle B. Nadler, Elizabeth Shah, Celeste Yu, Scott Bratman, Taylor Bird, June Roh, Elza C. de Bruin, Christopher Rushton, Sofia Birkeälv, Miguel Alcaide, Lucia Oton, Sergii Gladchuk, Yilun Chen, Anthony George, Girish Putcha, Samuel Woodhouse, Philippe L. Bedard, Lillian L. Siu, Hal K. Berman, David W. Cescon

Background: Genomic structural variation (SV) is a recognized property of cancer cells, contributing to genomic instability and oncogenesis. SV breakpoints and rearrangement patterns are often tumor-specific and can reflect underlying tumor biology. The landscape and implications of SV in breast cancer has been incompletely characterized. Furthermore, the utility of using SVs for prospective circulating tumor DNA (ctDNA) detection and monitoring in early breast cancer (EBC) has not been evaluated.
Methods: SV was evaluated through whole genome sequencing (WGS) analysis in two independent breast cancer datasets: (1) The 100,000 genomes project [n=3044 patients; Genomics England, GEL], made possible through access to data and findings in the National Genomic Research Library via the Genomics England Research Environment and (2) a cohort of patients with EBC treated with neoadjuvant chemotherapy [ctDNA evaluation in early breast cancer (TRACER; n=210 patients) Princess Margaret Cancer Centre, Canada]. SV burden and type was evaluated in the GEL dataset using MANTA and in TRACER using an in-house pipeline (SV size cut-off of >50 bp). Survival correlates within the GEL dataset were evaluated using Cox proportional hazard models with cases censored after 6 years. The performance of an SV-based ctDNA assay was evaluated in BT-474 (cell line, contrived samples) and in FFPE tumors (TRACER, clinical samples) using shallow depth (~15X) WGS followed by SV detection (up to 16) via proprietary multiplex digital PCR. Longitudinal ctDNA detection was performed in the TRACER cohort.
Results:
SV was common across all breast cancer subtypes (GEL data; median SV burden: 108, range: 4-1448; median aggregate copy number of the top 16 SV: 57, range: 16-160). A higher proportion of inversions were seen in HER2-positive tumors, while deletions were common in TNBC. In a multivariate model (including clinical stage, subtype, and tumor mutational

burden), there was a significant association between higher SV count and worse overall survival (OS) in ER+/HER2- breast cancer (HR: 2.31, p=0.0207). Patients with ER+/HER2- breast cancer who experienced a clinical recurrence had higher SV copy number in the top 16 variants than those who did not (p<0.0001). In silico analyses demonstrated that personalized SV-based ctDNA panels (fingerprints) could be successfully designed for 97.1% of GEL cases. To assess the characteristics of an SV-based ctDNA assay, a limit of detection (LoD) study was performed with BT-474 using contrived cfDNA (70 ng). The LoD95 was estimated at 0.00052% tumor fraction (5 PPM) with variants detected as low as 1 in 10 million (0.00001% or 0.1 PPM in 31% of cases). A specificity of 100% was seen in 134 healthy donors using 24 different fingerprints (assessment of 1600 SVs). In an initial cohort of 55 patients (TRACER; ER+/HER2-:19, HER2+:23, TNBC:13), shallow WGS and fingerprint design were successful in all patients. The median number of SVs was 336 (range: 73-1345) with the SV type distributed in a similar fashion (more inversions in HER2+ tumors, deletions in TNBC). A trend towards higher SV copy number based on tumor-only WGS was also seen in those with ER+/HER2- disease who experienced subsequent recurrence (TRACER; SV in no recurrence vs. recurrence: 54.3 vs. 134.1, p=0.099). Shallow WGS and SV-based ctDNA assay design is underway for additional patients in the TRACER cohort.
Conclusion:
SVs are prevalent in breast cancer and associated with prognosis. Personalized SV-based panels permitted ultrasensitive ctDNA detection with high sensitivity and specificity in contrived samples, supporting assay feasibility for early breast cancer. Further analysis of the prognostic impact of SV-burden and type as well as on-treatment and adjuvant ctDNA detection in patients with EBC (TRACER) using SV tracking will be presented at the meeting.

PS9-06: Tracking structural variants in ctDNA using a high-sensitivity assay predicts relapse in the post-neoadjuvant setting: the multicenter ALIENOR trial

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Background: Patients (pts) with breast cancer (BC) not achieving a pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) are at higher risk of relapse. The multicenter ALIENOR study (NCT03357120), conducted in patients who did not achieve pCR after NAC, is aiming to assess the prognostic value of serial ctDNA monitoring with a novel personalized, tumor-informed ctDNA assay predicated on detection of structural variants (SVs) in plasma. Here, we report the first of two pre-planned analyses from this study (3-years from last patient enrolled).
Methods: Pts with cT2cN1, cT3cN0-1 or locally advanced (cT4cN0-3 or cT1-4cN2-3) M0 BC of any immunohistochemical subtype not reaching pCR after NAC (6 to 8 cycles) were eligible. Pts were enrolled into a 5-year plasma surveillance period (first plasma sample

taken 10 to 63 days after surgery, then every 6 months for up to 5 years). A clinical assessment was performed every 6 months. Tumor blocks from surgical specimens were centrally assessed by one investigator (GMG). Tumors with >20% invasive tumor cells and at least 100 ng of DNA were evaluable. Whole genome sequencing (WGS) was performed on tumor material and personalized multiplex dPCR assays designed tracking up to 8 structural variants (SVs) for use in ctDNA analyses on all available plasma time points (SAGA Diagnostics; Lund, Sweden).
Results: 119 pts were eligible for enrollment and 87 were available for ctDNA analyses. 79 of 87 pts (91%) underwent successful SV-personalized panel design (median of 7, range 4-8 SVs per patient): 463 plasma samples underwent ctDNA testing. BC subtypes included: 41 luminal HER2-, 20 HER2+ and 18 triple-negative. At a median follow-up of 54.5 months (95% CI: 53.3-56.2) from surgery, 21 of 79 pts relapsed (distant: 19 pts, locoregional: 2 pts, distant and locoregional: 1pt). Pts with detected ctDNA, whether at any time point in surveillance (n=32) or at a landmark pre-adjuvant time point (10 to 63 days post-surgery) (n=15) had shorter recurrence-free interval (RFI): HR 20.6 (95% CI 4.8-88.8; $p<0.0001$) and 2.5 (95% CI 1.0-6.2; $p<0.05$), respectively. The median lead-time from ctDNA detection to clinical relapse was 12.2 months (range 3.4-60.6). ctDNA was detected at any time-point in 90.5% (19/21) of pts who relapsed. Of 32 pts with ctDNA detected at any time point, 19 relapsed (59.4%) and 13 are still relapse-free at last follow-up. In this later group, 6 pts positive at landmark time point only, were subsequently cleared in all time points, 3 pts positive at the last visit time point only, are pending longer follow up, and 4 pts had multiple positive ctDNA time points including several with rising levels of ctDNA. Of 47 pts with no ctDNA detected, 2 relapsed (4.3%): one with local relapse; one with brain-only metastasis. The univariate analysis showed that clinical stage, BC subtype, RCB, and age were not statistically associated with RFI.
Conclusions: Serial ctDNA assessment using a novel SV-based assay is predictive of relapse with lead-times of up to 61 months over clinical relapse. These data support further evaluation of this assay in prospective trials. Landmark-only ctDNA detection followed by clearance on therapy might represent micrometastatic disease controlled or eradicated by adjuvant treatment. Longer follow-up is required in cases with ctDNA detection without clinical relapse to fully characterize clinical accuracy and lead-times associated with SV-based ctDNA surveillance.

PS9-07: Epigenomic Characterization of ER Transcriptional Activation via Liquid Biopsy

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Background:Endocrine therapies (ET) are a cornerstone treatment for estrogen receptor-positive (ER+) breast cancer (BC). ET's efficacy depends on the transcriptional addiction of

the cancer cells to estrogen receptor signaling, for which there is no current clinical test. ER IHC and ESR1 mutations are insufficient proxies for ER transcriptional dependency. A blood-based biomarker for dynamic measurement of the ER transcriptional dependency of a tumor would greatly improve our ability to identify patients who would most benefit from continued ET.

Methods:We profiled key epigenomic determinants of ER transcriptional activity in a broad array of ER+ BC cell lines, and then translated the resulting epigenomic signature into a form assayable in 1mL of plasma from BC patients. First, we profiled the epigenome of 8 breast cancer cell lines (MCF7, T47D, BT483, CAMA1, HCC1428, ZR-75-1, MDA-MB-361) in estrogen depleted media and in the presence of physiologically relevant levels of estradiol (E2, 0.1nM). Using ChIP-seq for histone modifications associated with active promoters and enhancers paired with enrichment-based DNA methylation we identified the epigenomic loci most associated with response to E2. The concurrent plating and treatment of these cell lines makes this a unique resource for the identification of enhancers, promoters, and DNA methylation marks associated with transcriptional response to estrogen signaling. We then employed the Precede multi-analyte liquid biopsy assay, which profiles these same epigenomic features from 1mL of patient plasma and used proprietary computational methods to combine information across analytes to create a patient-specific ER activity quantification algorithm robust to varying levels of circulating tumor DNA fraction (ctDNA%). We analyzed plasma from 38 patients with ER+ metastatic BC seen at the OHSU Knight Cancer Institute who had blood drawn within 1 week of tumor biopsy with ER and HER2 IHC. Additionally, we profiled 48 patient samples with the same characteristics sourced from commercial biobanks.

Results:We identified an epigenomic signature that profiles ER activity in patient plasma samples. Through a paired genome-wide analysis of all cell lines' response to E2, we identified 2320 E2-upregulated enhancers, 334 E2-upregulated promoters, and—intriguingly—0 DNA methylation loci that responded to E2 modulation. E2 responsive enhancers and promoters are linked to genes associated with estrogen response, cell cycle, and epithelial to mesenchymal transition. Many of these sites are located adjacent to known ESR1, FOXA1, and GATA3 binding sites. Computational refinement and aggregation of a subset of the E2-induced enhancers enabled the creation of an ER activity score that can be assayed in patient plasma and is robust to clinically relevant levels of ctDNA%. Patients with ER+ tumors displayed higher ER activity scores compared to patients with ER- tumors, and scores were consistently high for patients with ESR1 mutations. This plasma-based ER activity score is highly correlated with previously published RNA-based ER activity scores (Guan et al. 2019) which require biopsy tissue.

Conclusions:ER transcriptional pathway activation correlates with changes to the epigenome that are detectable via Precede multi-analyte liquid biopsy assay, enabling a measurement of breast cancer dependence on endocrine signaling from 1mL of plasma through an ER activity score. This ER activity score is biologically interpretable and correlates with previous RNA-seq based predictors of ER activity. While ESR1 mutant patients score consistently high, a subset of ESR1 wildtype patients also demonstrate high

ER activity scores, implying a potentially expanded patient population for treatment with novel anti-endocrine agents such as SERDs.

PS9-08: Ultra-sensitive detection of circulating tumor DNA (ctDNA) in patients (pts) undergoing neoadjuvant endocrine therapy for hormone receptor-positive (HR+) early breast cancer (BC)

Albert Grinshpun, Derek Dustin, Mingyang Cai, Melissa Hughes, Molly DiLullo, McKenna Moore, Erica L Mayer, Eric P Winer, Nancy U Lin, Sara M Tolaney, Otto Metzger, Rinath Jeselsohn

Introduction: Neoadjuvant endocrine therapy (NET) is often used in HR+, HER2-negative primary BC to decrease tumor extent prior to surgery. In neoadjuvant chemotherapy treatment of HER2-positive and triple negative BC, the residual cancer burden (RCB) is prognostic and utilized for treatment decisions of adjuvant therapy. However, in HR+, HER2-negative BC, RCB is not a surrogate for sensitivity to NET and long term outcomes. Moreover, despite the approval of a CDK4/6 inhibitor for high risk early-stage HR+ BC, there are currently no biomarkers predictive of sensitivity to this class of drug in this setting.
This study aimed to investigate the potential utility of baseline ctDNA and dynamic changes during NET as a biomarker to help guide systemic treatment decisions.
Methods: The phase II PELOPS trial (NCT02764541) enrolled pts with clinical stage I-III HR+, HER2-negative BC and randomized to NET plus palbociclib or NET alone for 24 weeks prior to surgery; plasma samples were collected at baseline (BL) and prior to surgery (PS). Cell-free DNA was analyzed using Guardant Reveal powered by Infinity, a tissue-free, epigenomic assay that utilizes comprehensive methylome and DNA sequencing data for ctDNA detection. Categorical group comparison was performed using Fisher's exact test, and Mann-Whitney test were used to evaluate ctDNA dynamics.
Results: A total of 104 plasma samples from 54 pts were analyzed with successful sequencing achieved in 95.0% (95/100) of samples. At BL and PS, the detection rates were 20/50 (40%) and 6/45 (13.3%), respectively. Overall, ctDNA was detected in 27.4% (26/95) of samples. Higher tumor fraction at BL (measured as methylation score) was associated with pathological stage at surgery (median 0.049% for stage III disease vs. 0% for stage I/II, $p = 0.0006$) and higher RCB scores following NET (median 0.049% for RCBIII vs. 0% for RCBI-II, $p = 0.049$). Using binary detection methods, BL ctDNA was detected in 0% (0/3) pts with RCBI, whereas BL ctDNA was detected in 20.8% (5/24) of pts with RCBII, and 61.1% (11/18) of pts with RCBIII (RCBI-II vs. RCBIII $p = 0.005$). Comparison of ctDNA between matched BL and PS samples showed that most pts (63.4%, 26/41) with undetectable ctDNA at BL remained with undetectable ctDNA post NET (PS). Six (14.6%, 6/41) pts, all with lobular BC and RCBIII, had persistent ctDNA at both timepoints. Within this subset of pts, 67% (4/6) developed metastatic disease recurrence within 5 years of surgery. Interestingly, there were no pts (0%, 0/41) that became ctDNA positive at PS, and 22% (9/41) pts experienced ctDNA clearance from BL to PS.

Somatic variants were identified in 27% (7/26) of ctDNA detected samples. There were alterations in five oncogenes at BL (PIK3CA, MAP3K1, TERT, RHOA, AKT1), and two alterations were acquired after NET (ATM, ESR1 D538G).
Conclusions: Ultra-sensitive tissue-free ctDNA testing can detect ctDNA in a substantial proportion of pts with primary HR+ BC. The presence of ctDNA at BL in this population was associated with larger pathologic tumor size and higher RCB scores following NET. Furthermore, persistence of ctDNA after NET was associated with high rates of recurrence. This study is the first investigation into the dynamics of ctDNA during NET using the Guardant Infinity platform, and our findings suggest that ctDNA has the potential to provide valuable insights into tumor burden, sensitivity to ET, and the emergence of endocrine resistance mutations. These results are limited by the sample size but warrant further investigation of the role of ctDNA as a tool to predict sensitivity to ET and long term outcomes. Such a tool could be valuable in tailoring treatment approaches, particularly given the ongoing development of novel ETs and the addition of CDK4/6i in the adjuvant setting.

RF1-01: Effect of a weight loss intervention (WLI) on metabolic and inflammatory biomarkers in women with obesity and breast cancer: Results from the Breast Cancer Weight Loss (BWEL) Trial (Alliance)

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Background: Unfavorable metabolic and inflammatory parameters are associated with increased risk of recurrence, comorbidities, and cancer-specific and all-cause mortality in early-stage breast cancer. The BWEL trial (Alliance for Clinical Trials in Oncology A011401; NCT02750826) evaluates the impact of a lifestyle based WLI on invasive disease-free survival in 3180 women with stage II-III Her-2 negative breast cancer and a body mass index (BMI) of at least 27 kg/m2. Here we report the impact of the WLI on metabolic and inflammatory biomarkers.

Methods: BWEL participants were randomized to a health education (HE) alone control arm (n=1489) or to a 2-year WLI plus HE (n=1491). The WLI was delivered through telephone-based health coaching and focused on caloric restriction and increased exercise. Participants underwent collection of fasting (confirmed by self-report) blood at baseline, 6 and 24 months. Serum levels of c-reactive protein (CRP), insulin, and leptin were assessed centrally using commercial ELISA assay kits at Yale School of Public Health. Samples were run in batches with all samples from an individual patient analyzed in a single batch. The absorbance was determined at the wavelength of 450 nm with a correct reference

wavelength of 600 nm using a spectrometry (Biotek Instrument Inc, Winooski, Vermont). 10% of samples were run in duplicate, and a pooled serum sample was run in each plate for the calculation of intra-assay variation. Non-fasting samples were omitted from metabolic analyses. Summaries are reported as means (± standard deviation). Comparison of the mean changes from baseline between the treatment groups was determined with a two-sample t-test.

Results: At baseline, average BMI was 34.5 (±5.7) kg/m2, average age was 53.4 years (±10.6), and 57% of participants were postmenopausal at the time of diagnosis. Most participants were White (80.3%); 12.8% identified as Black and 7.3% as Hispanic. Fasting serum samples were available from 2725 participants at baseline, 2234 at 6 months, and 1230 at 24 months. There were no significant differences in levels of metabolic or inflammatory biomarkers between groups at baseline (Leptin: 40.2 [±23.1] ng/ml WLI vs 39.3 [±23.7] ng/ml HE; CRP: 4.2 [±2.8] mg/L WLI vs 4.5 [±2.9] mg/L HE; Insulin: 16.5 [±14.9] mIU/ml WLI vs 16.5 [±14.3] mIU/ml HE). Participants in the WLI arm experienced significant reductions in all metabolic and inflammatory biomarkers vs HE at 6 and 24 months. Leptin changed by -8.4 (±16.1) ng/ml at 6 months and -1.8 (±18.70) ng/ml at 24 months in the WLI vs +1.9 (±16.1) ng/ml and +4.6 (±20.5) ng/ml in the HE arm (both $p<0.0001$). CRP changed by -0.3 (±2.1) mg/L at 6 months and -0.4 (±2.4) mg/L at 24 months in the WLI vs +0.01 (±2.1) mg/L and +0.01 (±2.3) mg/L in the HE arm ($p=0.0004$ and 0.0014, respectively). Insulin changed by -2.4 (±17.2)mIU/ml at 6 months and -0.8 (±11.6) mIU/ml at 24 months in the WLI group vs + 0.9 (±14.7) mIU/ml and +1.9 (±14.7)mIU/ml in the HE arm ($p<0.0001$ and $p=0.0005$, respectively).

Conclusions: A telephone-based WLI led to significant improvements in metabolic and inflammatory biomarkers known to be associated with cancer recurrence, comorbidities, and survival in a cohort of participants with obesity treated for early breast cancer. Further follow-up of the BWEL trial will evaluate whether changes in biomarkers predict improvements in cancer outcomes.
Support: U10CA180821, U10CA180882, UG1CA189823; U24CA196171; U10CA180820 (ECOG-ACRIN), U10CA180868 (NRG Oncology); U10CA180863, CCS 707213 (CCTG), UG1CA189974 (SWOG); https://acknowledgments.alliancefound.org.

RF1-02: Palbociclib plus letrozole versus weekly paclitaxel, both in combination w/ trastuzumab plus pertuzumab, as neoadjuvant treatment for patients w/ HR+/HER2+ early breast cancer: primary results from the randomized phase II TOUCH trial (IBCSG 55-17)

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Background: HR+/HER2+ breast cancer (BC) is a biologically heterogeneous group characterized by low pathological complete response (pCR) to chemotherapy combined with anti-HER2 therapy and relative endocrine resistance in the neoadjuvant setting. CDK4/6 inhibitors (CDK4/6i) have recently shown potential for chemotherapy de-escalation when combined with endocrine and anti-HER2 therapy.
Methods: TOUCH is an international, open-label, phase II trial for post-menopausal patients (pts) with cT>1 cm, cN0 or cN1, HR+/HER2+ BC, randomized 1:1 to Paclitaxel (T) 80 mg/m2 iv on day 1,8,15 q28d for 4 cycles (arm A) or Palbociclib (Pal) standard dose/schedule for 4 cycles plus Letrozole (L) 2.5 mg/day for 16 weeks (arm B). All received 5 cycles of trastuzumab sc + pertuzumab iv (HP) standard dose q3w. Randomization was stratified by: a) age and G8 geriatric score (<65 y vs ≥65 y and G8 >14 vs ≥65 y and G8 ≤14); b) cN0 vs cN1. The primary objective was the interaction between a gene-signature of E2F pathway activity (RBsig) and pCR (ypT0/ypTis ypN0), hypothesizing higher pCR for tumors with RBsig high in T+HP, and for tumors with RBsig low in Pal+L+HP.
RNA-sequencing was performed from pre-treatment FFPE biopsies (NEBNext® UltraTM II Directional RNA Library Prep Kit with the Twist Human Core Exome + Custom IntegraGen Enrichment V2 capture System, Paired End 100b reads).
RBSig score was calculated by first standardizing each normalized gene measurement across all sequenced specimens and adding up standardized values for each gene. RbSig was dichotomized at the median. Treatment-by-RBSig interaction was estimated using logistic regression.
Results:
From October 2018 to July 2022, 145 pts were randomized (73 in arm A, 72 in arm B) in 37 centers from 4 countries. Median age was 69 years (IQR 63,73), with 99 pts (68%) being ≥65y of age (65 with G8 score >14, 34 with G8 ≤14); 132 pts (91%) had a ECOG PS 0; at diagnosis 109 pts (75%) were cN0 with a median cT of 30 mm (IQR 20-40); 76 pts (56%) had G3 tumors. More pts completed Pal vs T (94.4% vs 79.5%) and >95% completed 5 cycles of HP. Surgery was performed in 98.6% of the pts, BCS being more frequent in arm B vs A (82% vs 67%). pCR and other secondary efficacy endpoints were similar between arms. Overall, pCR was 32.9% (95%CI: 22.3-44.9) in arm A vs 33.3% (95% CI: 25.5-41.4) in arm B. RBsig was successfully determined in 115 pts who completed at least one full dose of treatment. Median RBsig was lower in arm B. There was no interaction between pCR and RBsig (p=0.18): pCR in RBsig high vs low was 31% (95%CI: 16.1-50) vs 42% (95%CI: 23.3-63.1) in Arm A, and 38% (95%CI: 20.2-59.4) vs 26% (95%CI: 11.9-44.6) in Arm B. No new safety signal was observed. Grade 3-4 adverse events were seen in 31.5% vs 54.1% of pts in arm A vs Arm B (mainly diarrhea in Arm A and asymptomatic neutropenia in Arm B).
Conclusions:
Although the primary hypothesis was not supported, TOUCH shows for the first time that, when combined with dual anti-HER2 blockade, a de-escalated chemo-free backbone of Pal+L yields pCR rates similar to weekly T. The lack of clinical impact of the increased rate of neutropenia of Pal+L suggests it might represent an attractive alternative for postmenopausal pts with HR+/HER2+ BC, especially older ones. Translational studies are ongoing to uncover potential gene-signatures with prognostic/predictive significance.

RF1-03: Three-year event-free survival (EFS) of the multicenter phase II TRAIN-3 study evaluating image-guided optimization of neoadjuvant chemotherapy duration in stage II and III HER2-positive breast cancer (BOOG 2018-01)

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Background Neo-adjuvant treatment with 6-9 cycles anti-HER2 based chemotherapy leads to excellent long-term survival in patients with stage II-III HER2+ breast cancer, but comes with important side effects. The TRAIN-3 study previously showed that one in three patients with hormone receptor negative (HR-) tumors and one in six with hormone receptor positive (HR+) tumors have an early pathologic complete response (pCR) after only three cycles of neo-adjuvant chemotherapy. Here, we present the results of the primary endpoint, 3-year event-free survival (EFS).
Methods TRAIN-3 is a single-arm, phase 2 study in 43 hospitals in the Netherlands. Patients with stage II–III HER2-positive breast cancer received up to nine cycles of neoadjuvant systemic therapy with paclitaxel, trastuzumab, carboplatin and pertuzumab (PTC-Ptz) and were referred for surgery once a complete radiologic response was seen on MRI. Locoregional and endocrine treatment followed national guidelines. Patients with a pCR completed one year of trastuzumab and pertuzumab as adjuvant treatment. Patients with residual invasive disease completed the 9 cycles of PTC-Ptz followed by 14 cycles of T-DM1. EFS was estimated using Kaplan-Meier statistics on an intention-to-treat basis for each HR subgroup separately. Three-year EFS rates of 88% for HR- and 90% for HR+ tumors were taken as reference. The study would be declared successful if no more than 38 events in the HR- subgroup and 34 events in the HR+ subgroup occurred after 700 patient years of follow-up.
Results 235 patients with HR- tumors received 1-3 (n=91, 38.7%), 4-6 (n=76, 32.3%) or 7-9 (n=68, 28.9%) cycles of PTC-Ptz. 232 patients with HR+ tumors received 1-3 (n=69, 29.7%), 4-6 (n=71, 30.6%) or 7-9 (n=92, 39.7%) cycles of PTC-Ptz. After a median follow-up of 39.7 months (IQR 34.8-45.4), 3-year EFS was 92.1% (95% CI 88.5-95.8) in the HR- subgroup and 92.0% (95% CI 88.5-95.6) in the HR+ subgroup. A total of 19 and 21 events were reported in patients with HR- and HR+ tumors respectively, meeting the primary endpoint. Events included 9 vs 8 distant recurrences, 7 vs 6 locoregional recurrences, 2 vs 4 non-breast second primary malignancies, 0 vs 3 second primary breast tumors, and 1 vs 0 non-breast cancer related death, in HR- and HR+ patients respectively. EFS rates in patients treated with 1-3 cycles of neoadjuvant systemic therapy were 96.0% (95% CI 91.6-100) in HR- and 97.1% (95% CI 93.3-100) in HR+ patients. Corresponding figures were 90.8% (95% CI 84.5-97.5) and 92.8% (95% CI 87.0-99.1) in patients treated with 4-6 cycles and 87.6% (95% CI 79.1-97.0) and 87.1% (80.3-94.6) in patients treated with 7-9 cycles.
Conclusions In patients with stage II-III HER2+ breast cancer, MRI-guided treatment

optimization is associated with excellent 3-year EFS. These results confirm our earlier findings on pCR rate that one in three patients with HR- disease and one in six patients with HR+ disease can be treated effectively with only three cycles of neoadjuvant chemotherapy. This approach offers a novel therapeutic option that provides a significant reduction in toxicity for this patient population.

RF1-04: Long-Term Follow-up and updated analysis from S0221, Comparing Alternative Dose-Schedules of Adjuvant Anthracycline/Taxane Therapy in High-Risk Early Breast Cancer

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Background: While the landscape of metastatic breast cancer treatment has evolved over the years with incorporation of targeted agents and antibody drug conjugates, chemotherapy continues to be the mainstay of adjuvant treatment for high-risk early breast cancer. S0221 was a previously reported phase III randomized trial performed by the North American Breast Cancer Intergroup (now known as the National Clinical Trials Network [NCTN]) investigating alternative dosing schedules of chemotherapy for early breast cancer.
Methods: S0221 investigated weekly (arms 2 and 4) doxorubicin (A)/cyclophosphamide (C) + granulocyte colony stimulating factor (GCSF; filgrastim or pegfilgrastim) versus (vs.) every 2 weeks (Q2W) (arms 1 and 3) schedule AC, followed by paclitaxel (P) given Q2W or weekly for 12 weeks as post-operative adjuvant therapy in node-positive or high-risk node-negative breast cancer. Weekly AC was given as doxorubicin 24mg/m2 IV once per week and cyclophosphamide 60mg/m2 orally once per day with GCSF. Between December 2003 and November 2010, 2716 patients were randomized in a 2x2 factorial design to: 1) 15 weeks of weekly AC vs. 6 cycles of Q2W AC and 2) P weekly vs. P Q2W with GCSF support and this accounted for arms 1-4. After enrollment of 2716 patients, randomization to the two AC arms was stopped for futility and the trial was modified to study only the P schedules. Between January 2011 and January 2012, an additional 578 patients were assigned to 4 cycles of Q2W AC and randomized to P weekly vs. Q2W accounting for arms 5-6. Here, we present updated survival outcomes of four arms on the original protocol and report, for the first time, analysis of 578 patients from two additional arms on the revised protocol. Updated survival was assessed using log-rank tests and Cox regression models.
Results: At a median follow-up of 12.1 years, among the patients treated in the original protocol, there were no significant differences among the four treatment arms for disease free survival [DFS] (p=0.91) or overall survival [OS] (p=0.34). When stratified by disease subtype, the human epidermal receptor-2 (HER2) positive cohort had the highest 10-year DFS rate (77.7%) compared to HR-positive/HER2-negative (70.6%) or HR-negative/HER2-negative (70.3%) cohorts (p value = 0.0005). The HER2-positive cohort also had a superior 10-year OS rate of 82.3% compared to HR-positive/HER2-negative (78.1%) or HR-negative/HER2-negative (74.9%) cohorts (p=0.0044). Among the 578 patients assigned AC for 4 cycles and randomized to P weekly vs. Q2W P, there were no overall differences in DFS (p=0.32) or OS (p=0.42). There was no difference in 10-year DFS rate between original

(71.3%) or revised (74.4%) protocol (HR 0.80; 95% CI 0.75-1.05). Patients were also stratified in terms of sex (23 men enrolled) and race (379 Blacks enrolled). While women have superior DFS and OS compared to men, due to small number of men and wide CI, these data should be interpreted with caution. Black patients had worse DFS and OS (10-year DFS rate of 64.2% vs. 72.8%; 10-year OS rate of 70.4% vs. 79.0%) compared to non-Blacks. In terms of toxicity, cardiac toxicity profile was more favorable in arms 2 and 4 (0.5-0.7%) that used weekly AC compared to the other four arms that incorporated AC Q2W (1.1-3.3%). There was more skin toxicity with weekly AC schedule with 15.7-16.1% events compared to 2.4-4.0% events in the other four arms. There was no difference in infectious risk or changes in metabolic profile but there were more neurological AEs, and more pain with arms that used Q2W paclitaxel compared to weekly paclitaxel.
Conclusion: As there were no significant outcome differences in DFS or OS between the studied schedules with extended follow-up in the original cohort or in the additional 578 patients treated on the revised protocol, either paclitaxel schedule may be recommended, with selection based on toxicity, cost, or patient preference rather than efficacy.

[AA1]Character limit allowed 3400

RF1-05: DNA methylation patterns are similar in benign tissue from ipsilateral and contralateral breast while different from matched breast cancer, and healthy controls.

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Introduction: Epigenetic changes play a crucial role in cancer development. Among these, DNA methylation is one of the most significant due to its impact on gene expression and genome stability. Hyper-methylation generally suppresses transcription and hypo-methylation promotes it. In breast cancer research, tumor-adjacent tissue is often used as a reference for characterizing epigenetic alterations, but numerous studies suggest that the tissue adjacent to a tumor shares genomic characteristics with the tumor itself. The extent to which epigenetic profiles overlap between tumor, histologically normal tissues from the involved and uninvolved breast, and benign tissues from cancer-free individuals is unknown. Our study aims to address this gap by systematically comparing the DNA methylation profiles across these different tissue types.

Methods: A total of 72 cancer patients were selected from Northwestern University, while data from 182 cancer-free women was obtained from tissue donated to the Komen Tissue Bank. From each individual cancer patient, four regional samples were collected: tumor tissue (TU), tumor-adjacent normal tissue (AN), benign tissue from the opposite quadrant of the involved breast (OQ), and benign tissue from the contralateral uninvolved breast (CUB). From cancer-free patients, normal breast tissue was collected (CFN). From these, we created a "tumor proximity axis": CFN→CUB →OQ→AN→TU.
Methylation profiles were assayed using Illumina's Infinium Methylation EPIC v1.0 BeadChip.
Differential methylation analysis was conducted in two sets of four pairwise comparisons: (1) comparing tumor samples to the four benign tissue categories, and (2) comparing tissue categories that are adjacent along the tumor proximity axis. The differentially methylated CpGs were analyzed for enrichment of transcription factor binding sites (TFBS) and genes.

Results: Following data processing and quality control, there were 72 TU, 63 AN, 70 OQ, 72 CUB, and 182 CFN samples for analysis. Differential methylation analysis showed that TU samples had distinct methylation profiles, with more hypomethylation events compared to hypermethylation events relative to benign tissues. Case-benign tissues (AN, OQ, CUB) exhibited similar methylation profiles, distinct from CFN.
Transcription factor binding site (TFBS) enrichment analysis revealed that case-benign samples, even those distant from tumor, i.e. CUB, showed breast cancer-related methylation changes. Hypomethylated sites in CUB compared to CFN were enriched for TF binding sites TP63, GATA3, ESR1, PR, AR, NR3C1, and GREB1. TU hypermethylation events were enriched for Polycomb-repressive complex 2 (PRC2) binding, including EZH2, SUZ12, and JARID2. ER+ and ER- tumors had distinct methylation profiles. Both ER+ and ER- tumors showed hypermethylation enrichment for PRC2-related binding motifs. However, hypomethylation events differed, with ER+ tumors enriched for hormone receptor-related pathways and ER- tumors enriched for hematopoiesis/immune-related pathways. We did not find any differential methylation between benign tissues from patients with ER+ vs. ER- tumors.

Conclusions: DNA methylation changes profoundly at two points on the tumor proximity axis: CFN to CUB and AN to TU. Specifically, all case-benign tissues, including CUB, exhibited changes associated with breast cancer when compared to CFN. Although the methylation profiles of ER+ and ER- tumors differed, benign tissues showed no differences as a function of ER status of the tumor. These findings demonstrate that benign case tissue is not epigenetically "normal" and provide insights for further investigation into the process of tumorigenesis.

RF1-06: Association of polygenic-based breast cancer risk prediction with patient management

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Background:A breast cancer (BC) risk predictor that combines the Tyrer-Cuzick (TC) risk model with a polygenic risk score ("combined risk score" or CRS) has been shown to significantly improve risk prediction over TC alone. Guidelines recommend that individuals predicted to have ≥20% remaining lifetime risk of BC undergo enhanced management, including annual screening mammography (SM) as early as age 30, annual breast MRI, and genetic counseling (GC). However, little is known about whether and how clinicians manage patients based on risk predicted by CRS. Here, we describe the uptake of mammography, breast MRI, and GC following receipt of CRS results.
Methods:De-identified administrative claims data from the Optum Labs Data Warehouse were linked with de-identified TC and CRS results originally provided to ordering clinicians between August 1, 2017 and November 30, 2021. Patients were included in the cohort if they had continuous medical and pharmacy enrollment for ≥360 days prior to and ≥360 days after the CRS result and were ≥18 years of age. Patients were excluded if they had a history of breast malignancy, BC, or any metastatic cancer during baseline; evidence of BC prevention measures (tamoxifen, raloxifene, aromatase inhibitors, pre-menopausal ovarian function suppression, or mastectomy) prior to receiving CRS results; or evidence of any cancer diagnosis in the ≥360 days after receiving CRS results. Patients were divided into

four subgroups based on their lifetime risk predicted by CRS and by TC alone (high risk (+): ≥20%, average risk (-): <20%): CRS+ TC+, CRS+ TC-, CRS- TC+, and CRS- TC-. Patient management claims were assessed in the ≥360 days after the CRS test date.
Results:The study cohort consisted of 8,662 patients: 2,443 (28.0%) CRS+ TC+, 696 (8.0%) CRS+ TC-, 856 (9.9%) CRS- TC+, and 4,687 (54.1%) CRS- TC-. Mean lifetime risk of breast cancer predicted by the CRS in each group was 29.9% (SD 7.9%), 24.5% (SD 4.3%), 16.4% (SD 2.6%), and 11.2% (SD 4.4%), respectively. Among the 3,309 patients with ≥20% lifetime risk predicted by TC (TC+), 856 (25.9%) had a lifetime risk <20% predicted by the CRS (CRS-). Conversely, among the 5,383 patients with lifetime risk <20% predicted by TC (TC-), 696 (12.9%) had a lifetime risk ≥20% predicted by the CRS (CRS+). Following receipt of CRS results, compared to the CRS- TC-group, the rate of SM in patients under age 40 years was 2.4, 2.2, and 1.9 times higher in the CRS+ TC+, CRS+ TC-, and CRS- TC+ groups, respectively; the rate of breast MRI was 13.8, 8.3, and 7.9 times higher in the CRS+ TC+, CRS+ TC-, and CRS- TC+ groups, respectively; and the rate of GC was 3.2, 2.9, and 2.4 times higher in the CRS+ TC+, CRS+ TC-, and CRS- TC+ groups, respectively.
Conclusions:For a substantial proportion of patients, the CRS predicted a different risk level compared to TC, suggesting that these patients should be managed differently based on their CRS result. Patients with a ≥20% lifetime risk for BC were more likely to undergo enhanced management compared to those with a lifetime risk <20%, regardless of whether their risk was based on the CRS or on TC. These results suggest that clinicians recommended management aligned with guidelines for those with ≥20% lifetime risk, even when such risk was predicted by the CRS.

RF1-07: Multifactor analysis for pathologic complete response (pCR) in a chemotherapy-free regimen of durvalumab, trastuzumab, and pertuzumab (DTP Trial) in HER2-enriched early breast cancer

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Background: The treatment landscape for HER2-positive breast cancers has significantly changed in recent years with the expansion of anti-HER2 therapies, aiming to de-escalate or potentially eliminate use of standard cytotoxic agents. Previously, we demonstrated a 67.6% pathologic response rate (Residual Cancer Burden, RCB 0/1) using a chemotherapy-free regimen of durvalumab, trastuzumab, and pertuzumab in a biologically selected subset of HER2-enriched tumors. Here, we present preliminary analysis of multiple clinical and immune factors that contribute to achieving pCR. Methods: Between 07/2020 and 02/2024, 39 patients with previously untreated stage I-III, ER/PR-negative, HER2-enriched breast cancer (BluePrint®, Agendia) received durvalumab [1120 mg intravenously (IV), every 3 weeks (Q3W)], trastuzumab (8 mg/kg IV loading dose, 6 mg/kg IV Q3W), and pertuzumab (840 mg IV loading dose, 420 mg IV Q3W) for 6 cycles. Multifactor correlation analysis with pCR was performed using clinical characteristics (age, T, N, stage, nuclear grade, BMI), biomarker datasets including patients' baseline HER2DX signatures (Prat et al, Lancet Oncology 2020) (immune, luminal, proliferation, and HER2 amplicon), and immunohistochemical assays [% tumor infiltrating lymphocytes (TILs), %Ki67, and PD-L1 Combined Positive Score (CPS)]. Statistical analyses, including Point Biserial correlations, Chi-Square association tests, and logistic regression, were performed using SAS 9.4 software with significance set at $p<0.05$. Results: Of 39 patients, 2 patients were not

evaluable – one due to an unrelated myocardial infarction and the other due to loss to follow-up. Six patients showed residual disease after DTP therapy and subsequently received standard salvage TCHP chemotherapy. Primary end-point as pathological response rate was 67.6% (N=25/37) with 18/37 patients achieving RCB0 and 7/37 patients achieving RCB1 status. Most adverse events were mild (grade 1-2), with only 5 patients experiencing grade 3 adverse reactions. Our analyses showed pCR was negatively associated with tumor size (p=0.007), nodal status (p=0.036), and advanced clinical stage (p=0.018) but positively associated with % TILs (p=0.02) and PD-L1 CPS score (p=0.038). We also found the HER2DX luminal signature to be associated with pCR (p=0.032). Additional analyses on tumor microenvironment and immune cell crosstalk are currently ongoing with paired baseline and end-of-treatment single cell RNA-sequencing as well as Xenium spatial transcriptomics. Conclusion: While immune markers such as TILs and PD-L1 scores are not typically measured in HER2-positive breast cancers, this data opens up the possibility of rationally leveraging targeted therapies in this subset of immune and HER2-sensitive breast cancers, thus potentially avoiding chemotherapy in the future for this biologically selected group. This regimen holds promise as an effective, relatively non-toxic, and biologically-driven alternative to standard chemotherapy for patients with HER2-enriched subset of early breast cancer. Clinical trial information: NCT03820141. Research Sponsor: Houston Methodist Hospital and Astrazeneca

RF2-01: Factors Influencing Additional Nodal Disease and Pathologic Nodal Upstaging with Axillary Dissection in Patients with Residual Node-Positive Breast Cancer After Neoadjuvant Chemotherapy Enrolled on Alliance A011202 Clinical Trial

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Background: The Alliance for Clinical Trials in Oncology A011202 randomized phase III trial enrolled patients (pts) with clinical T1-3 N1 breast cancer (BC) with residual node-positive disease on sentinel lymph node (SLN) surgery after treatment with neoadjuvant chemotherapy (NAC) to assess whether the recurrence-free interval with radiation to the undissected axilla and regional LNs (AxRT group) is non-inferior to axillary lymph node dissection (ALND) with RT (ALND group). Herein we report on the nodal burden in both groups of pts prior to randomization, and the percentage of pts who had additional positive nodes in the ALND group.
Methods: The pathologic findings from surgery after completion of NAC of all eligible pts were summarized using descriptive statistics. A positive SLN was defined as metastasis ≥0.2 mm present in a node harvested at SLN surgery. Fisher's exact test was used to assess whether the post-chemotherapy pathologic (ypN) category increased with the number of positive LNs found on ALND.
Results: Of 1627 eligible pts registered on A011202, 785 (48.2%) were randomized to ALND and 842 (51.8%) to AxRT. Median age at study entry was 51 years (range: 19-87). Clinical T category prior to NAC was: cT1 in 18.7%, cT2 in 58.9% and cT3 in 22.4% of ALND; cT1 in 20.2%, cT2 in 56.5% and cT3 in 23.3% of AxRT group. Tumor subtype distribution was: 12.1% ER-/PR-/HER2-, 18.6% HER2+, 69.3% ER+ and/or PR+ HER2-. Disease was grade III in 41.5% of ALND group and 38.6% of AxRT group.
The median number of LNs examined from SLN surgery was 4 (range: 1-8) for both ALND

and AxRT arms. There were 99 pts (6.1%) who had 1 LN examined, 240 (14.8%) had 2 LNs examined, and 1288 (79.2%) had 3-8 LNs examined. Among the pts with 2-8 LNs examined from SLN surgery, 815 (53.3%; ALND: 53.7%; AxRT: 53.0%) had 1 positive SLN; 409 (26.8%; ALND: 26.6%; AxRT: 26.9%) had 2 positive SLNs; and 304 (19.9%; ALND: 19.7%; AxRT: 19.8%) had ≥3 positive SLNs. Nodal disease from SLN surgery was macroscopic in 83.6% (ALND: 84.9%; AxRT: 82.3%).
In the ALND arm, 361 pts (46.0%) had additional positive nodes on ALND. This was significantly higher in pts with macrometastatic disease in the SLNs (47.5%) compared with those with micrometastatic disease in SLNs (37.1%) (p=0.043), but no significant differences were found between tumor subtypes (p=0.075). The percentage of pts with additional positive nodes on ALND increased as the number of positive nodes from SLN surgery increased: 33.3% with 1 positive SLN, 52.6% with 2 positive SLNs and 75.9% with ≥3 positive SLNs.
The number of additional positive nodes on ALND was 1 for 130 (36.0%) pts, 2 for 70 (19.4%) pts, and ≥3 in 161 (44.6%) pts. ALND resulted in an increase in ypN category in 24.7% (194/785) of pts; 148 (18.8%) went from ypN1 to ypN2, 29 (3.7%) from ypN1 to ypN3, and 17 (2.2%) from ypN2 to ypN3.
The increase in ypN category for the HER2+ BC [17.1% (25/146)] was significantly lower than that seen in the ER+ and/or PR+ HER2- BC [27.3% (150/549)] (p=0.013) and trended toward being lower than in ER- PR- HER2- BC (26.8% (19/71) (p=0.108).
Conclusion
In patients with residual nodal positive disease after NAC, the rate of additional node-positive disease on completion ALND is high (46%) and increases with presence of SLN macrometastases and as the number of positive SLNs increases. ALND findings resulted in ypN category increase in 24.7% overall; this increase was less frequent in HER2+ disease. Additional data will be critical to understanding the value of ALND in the setting of positive SLNs after NAC in terms of impact on adjuvant treatment recommendations and outcomes.
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RF2-02: Axillary surgery after neoadjuvant systemic therapy (NST) for early-stage breast cancer – Treatment algorithms and prognostic impact of residual micrometastases in five neoadjuvant studies

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Background: For patients (pts) undergoing NST, the axillary status before– and after NST is of major importance for systemic and locoregional treatment. The relevance of nodal micrometastases (ypN1mi) for postneoadjuvant treatment decisions needs to be addressed. A prior analysis in this data set suggested that ypN1mi after NST may have an impact on survival, but the results were limited because of the uncertainty in which clinical situation they had been detected.
Methods: Data from 3199 pts with early BC, who were enrolled in five neoadjuvant AGO-B /

GBG trials (GeparSepto, GeparOcto, GeparNuevo, GeparX, GeparOla) conducted between 2012 and 2019 were included. Initial clinical nodal stage (cN), occurrence of ypN1mi, and axillary intervention were retrospectively analyzed and correlated to outcome.
Results: 3126/3199 (97.7%) pts underwent breast surgery and information was available on initial cN status and axillary intervention. TNBC, HER2+ and HR+/HER2- BC was reported in 38.9%, 29.1% and 32.0%, respectively.
In 1803 cN0 pts we had evaluable information: at baseline pts received sentinel lymph node biopsy (SLNB-BL) only (1155; of which 846 had pN0, 306 pN+ and 3 pNx) or no axillary intervention (NIL) (641) or upfront axillary lymph node dissection (ALND) (7). Of the 641 pts who had no axillary intervention at baseline: after NST pts received SLNB (SLNB-post) only (507) or ALND (79) or targeted axillary dissection (TAD) only (9) or completion ALND as a consequence of SLNB-post/TAD (ALND-c) 17 or NIL (28). For all pts without axillary intervention at baseline, incidence of ypN0 was: 76.6%, 96.8%, 95.8% for HRpos/HER2-, HER2+, TNBC respectively. In case of ypT0, the incidence of ypN0 was: 89.7%, 98.7%, 98.5% for HRpos/HER2-, HER2+, TNBC respectively.
In 1321 cN+ pts we had evaluable information: at baseline pts received no axillary intervention (1105) or SLNB-BL (202; of which 50 had pN0; 151 pN+; 1 pNx) or upfront ALND (14). Of the 1105 pts without axillary intervention at baseline: 876 pts received ALND after NST or limited surgery only (119; of which SLNB-post 96; clipped node resection 5; TAD 18) or c-ALND following limited surgery (100) or NIL (10). For all pts without axillary intervention at baseline, incidence of ypN0 after NST were: 35.8%, 84.4%, 64.4% for HRpos/HER2-, HER2+, TNBC respectively. In case of ypT0, incidence of ypN0 was: 83.1%, 98.8%, 88.0% for HRpos/HER2-, HER2+, TNBC respectively.
Current major guidelines recommend surgical axillary staging to be performed preferably after NST irrespective of cN status. Excluding all pts with LN intervention at baseline, pts with cN0 at baseline but with ypN1mi after NST had a significantly worse iDFS with a hazard ratio (HR) of 4.01 (1.47, 11.0) p=0.007, DDFS HR 5.34 (1.94, 14.7) p=0.001, and OS 5.74 (1.36, 24.2) p=0.017 compared to ypN0 after NST . 5 year LRR rates were 4.1%; 7.0%; 10.0% for ypN0, ypN1, ypN2-3 respectively with no LRR in ypN0i+ or ypN1mi. The results in the cN0 overall cohort did not differ.
In pts with cN+ before NST, the prognostic relevance of ypN1mi seemed less pronounced. Also excluding all pts with LN surgery at baseline, pts with cN+ at baseline but with ypN1mi after NST had a significantly worse iDFS HR 1.87 (1.10, 3.18) p= 0.021 and a trend for worse DDFS HR 1.67 (0.926, 3.03) p= 0.088 and OS HR 1.91 (0.919, 3.96) p=0.083 compared to ypN0 after NST. 5-year LRR rates were 4.8%; 8.6%; 8.6%; 13.0% for ypN0, ypN1mi, ypN1, ypN2-3 respectively with no LRR in ypN0i+. The result in the cN+ overall cohort did not differ.
Conclusion: Results from 3199 pts included in our analysis demonstrate a prognostic impact of ypN1mi in pts with initially cN0 and axillary intervention performed exclusively after NST. In pts with initially cN+, a remaining smaller amount of tumor residuals seems less impactful. Outcome results from prospective trials with less axillary intervention in certain subgroups after NST are needed.

RF2-03: Diagnostic performance of axillary ultrasound after neoadjuvant chemotherapy in initially node-positive breast cancer patients – results from the prospective AXSANA registry trial (NCT04373655)

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Fröhlich S, Wihlfahrt K, Berger T, Lux MP, Kolberg HC, Rubio IT, Gasparri ML, Kontos M, Bonci EA, Niinikoski L, Murawa D, Pinto D, Peintinger F, Ruf F, Basali T, Rief A, Schlichting E, Nina H, Valiyeva Qanimat H, Vanhoeij M, Rebaza L, Aktas Sezen B, Kadayaprath G, Dostalek L, Kothari A, Perhavec A, Ivanov T, Zippel D, Thongvitokomarn S, Kühn T and the AXSANA Study Group

Background: Axillary ultrasound (AUS) is the most widely used imaging procedure to assess the lymph node status in breast cancer patients before surgery. While AUS provides excellent results for predicting axillary involvement in patients undergoing primary surgery, its performance after neoadjuvant chemotherapy (NACT) so far has not been analyzed in large prospective multicentric studies, especially in patients who convert from node-positive to node-negative disease.
Methods: The AXSANA study is a prospective, non-interventional, international registry study investigating different surgical axillary staging procedures with regard to disease-free survival, axillary recurrence rate, and quality of life in patients presenting initially with node-positive breast cancer who convert to a clinically node-negative status after NACT. In the current analysis, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of AUS after NACT are analyzed for all patients included in the AXSANA study between June 1, 2020, and May 31, 2024, whose surgical treatment after NACT was completed. All datasets were systematically monitored.
Results: Among 3,841 eligible patients, 3,429 women (89.3%) underwent AUS after NACT before surgery. The result of the AUS was unclear in 168 of these patients (4.9%), suspicious in 1,061 (30.9%), and unsuspicious in 2,200 (64.2%). Axillary pathological complete response was achieved in 1,794 of 3,261 patients (55.0%) with either suspicious or unsuspicious AUS results after NACT. Sensitivity was 46.4% (95% CI 43.8%-49.0%), specificity 78.8% (95% CI 76.9%-80.7%), PPV 64.2% (95% CI 61.2%-67.1%), and NPV 64.3% (95% CI 62.2%-66.3%).
Conclusions: To our knowledge, this analysis provides the largest dataset from a prospective cohort study to investigate the diagnostic performance of AUS after NACT in initially node-positive breast cancer patients. Our data clearly show that the pathological nodal status after NACT cannot reliably be determined with AUS alone. Consequently, surgical axillary staging remains indispensable to assess the axillary lymph node status after chemotherapy in node-positive breast cancer patients that convert to ycN0. Axillary lymph node dissection based on suspicious AUS results after NACT may lead to surgical overtreatment in many patients.

RF2-04: Ultrahypofractionated versus conventional fractionated sequential boost after whole-breast radiation therapy in breast cancer patients. One-year cosmetic outcomes of a randomized, controlled, phase 3 trial (ULTIMO).

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Objective: There is a growing body of evidence for using ultrahypofractionated (UHF) radiation therapy (RT) for whole-breast RT (WBRT), demonstrating excellent oncological and cosmetic outcome (CO) [1,2]. The question arises, whether the boost given to the lumpectomy cavity could also be given in an UHF way. The ULTIMO trial compares side effects, quality of life and CO at 3 years in breast cancer patients referred for postoperative

WBRT with an indication for a boost to the lumpectomy cavity. Patients were randomized to receive either an UHF single boost dose of 6 Gy or a conventionally fractionated (CF) boost of 10 Gy in 5 fractions. Assuming an alpha/beta value of 3, these schemes are equivalent for tumor control, but with regard to late side effects an additional 2 Gy is given with the 6 Gy UHF boost. Therefore, a possible difference in late side effects including CO should be investigated. Hereby, we report the COs at 1 year.

Methods: From April 2022 to November 2022, 132 patients were randomized. CO was assessed at baseline (pre-RT) and at 1 year after RT using different modalities; 1/digitalized photographs in 2 positions (i.e., arms up and alongside the body, respectively) for a computer-based (i.e., BCCT.core arms down/up [3]) evaluation and a panel-based evaluation; 2/patient-reported outcome measures (PROMs) (i.e., EORTC QoL questionnaire C30 and BR23 [4]). Computer- and panel-based CO was scored using a 4-point Likert scale (0 = Excellent, 1 = Good, 2 = Fair, 3 = Poor). PROMS analyzed for the current analysis included: the patient's opinion on the breast appearance (0 = no difference, 3 = large difference), and patients' overall satisfaction with the treated breast (0 = very satisfied, 1 = reasonably satisfied, 2 = not dissatisfied, 3 = dissatisfied, 4 = very dissatisfied). Side effects were scored using the CTCAE 5.0.
Change in scores was calculated by subtracting the baseline score from the 1-year score. Mann-Whitney U tests compared delta changes between groups, and Wilcoxon signed-rank tests assessed within-group changes.

Results: Follow-up data at 1 year was available for 107 of the 132 patients, 54 received an UHF boost and 53 patients a CF boost. No significant differences were found in baseline COs between groups (BCCT.core down, $p = 0.168$; BCCT.core up, $p = 0.394$; panel-scored CO, $p = 0.422$; PROM satisfaction, $p = 0.459$). However, significant more patients in the UHF group rated their post-surgery CO (i.e., PROM appearance of the breast) worse at baseline ($p = 0.021$).
Overall, an Excellent-Good cosmetic result was scored more often than a Fair-Poor result for both treatment groups at 1 year post-RT: 79% BCCT.core down, 82% BCCT.core up, 88% panel-scored CO for the CF boost vs. 65% BCCT.core down, 63% BCCT.core up, 88% panel-scored for the UHF boost.
Seventy-five percent of patients in the CF group vs. 67% in the UHF group reported to be very to reasonably satisfied with the treated breast after 1 year. Within-group comparisons showed no significant changes in COs from baseline to 1 year post-RT for both CF boost (BCCT.core down, $p = 0.325$; BCCT.core up, $p = 0.716$; panel-scored CO, $p = 0.540$; PROM appearance, $p = 0.408$; PROM satisfaction, $p = 0.962$) and UHF boost (BCCT.core down, $p = 0.203$; BCCT.core up, $p = 0.211$; panel-scored CO, $p = 0.253$; PROM appearance, $p = 0.584$; PROM satisfaction, $p = 0.659$).
The delta changes in COs did not significantly differ between the two groups (BCCT.core down, $p = 0.952$; BCCT.core up, $p = 0.264$; panel-scored CO, $p = 0.506$; PROM appearance, $p = 0.641$; PROM satisfaction, $p = 0.892$).
No high-grade late adverse events (CTCAE ≥ grade III) were reported.

Conclusion: An UHF single sequential boost dose of 6 Gy resulted in similar cosmetic outcomes at 1 year post-RT as compared to a CF sequential boost dose of 10 Gy in 5 fractions. No significant differences were observed in the change of cosmetic scores between the two boost fractionation schemes, however the 3-year results have to be awaited.

RF2-05: Validation of the association between TILs, ER status and benefit of radiotherapy in node positive, breast cancer patients: a DBCG study

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Background: Studies of the Danish Breast Cancer Group (DBCG)82bc cohort of high-risk breast cancer (BC) patients randomized to +/- postmastectomy radiotherapy (RT) showed that high levels of tumor-infiltrating lymphocytes (TILs) in treatment-naïve tumor predicts improved overall survival (OS), especially for patients with estrogen receptor-negative tumors (ER-) (doi: 10.1080/0284186X.2021.1989629). The association was mediated through distant tumor control rather than local control. We aimed to validate these findings in modernly treated, irradiated BC patients and to investigate the predictive value of TILs in terms of benefit from RT.
Methods and Materials: Patients originated from the DBCG-IMN2 cohort (N=980), including node-positive (N+) BC patients with T1-T3 disease, treated between 2007-2014 with breast-conserving surgery or mastectomy, axillary dissection, and loco-regional RT. Irradiation of the internal mammary nodes (IMN-RT) was administered in 460 right-sided patients (47%), and 520 left-sided patients (53%) did not receive IMN-RT (IMN-RT allocated per laterality).
All patients had systemic adjuvant therapy including taxanes, trastuzumab and letrozole. Formalin-fixed, paraffin-embedded treatment-naïve tumor tissue was collected from 980 patients. Stromal TILs were estimated on whole slide Hematoxylin & eosin-stained sections following international guidelines. Tumors were categorized into "low" and "high" TILs groups using a 30% cutoff.
Endpoints included loco-regional recurrence (LRR), distant metastasis (DM), and OS. Kaplan-Meier and Aalen-Johansen estimators along with univariate Cox regression analysis were used for statistical evaluation.
Results: Of the 980 patients, 798 (81%) had estrogen receptor-positive (ER+) tumors, whereas 182 (19%) had ER- tumors. TILs were successfully scored in 911 tumors, and among these, 737 (81%) had low TILs and 174 (19%) high TILs.
Patients with ER-/low TILs tumors (N=88) had significantly worse OS compared to ER-/high TILs (N=76) with an adjusted Hazard Ratio (adj. HR) of 0.3 (95% CI: 0.2-0.6), and absolute reduction in OS at 10-years of 30% (47% vs. 77%). No significant difference in OS was observed among patients with ER+ tumors (adj. HR 0.9 (0.6-1.5)), and the interaction test between ER status and TILs was significant (p=0.006). A similar association was found for DM (adj. HR 0.3 (0.2-0.6)), where patients with ER-/low TILs tumors had a 24% absolute higher 10-year risk (42% vs. 18%) compared to ER-/high TILs. No significant difference in DM was observed among ER+ tumors (adj. HR 0.6 (0.4-1.1)). TILs did not impact LRR in patients with either ER- tumors (adj. HR 1.3 (0.3-5.5)) or ER+ tumors (adj. HR 3.2 (0.9-11.0)).
The association between RT, TILs and OS as previously described in the DBCG82bc study was further validated since a predictive value of TILs in terms of IMN-RT was seen. Patients with low TILs and right-sided tumors, receiving IMN-RT, had an absolute improvement in OS at 10-years of 9% (79% vs. 70%) compared to patients who did not receive IMN-RT (adj. HR 0.7 (0.5-0.9)). For patients with high TILs, an absolute OS improvement at 10-years of 3% was non-significant (79% vs. 76%). Subdividing by ER status did not affect these results.
Conclusions: This study validates previous findings in the DBCG82bc cohort, indicating a robust association between TILs and ER status in irradiated, N+ BC patients. It further supports that poorer OS in irradiated patients with low TILs is mediated through

compromised distant tumor control rather than through local failure. The data suggests a predictive value of TILs in terms of IMN-RT, where the addition of IMN-RT was associated with improved OS in patients with low TILs. The results emphasize the potential of TILs as a biomarker in tailoring more personalized RT strategies for BC patients.

RF2-06: A Nationwide Double-Blind Phase III RCT Comparing Olanzapine and Prochlorperazine for Refractory Chemotherapy-Induced Nausea in NCORP Community Practices

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Background: Despite following ASCO guidelines for antiemetic use during chemotherapy, nausea remains a clinically significant issue and persists as a major concern for patients, surpassing vomiting in significance. Current ASCO guidelines recommend olanzapine or a dopamine antagonist like prochlorperazine for refractory chemotherapy-induced nausea and vomiting (CINV). However, there is a lack of direct empirical comparisons to establish their relative efficacy.

Methods: URCC 16070 enrolled chemotherapy-naïve breast cancer patients starting a high/moderate emetogenic chemotherapy regimen. They were prescribed antiemetics per ASCO guidelines. Screening and assessments occurred during Cycle 1 (N=1,363), followed by randomization for Cycle 2. Patients experiencing ≥ moderate nausea (≥3 on a 1-7 scale) in Cycle 1 and willing to continue (n=310) were randomized in a 1:1:1 ratio to three arms: 1) ASCO regimen plus olanzapine (OLZ), 2) ASCO regimen plus prochlorperazine (PC), or 3) ASCO regimen plus placebo (PBO). Nausea (on a 0-10 scale) and vomiting were assessed via the MASCC Antiemesis Tool (MAT); change in average and maximum nausea are the primary outcomes. A clinically meaningful difference was defined as a ≥2.0 point difference in MAT max nausea between the groups. Quality of life (QOL) was measured using FACT-G. Intent-to-treat analyses (ANOVA and Cohen's Δ effect size calculation for continuous data, chi-square for categorical data) evaluated whether olanzapine or prochlorperazine improved nausea compared to placebo, testing between-arm differences in nausea change from Cycle 1 to 2 at p=0.025.

Results: Among the 310 randomized patients, the median age was 50.7 years, and 80.7% were White, 12.3% Black, 5.8% Asian, and 4.2% Hispanic/Latino, with no significant between-arm differences. The change in MAT maximum nausea was statistically significant for the OLZ (max change = −2.95: Cohen's Δ = 0.58) and PC arms (max change = −2.46: Cohen's Δ = 0.37) compared to the placebo (max change = −1.58; p <0.001 vs OLZ, p = 0.036 vs PC). Similar changes were noted for MAT average nausea in the OLZ (avg change = −2.59: Cohen's Δ = 0.57) and PC arms (avg change = −1.94: Cohen's Δ = 0.28) against the placebo (avg change = −1.29; p <0.001 vs OLZ, p = 0.043 vs PC). As expected, overall QOL decreased in all groups from pre-chemo to post-chemo (FACT-G mean: pre-chemo = 86.9, Cycle 1 = 78.5, Cycle 2 = 77.6). The decrease in QOL in the OLZ arm was significantly less compared to placebo (FACT-G mean between-group difference = +4.91; p = 0.006), while the change in the PC arm was not significantly different from placebo (FACT-G mean between-group difference = −0.87; p = 0.61). Clinically meaningful between-group differences in the FACT-G Physical (mean difference = +2.28; p = 0.008) and Functional (mean difference = +1.81; p = 0.009) subscales were also observed for the OLZ group compared to placebo. Vomiting

rates decreased in the OLZ group (Cycle 1: 17.4% vs Cycle 2: 2.0%) and the PC group (Cycle 1: 16.4% vs Cycle 2: 3.7%) versus the placebo (Cycle 1: 13.2% vs Cycle 2: 8.1%; p=0.12) but lacked significance due to low numbers. In terms of direct comparison, OLZ exhibited superiority over PC in terms of QOL (FACT-G mean difference = +5.80; p = 0.006), but not for nausea control (MAT max nausea: mean difference = +0.67; p = 0.142; MAT avg nausea: mean difference = +0.53; p = 0.054).

Conclusions: This URCC NCORP phase 3 RCT establishes OLZ as a highly effective option for managing refractory nausea during moderate to highly emetogenic chemotherapy. Both statistically and clinically significant reductions in nausea, as well as clinically meaningful improvements in QOL, particularly in physical and functional domains, highlight OLZ's substantial impact. For patients who may not tolerate OLZ, PC remains a viable alternative. These results support OLZ's incorporation into antiemetic protocols and underscore its role in enhancing patient outcomes. Funding: R01CA200579, UG1CA189974

RF3-01: TBCRC 056: A Phase II Study of Neoadjuvant Niraparib with Dostarlimab for Patients with BRCA- or PALB2-mutated Breast Cancer: Results From the ER+/HER2- Cohort

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Background: PARP inhibitors (PARPi) have an approved role in treating patients (pts) with germline BRCA1/2 mutated (gBRCAm) breast cancer in the advanced and early settings, with activity also seen in pts with gPALB2m. Preclinical data suggest PARPi exposure leads to intratumoral activation of the proinflammatory cGAS/STING pathway, recruiting CD8+ T cells, and sensitizing gBRCAm cancers to immune-targeted therapy. Preoperative treatment with PARPi monotherapy has demonstrated notable pathologic complete response (pCR) rates. TBCRC 056, an open-label randomized phase II study, evaluates the PARPi niraparib (N) with the anti-PD-1 antibody dostarlimab (D) in the neoadjuvant treatment of gBRCAm or gPALB2mHER2-negative breast cancer, with cohorts for both triple negative breast cancer (TNBC, Arms A and B) and hormone receptor positive (HR+) disease (Arm C). Results from Arm C are being presented.
Methods: Eligible pts in Arm C had a gBRCAm or gPALB2m and a tumor size of >1.0 cm, ER and/or PR+ (>10%). All pts in Arm C received 18 weeks of combination N 200 mg orally once daily and D 500 mg IV every 3 weeks (1 cycle = 3 weeks), with mandatory baseline (BL) and week 3 core tumor biopsies. After 18 weeks, pts either underwent surgery or received additional preoperative systemic therapy (per physician's choice) if residual disease was present on imaging. The primary study objectives are to evaluate pCR at surgery as well as change in stromal tumor infiltrating lymphocytes (sTILs) from BL to 3 weeks in Arms A and B. Evaluation of pCR and change in sTILs in Arm C are secondary study objectives, with pCR to be reported with a two-sided exact 90% confidence interval without hypothesis testing. Change in sTILs between BL and 3 weeks was evaluated using a Wilcoxon signed-rank test, and the association between sTILs and pCR was assessed using a Wilcoxon rank sum test.
Results: Accrual to Arm C was completed after 18 pts with HR+/HER2- breast cancer were enrolled and treated between April 2021 and May 2024 (5 gBRCA1m (27.8%), 13 gBRCA2m

(72.2%), 0 gPALB2m). Median age was 41.8 years (range 28.8-61.9), 14 were self-reported White (77.8%), 4 (22.2%) Black, and none Hispanic. Stage distribution was 38.9% stage I, 44.5% stage II, and 16.7% stage III. All 18 pts had ER+ >10% disease. Sixteen of 18 pts have completed protocol therapy through surgery, with results pending for 2 pts. Among evaluable pts, 10 (62.5%) completed 6 cycles of D with mean number of cycles received 4.7. Six pts discontinued D early (1 progressive disease, 1 withdrawal of consent, 4 unacceptable adverse events (AE)). Thirteen pts (81.2%) completed 6 cycles of N with mean number of cycles received 5.4. Three pts discontinued N early (1 progressive disease, 1 withdrawal of consent, 1 unacceptable AE). The most common AE (> grade 2) with D+N were rash (25.0%), elevated liver function tests (18.8%), diarrhea (12.5%), and hypertension (12.5%). Of the 16 pts evaluable for response at this time, 3 had pCR at surgery (18.8%, (90% CI 5.3% - 41.7%)), 11 (68.8%) had residual disease (3 RCB-I, 4 RCB-II, 4 RCB-III), and 2 (12.5%) received additional chemotherapy before surgery. Among 12 pts with evaluable sTILs at BL and 3 weeks, mean sTILs at BL was 11.9% (range 1.0% – 35.0%), mean sTILs at week 3 was 23.8% (range 5.0% - 90.0%), with a mean absolute increase of 11.9%; Wilcoxon signed rank p=0.09). Higher sTILs (as a continuous variable) at BL was associated with pCR (odds ratio 1.09, 95% CI 1.00 – 1.19, Wilcoxon rank sum p = 0.03).
Conclusions
In pts with gBRCAm HR+/HER2- breast cancer, 18 weeks of PARPi and anti-PD1 resulted in a pCR rate of 18.8%, and a mean absolute increase of sTILs of 11.9% at 3 weeks. Further data from the TNBC cohort of this study are awaited. Given the reported activity of PD-1 inhibitors in the preoperative management of HR+ breast cancer, additional exploration of targeted non-chemotherapy approaches is of great interest in this pt population.

RF3-02: Efficacy of adjuvant avelumab by PD-L1, tumor infiltrating lymphocytes and residual cancer burden in high-risk triple negative breast cancer: secondary and exploratory endpoints of the phase III A-BRAVE trial.

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Background: The A-BRAVE trial showed an improvement in long-term outcome with the anti-PD-L1 avelumab administered as adjuvant therapy for high-risk early TNBC patients. Here, we report the efficacy of avelumab according to PD-L1, tumor infiltrating lymphocytes (TILs), and residual cancer burden (RCB).
Methods: The phase III A-BRAVE trial randomized 466 patients with high risk early TNBC to 1-year avelumab vs observation after completion of standard surgery and neoadjuvant/adjuvant chemotherapy. High risk was defined as high disease burden in case of primary surgery (n=83 Stratum A) or invasive residual disease (breast and/or nodes) after neoadjuvant chemotherapy (n=383 Stratum B). Avelumab vs observation improved outcomes (Conte P ASCO 2024): +5.1% in 3-yr DFS in ITT and Stratum B (non-significant, co-primary endpoints); +8.5% in 3-yr OS in ITT (p=0.035) and Stratum B (p=0.070); +7.5% in 3-yr DDFS in ITT (p=0.028).
Here, we report DFS (secondary endpoint), DDFS and OS by PD-L1 status in ITT, as well as

DFS, DDFS and OS by post-neoadjuvant chemotherapy TILs and RCB in Stratum B. PD-L1 was evaluated on surgical tumor samples (Stratum A) and diagnostic core-biopsies (Stratum B) with the IHC 73-10 RUO assay (Agilent Technologies); the % of positive stromal cells/total stromal cells was calculated with digital pathology. PD-L1 high was defined according to a previously published >21% cut-off (Dieci MV, Eur J Cancer 2020). In Stratum B, surgical samples were evaluated for TILs (centrally) and RCB (locally) according to guidelines. Only p values <0.05 are shown.

Results: PD-L1 expression was prognostic: every 1% increment was associated with improved DFS (HR 0.99, 95%CI 0.97-1.00, p=0.014), DDFS (HR 0.98, 95%CI 0.97-0.99, p=0.005) and OS (HR 0.99, 95%CI 0.97-1.00, p=0.049). PD-L1 high vs PD-L1 low patients showed better outcome: 3-yr DFS 80.6% vs 64.3%, p=0.004; 3-yr DDFS 85.1% vs 69.8%, p=0.002; 3-yr OS 89.6% vs 79.5%, p=0.032. No significant interaction between PD-L1 and treatment arm was observed for any efficacy endpoint. However, the benefit of avelumab vs control was more evident for PD-L1 low: 3-yr DFS 67.3% vs 61.1%, 3-yr DDFS 73.6% vs 65.9%, 3-yr OS 84.6% vs 74.1% in PD-L1 low; 3-yr DFS 77.1% vs 84.4%, 3-yr DDFS 85.7% vs 84.4%, 3-yr OS 91.4% vs 87.5% in PD-L1 high.
In Stratum B, RCB was prognostic. Outcomes by RCB I, II and III were: 3-yr DFS 76.7%, 67.5%, 32.4%; 3-yr DDFS 76.5%, 73.6%, 39.7%; 3-yr OS 83.1%, 81.7%, 46.6%, p<0.001 for all endpoints. No significant interaction for any efficacy endpoint was observed between RCB and treatment arm. The benefit of avelumab vs control by RCB category was: RCB I (n=30, 3-yr DFS 84.6% vs 70.6%; 3-yr DDFS 84.6% vs 70.6%; 3-yr OS 84.6% vs 82.4%), RCB II (n=224, 3-yr DFS 67.3% vs 67.6%; 3-yr DDFS 75.8% vs 71.3%; 3-yr OS 87.4% vs 75.7%), RCB III (n=55, 3-yr DFS 44.6% vs 19.2%, p=0.028; 3-yr DDFS 55.0% vs 23.1%, p=0.003; 3-yr OS 57.5% vs 33.6%, p=0.026).
TILs on residual disease were significantly prognostic in Stratum B: every 1% increase was associated with: HR 0.98 95%CI 0.97-0.99, p=0.002 for DFS; HR 0.98 95%CI 0.97-0.99, p=0.004 for DDFS; HR 0.98, 95%CI 0.96-1.00, p=0.010 for OS. Results were similar after correction for RCB.
There was no significant interaction for any efficacy endpoint between TILs and treatment arm. The benefit of avelumab vs control was more evident in TILs<10% (3-yr DFS 57.7% vs 50.0%; 3-yr DDFS 64.7% vs 53.6%; 3-yr OS 74.6% vs 61.8%) than TILs>10% (3-yr DFS 72.5% vs 75.4%; 3-yr DDFS 81.5% vs 79.2%; 3-yr OS 89.6% vs 85.6%).
Conclusions: Efficacy of avelumab for high-risk TNBC did not significantly differ by PD-L1 in the ITT, or by TILs and RCB in Stratum B. However, these biomarkers help identifying subgroups of patients at poorer prognosis deriving the greatest magnitude of benefit from this treatment.

RF3-03: Nivolumab + Ipilimumab (NIVO+IPI) compared to capecitabine for triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy – Final results of BreastImmune-03, a multicenter randomized open-label phase II trial.

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Background - Early triple negative breast cancer (TNBC) with moderate to extensive residual disease after neoadjuvant chemotherapy and surgery is associated with high risk of recurrence. Before the era of (neo)adjuvant anti-PD-1 antibody treatment, capecitabine was a treatment option in the adjuvant setting. BreastImmune-03 trial was designed to evaluate the efficacy of post-operative nivolumab + ipilimumab (NIVO+IPI) compared to capecitabine for patients (pts) with class II or III Residual Cancer Burden (RCB) after standard neoadjuvant chemotherapy.
Methods - BreastImmune-03 was a multicenter, randomised, open-label phase II trial comparing a dual immunotherapy regimen (NIVO+IPI: nivolumab (360 mg intravenously [IV], every 3 weeks for 8 doses) and ipilimumab (1 mg/kg IV, every 6 weeks for 4 doses)) versus capecitabine (1000 mg/m^2 BID, 14 days on / 7 days off for 8 cycles) as adjuvant treatment in TNBC with RCB II-III after neoadjuvant chemotherapy and surgery. Randomization (1:1) was stratified by center, ECOG performance status (PS), and RCB class. A total of 114 pts (46 events) were needed to detect an improvement from 60 to 80% of the 2-year disease free survival (DFS) rate (Hazard ratio [HR]: 0.44; 80% power, alpha 5%). Secondary endpoints include overall survival (OS), local-regional recurrence rate (LRR), distant metastasis-free survival (DMFS), safety according to CTCAE 5.0, and quality of life scales.
Results - From July 2019 to October 2021, 95 pts were randomized: 50 in the capecitabine arm and 45 in the NIVO+IPI arm (median age: 47 years [29-82], ECOG PS 0: 80%, RCB III: 40%) across 17 French centers. Due to an unexpectedly high rate of biological myocarditis in the NIVO+IPI arm (11% versus 2% in capecitabin arm), enrollment was prematurely closed based on independent data monitoring committee recommendation. With a median follow-up of 34.3 months (interquartile range: 33-36), 39 events (relapse or death) were reported: 17 (38%) in the NIVO+IPI arm versus 22 (44%) in the capecitabine arm (HR: 0.84; 95% CI: 0.45-1.59; log-rank test p-value: 0.59). There was no difference in terms of OS (HR: 0.98; 95% CI: 0.44-2.20; log-rank test p-value: 0.97), nor in LRR and DMFS. Notably, 38% of pts discontinued treatment prematurely due to treatment-related adverse events (AE) in the NIVO+IPI arm, compared to 14% in the capecitabine arm. The most common AE (>10%) in the NIVO+IPI arm were myocarditis (grade 3 only), hyperthyroidism (28.9%), hypothyroidism (22.2%), diarrhea (11.1%) and fatigue (31.1%).
Conclusion – Although BreastImmune-03 trial was prematurely ended, a 6-month postoperative NIVO+IPI combination does not seem to improve clinical outcomes in TNBC pts with RCB II-III compared to capecitabine. In addition, immune-mediated AE limit the use of the NIVO+IPI combination in this setting. A centralized collection of tumor and blood tissue has been performed and translational correlative studies are ongoing.
Clinical trial information: NCT03818685

RF3-04: NRG-BR004: A Randomized, Double-blind, Phase III Trial of Taxane/Trastuzumab/Pertuzumab with Atezolizumab or Placebo in First-line HER2-positive Metastatic Breast Cancer

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Background: The CLEOPATRA trial established trastuzumab, pertuzumab, and docetaxel (THP) as a standard of care for first-line metastatic, HER2-positive breast cancer with

median progression-free survival (PFS) of 18.7 months and median overall survival (OS) of 57 months. NRG-BR004 was a phase III, placebo-controlled trial designed to determine whether the addition of the PD-L1 inhibitor atezolizumab to THP would improve PFS, relative to THP/placebo in patients with newly documented HER2-positive measurable metastatic breast cancer.

Methods: In this double-blinded phase III trial, patients with newly documented HER2-positive measurable metastatic breast cancer were randomly assigned (in a 1:1 ratio) to receive atezolizumab (1200 mg IV) or placebo on days 1 and 22 every six weeks until progression or for two years, combined with 1) a taxane regimen selected by investigator (weekly paclitaxel 80 mg/m2 IV on days 1, 8, 15, 22, 29, and 36, or docetaxel 75 mg/m2 IV on days 1 and 22 every six weeks) administered until progression or toxicity required discontinuation and 2) trastuzumab with pertuzumab on days 1 and 22 every six weeks until progression or toxicity required discontinuation. The primary endpoint, PFS, was assessed by investigators using RECIST 1.1 criteria.

Results: One hundred ninety out of the planned 600 patients were randomized over 37 months from May 1, 2019, to May 20, 2022. An imbalance in Grade 5 AEs coupled with continued poor accrual and the changing landscape in HER2+ metastatic breast cancer resulted in the permanent closure to further enrollment in May of 2022. A decision was made to discontinue atezolizumab/placebo in patients receiving the investigational component of the trial therapy and to unblind investigators and patients. Treatment with the standard components of therapy continued at investigator discretion. The study continued to collect information on PFS events, deaths, and late immune adverse events through April of 2024.

The primary analyses were performed in September 2024. Median follow-up for PFS was 31.1 months and 35.6 months for OS. Characteristics for the randomly assigned patient population included median age of 52 years, 66% had estrogen-receptor positive disease, 79% had visceral metastases, 4% had brain metastases, 82% had presented with de novo metastatic disease, and only 14% were PD-L1 positive. Characteristics were balanced between the two arms. The mean and median number of cycles of trastuzumab, pertuzumab, and taxanes were similar in the atezolizumab and placebo arms.

Total grade 2, 3, and 4 adverse events were similar across both arms. Grade 5 events totaled 6 in the atezolizumab arm and 0 in the placebo arm at the time of closure of the study and discontinuation of atezolizumab/placebo.

Two-year PFS was 54.0% and 45.6% in the atezolizumab-THP arm and placebo-THP arm (hazard ratio 0.73 [0.49, 1.09]), respectively. Three-year OS was 86.4% and 81.7% in the atezolizumab-THP arm and placebo-THP arm (hazard ratio 0.8 [0.39, 1.63]), respectively. Stratified log-rank test was used to compare the distribution of PFS and OS between the two treatment arms with estrogen receptor status and prior neoadjuvant or adjuvant trastuzumab as the stratification factors.

Conclusions

Accrual to BR004 was closed to further enrollment in May 2022 and atezolizumab/placebo was discontinued due to poor accrual and the imbalance in Grade 5 events between the two arms, 6 deaths in the atezolizumab-THP arm versus 0 in the placebo-THP arm. The results suggest checkpoint inhibitors may have activity in metastatic HER2+ breast cancer, but further studies would be necessary to establish efficacy and improve safety.

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RF3-05: A Phase 2 Study of Neoadjuvant HER2-targeted Therapy +/- Immunotherapy with Pembrolizumab (neoHIP)

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Background: Immune checkpoint inhibition (ICI) is synergistic with HER2-directed therapy in pre-clinical models. Clinically, pembrolizumab (K)-mediated ICI in addition to HER2-directed therapy with trastuzumab (H) was safe and demonstrated modest activity in H-resistant HER2-positive (HER2+) metastatic breast cancer. Because ICI may confer more robust activity when administered earlier in the course of disease, H and K administered in the curative-intent, treatment-naive setting may allow for de-escalation of chemotherapy; confer life-long, tumor-specific immunity; and ultimately improve cure rates. Moreover, the synergy of H and K with paclitaxel (T) may overcome the need for dual HER2-blockade with H plus pertuzumab (P). In this randomized, multicenter, phase 2, open-label trial, the efficacy and safety of neoadjuvant THP vs THP-K vs TH-K were explored.
Methods: In this multicenter, prospective, randomized phase 2 study, treatment-naive patients aged ≥18 years with stage II-III HER2+ breast cancer were randomized and stratified by clinical nodal status (positive vs. negative) and hormone receptor status (positive vs negative). Arm A received weekly T at 80 mg/m2 for 12 weeks, H at 8mg/Kg (loading dose) followed by 6mg/Kg every 3 weeks x 3 doses, P at 840mg (loading dose) followed by 420mg/Kg every 3 weeks x 3 doses (THP); Arm B received THP plus K at 200mg every 3 weeks x 4 doses (THP-K); and Arm C received TH-K every 3 weeks x 4 doses. Definitive surgery occurred 3-6 weeks after the last dose, followed by postoperative treatment at the treating physician's discretion per local standards. The primary endpoint is pathologic complete response (pCR; ypT0/Tis ypN0). Secondary endpoints include pCR rates defined by ypT0ypN0 and ypT0/Tis, residual cancer burden (RCB), event-free survival (EFS), and safety.
Results: 138 patients with untreated, stage II-III HER2+ breast cancer were enrolled between January 4, 2019, and March 25, 2024: 58 to Arm A, 58 to Arm B, and 22 to Arm C (enrollment to Arm C was terminated after a prespecified interim efficacy analysis). In Arm A, 4 patients were discontinued before surgery. The primary endpoint, pCR (ypT0/Tis ypN0) was achieved in 28 of 54 (51.9%) and 39 of 58 (67.2%) evaluable patients in Arms A and B, respectively. After a prespecified interim analysis, enrollment to Arm C was discontinued due to a pCR rate <40%. Secondary endpoints for alternative definitions of pCR rates included 46.3% vs 51.7% for ypT0ypN0 and 53.7% vs 68.9% for ypT0/Tis in Arms A and B, respectively. Among the 54 patients in Arm A evaluable for RCB: 28 (51.9%) of 54 were classified as RCB-0, 9 (16.7%) as RCB-1, 13 (24.1%) as RCB-2, and 4 (7.4%) as RCB-3. Among the 58 patients in Arm B evaluable for RCB: 39 (67.2%) of 58 were classified as RCB-0, 4 (8.6%) as RCB-1, 12 (20.7%) as RCB-2, and 2 (3.5%) as RCB-3. At this interim analysis (data cut off, September 30, 2024), the median follow-up is 24 months. In the THP arm, 25.9% of patients experienced grade 3 adverse events (AEs), compared to 22.4% in the THP+K arm. None of the grade 3 AEs in Arm A were immune-related events (irAEs). Of the 116 patients enrolled to Arms A and B, 57.8% were White/non-Hispanic, 22% Hispanic, 11.5% Asian, 5.2% Black/African American, 0.9% American Indian, and 2.5% other. On Arm

A, 15 of the 30 (50%) White/non-Hispanic subjects achieved pCR; similarly, 7 of the 14 (50%) Hispanic subjects achieved pCR. However, on Arm B, 27 of the 35 (77.14%) White/non-Hispanic subjects achieved pCR while only 6 of the 12 (50%) Hispanic subjects achieved a pCR.
Conclusion: This is the first study to demonstrate the synergy between ICI and dual HER2-directed therapy in curative intent setting. Thus, early administration of the combination may allow for de-escalation of cytotoxic chemotherapy and improve long-term outcomes in high risk, HER2+ disease. Additionally, the impact of response to specific therapies by racial groups warrants further investigation.

RF3-06: Mepitel Film for the Reduction of Radiation Dermatitis in Post-mastectomy Radiation Therapy: Results from Alliance A221803: A Multicenter Phase III Randomized Clinical Trial

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Background: Radiation dermatitis (RD) is a frequent side effect and source of morbidity in patients receiving radiation treatment (RT) for breast cancer (BC), particularly for those undergoing post-mastectomy radiation (PMRT). The Alliance A221803 phase III randomized trial investigated the potential of Mepitel Film (MF) to mitigate RD in this at-risk patient population. We hypothesized that MF would reduce the severity of RD compared to institutional standard of care (SoC).
Methods: Patients undergoing conventionally fractionated PMRT for non-inflammatory BC were randomized in a 2:1 fashion to receive either MF or institutional SoC. Stratification factors included patient body mass index (BMI) (< 25; 25-29.99; and ≥ 30), planned RT bolus (yes; no), planned RT boost (yes; no), and the presence or absence of reconstruction. The primary endpoint was the difference in area under the curves (AUCs) estimated from the repeated measures (means) mixed model using the patient-completed symptom scale component of the modified Radiation-Induced Skin Reaction Assessment Scale (mRISRAS) weekly during RT, 1-2 weeks after the end of RT (EoT), and at 3 months post-RT between the two arms controlling for stratification factors. The primary analysis included the first 192 assessable patients. Secondary endpoints included non-blinded provider component of the mRISRAS score, the combined (patient symptom scale and provider component) mRISRAS score, and acute adverse events (AEs) by CTCAE V5.0. This trial is registered on ClinicalTrials.gov (NCT04989504).
Results: Between July 26, 2022 and January 4, 2024, 216 patients enrolled, with 143 assigned to MF and 73 to SoC. Stratification factors were balanced. Forty-five (21%) out of 216 enrolled patients were planned to have boost, 140 (65%) reconstruction, 75 (35%) planned bolus, and BMI was < 25 (26%), 25-29.99 (31%), and ≥30 (43%). Eight patients in the SoC arm withdrew consent prior to RT, leaving 65 patients in the SoC and 143 patients in the MF arm in the modified intention-to-treat analysis. Median age was 53 years. Eight or 9 survey timepoints were collected depending on receipt of boost, with a median of 8 mRISRAS surveys completed per patient. The estimated AUC was 45.10 in the SoC arm and 33.88 in the MF arm, with a difference in AUC (MF-SoC) of -11.22 (95% CI: -19.90, -2.54; p=0.012), meeting the primary endpoint of a significant reduction in patient-reported RD with MF compared with SoC. The improvement was observed across all stratification factors. In a pre-specified analysis, a statistically significant arm × timepoint interaction

effect was seen, showing that patient-reported mRISRAS scores were lower with MF during RT at week 4, 5, and 6 of RT as well as at 7-14 days after the EoT (p = 0.0267). The combined patient and provider mRISRAS scores also identified less RD with MF, with a difference in AUC of -16.10 (p =0.011). The reduction in AUC with MF for unblinded provider mRISRAS scores was not statistically significant (AUC difference [MF-SoC -3.65 [95% CI: -9.35, 2.05, p=0.21]). Grade 2 at least possibly related or grade 3+ regardless of attribution RD was reduced with MF (44.6% in SoC, 25.2% in MF, p=0.005), whereas there was no significant difference in acute acneiform rash (1.54% in SoC vs. 1.4% in MF, p=0.94), pruritus (6.2% in SoC, 9.1% in MF, p=0.47), or skin infection (0% in SoC, 4.2% in MF, p=0.09). No grade 4 or 5 adverse events (AEs) were reported. Long-term follow-up is ongoing for evaluation of chronic AEs and reconstruction outcomes.
Conclusions: Mepitel Film significantly reduces RD compared to SoC in patients undergoing RT for breast cancer. These findings support the use of MF as a new SoC option for RD prevention in patients undergoing PMRT.
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RF3-07: ROSCO: Response to Optimal Selection of neoadjuvant Chemotherapy in Operable breast cancer: Randomised phase III, stratified biomarker trial of neoadjuvant 5-Fluorouracil,Epirubicin & Cyclophosphamide vs Docetaxel & Cyclophosphamide chemotherapy

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Biomarkers for specific cytotoxic chemotherapy sensitivity could better inform drug selection. Both CEP17 duplication and abnormal Topoisomerase 2 copy number appear associated with anthracycline sensitivity in the adjuvant setting (Bartlett et al 2015). This data is however not currently applied clinically. Taxanes are a routine component of adjuvant and neoadjuvant chemotherapy either in combination or sequenced with anthracyclines, or increasingly used in the absence of anthracyclines. ROSCO: ISRCTN15094808 was designed to prospectively evaluate the clinical utility of these two biomarkers for initial neoadjuvant chemotherapy selection.

Between November 2015 and May 2023, 990 consenting patients with early breast cancer considered suitable for neoadjuvant chemotherapy were randomised to four cycles of either Epirubicin and Cyclophosphamide with optional 5 Fluorouracil ((F)EC), or Docetaxel and Cyclophosphamide (TC). Patients with Grade 1 or 2 ER Rich, PR Rich, HER-2 negative tumours and all T1 N0 tumours were excluded. All HER-2 positive cancers were treated with concurrent anti HER-2 antibodies. Participants were stratified by centrally assessed biomarker status as biomarker normal (BM normal) with both CEP17 and TOP2A normal, or biomarker abnormal (BM abnormal) with CEP17 duplication and/or TOP2A abnormal. Surgery was performed after 4 cycles of chemotherapy; where pathological complete response (pCR) was not achieved, crossover to the alternative treatment arm for a further 4 cycles was given in an adjuvant setting. Crossover before surgery was permitted where interim biopsy after 4 cycles confirmed viable residual disease. The primary endpoint of the

study is pCR ypT0/Tis ypN0 after initial neoadjuvant chemotherapy.

Of the 990 patients consented, 496 were randomised to TC and 494 to (F)EC. 24 patients with no cancer seen in interim biopsy received further neoadjuvant chemotherapy and also had pCR at final surgery, these are considered in the primary analysis as non pCR.

Data from 950 patients are evaluable for the primary endpoint. Overall pCR was 245 (26%): the TC arm was 131 (27%) and (F)EC arm was 114(24%). Overall BM was normal in 233 (24%) with BM abnormal in 756 (76%).

With TC the pCR percentages for BM normal and abnormal were very similar: 30% and 27% respectively. For (F)EC BM normal, pCR is 17% and for FEC BM abnormal, it is 26%. Final data cleaning is ongoing, testing for treatment by biomarker interaction will be presented.

Higher response to (F)EC in the biomarker abnormal group was observed across all pathological subtypes tested.

Sensitivity analysis excluding 35 TC and 22 (F)EC patients where crossover chemotherapy was given off protocol neoadjuvantly despite a negative core biopsy or where patients withdrew prior to the primary endpoint was conducted. The pCR proportions observed in the treatment by biomarker groups were not impacted.

Preliminary analysis of this large prospective evaluation of CEP17 and TOP2A as potential predictors of anthracycline sensitivity conducted in a neoadjuvant context shows that evaluation of these biomarkers shows no predictive value for sensitivity to TC but demonstrates differential pCR to (F)EC. Suggesting women with BM abnormal cancers are likely to benefit more from inclusion of anthracyclines. Anthracycline-free chemotherapy may be considered as an option for women with BM normal cancers.

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